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Evaluation of plasma cotinine, serum vitamin b_{12} and serum ferritin between smokers and nonsmokers with and without recurrent aphthous stomatitis -A cross sectional study

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Abstract

Background: Recurrent Aphthous Stomatitis (RAS) is a chronic inflammatory ulcerative, relapsing, remitting oral mucosal disease affecting 50% of the population. Despite of numerous studies, the precise etiopathogenesis of RAS has not been delineated, studies suggested that the abnormalities in hematinic components such as serum ferritin and serum vitamin B_{12} in particular were found to be twice as common whereas Cotinine have a positive influence in RAS. Cotinine being the major degradation product of nicotine metabolism and it can be assayed in various biological Fluids. In the present study, it has been assessed in plasma, because plasma cotinine concentration is highly associated with tobacco smoke exposure from both direct smoke inhalation and environment exposure.

Aims and objectives: The present research was undertaken to compare the level of Plasma Cotinine, Serum B_{12} and Serum Ferritin between smokers and nonsmokers with and without RAS, to enlighten their

role and influence in severity of Recurrent Aphthous Stomatitis.

Materials and method: The study population comprises of 40 patients, divided into 4 groups, of which 10 in each group, Group 1-Healthy patients, Group 2- Patients with Recurrent Aphthous Stomatitis, Group 3-Smokers without RAS, Group 4-Smokers with RAS. Informed consent was obtained from all patients for this study,3ml of blood samples were collected and analysed for Plasma Cotinine levels by using 96 well Micro Plate Cotinine-Specific-Enzyme Linked Immunosorbent Assay method and serum vitamin B_{12} and serum ferritin levels using Chemiluminescent Assay Method.

Results: Significant decrease in levels of serum vitamin B_{12} and serum ferritin were observed in patients with RAS when compared to healthy volunteers, in contrast these parameters are comparatively increased in smokers and smokers with RAS. The study also depicts that plasma cotinine was significantly reduced in smokers with RAS when compared to smokers, thereby unveiling the fact that severity and prevalence of RAS is more common among patients with recent cessation or those who discontinued regular smoking habits.

Conclusion: The present study sheds lights by showing that increase in the level of plasma cotinine, serum B_{12} and serum ferritin, decreases the severity of RAS between. smokers and nonsmokers.

Keywords: Cotinine, Ferritin, Nicotine, Sutton Disease, Vitamin B_{12}.

Introduction

The term aphthae is derived from the Greek word aphthi, which insinuate “to set on fire “or “to inflame” and was delineated by Great philosopher Hippocrates as pain pertinent to a typical disorder of the oral mucosa during his time (Recurrent Aphthous Stomatitis).[1] Recurrent Aphthous Stomatitis is a chronic inflammatory ulcerative, relapsing, remitting oral mucosal disease that affects 50% of the population, with three archetypal forms namely minor, major and Herpetiform types.[2,3]

Despite multitudinous research, the authentic element of Recurrent Aphthous Stomatitis. remains obscure, but abnormalities in Hematinic factors such as Serum Ferritin and Serum Vitamin B_{12} have been found to be the primary source. [4] Although the mechanism of RAS is not fully understood, many investigators observed lower prevalence rate of RAS in smokers when compared to nonsmokers, increase in the incidence of RAS following the termination of smoking, and alleviation of lesions upon renewing of cigarette smoking. [5-12]

Cotinine is the prime degradation product of nicotine metabolism and it can be assayed in various biological fluids. Due to its relatively longer half-life in circulation, Cotinine is used as a most common biomarker to assess nicotine exposure and abstinence. [13,14]

In the present study, cotinine level has been assessed in plasma, because plasma cotinine concentration is highly associated with tobacco smoke exposure from both direct smoke inhalation via cigarette depletion and environment exposure via secondhand smoke. [15-17] Number of studies have reported that pure nicotine (not the other components of tobacco) is a safe component, although it depends on the dose. [18,19] Until date, tobacco has had only a preventative role against aphthous ulcers, therefore the role of inundated nicotine in tobacco as a therapeutic factor should be taken in to account. [20]

Cotinine Levels in Plasma increases with increase in cigarette smoke exposure. The blood Plasma Cotinine level that is accepted as defining a smoker is > 25 ng ml^{-1}. Levels above 25 ng ml^{-1} correlate well with the

number of cigarettes smoked per day. Cotinine Levels between 0 and 25 ng ml^{-1} occur in individuals exposed to cigarette smoke passively. [8]

Assessment of Plasma Cotinine level should be done within 24hrs, after smoking. According to Centre for Disease Control and Prevention, Smokers are classified as:

- Current Smokers - Smoked ≥ 100 cigarettes over their lifetime and smoked at least 10 hrs before the time of interview.
- Never Smoker - Never smoked any cigarettes in their lifetime.
- Former Smoker - Smoked ≥ 100 cigarettes over their lifetime and were not smoking at the time of interview or 24hrs prior to the interview. [21]

Hence, the present study is carried out to compare the level of Plasma Cotinine, Serum B_{12} And Serum Ferritin to enlighten the etiological factor of Recurrent Aphthous Stomatitis as well as to assess whether the Plasma Cotinine levels have a positive influence in Recurrent Aphthous Stomatitis and to determine the role of Serum Ferritin and Serum Vitamin B_{12} in Recurrent Aphthous Stomatitis Versus Controls. In essence we embark on a journey to find if there is any positive from the often disreputed smoking habit.

Materials and method

This study was carried out in the Department of Oral Medicine and Radiology, Sree Mookambika Institute of Dental Sciences, Kulasekharam, and Department of Biochemistry, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari district, Tamil Nadu. Clinical trial registration was made with Clinical Trial Registry of India (CTRI) (REF/2017/11/015768). Total number of subjects – 40. The patients were divided into 4 groups.

Group I- Healthy Volunteers (10).

Group II- Recurrent Aphthous Stomatitis with no smoking habit (10).

Group III- Smokers without Recurrent Aphthous Stomatitis (10).

Group IV- Smokers with Recurrent Aphthous Stomatitis (10).

Materials Required: Cotinine Micro –Plate Enzyme Immunoassay Kit, Serum Ferritin Kit and Serum Vitamin B_{12} Kit.

Inclusion and exclusion criteria: inclusion criteria

Group -I	Group-II	Group-III	Group-IV
Healthy volunteers aged between 18- 50 years.	History of regularly recurrent episodes of Minor oral aphthous ulceration over the preceeding 6 months or longer.	Patients with a history of smoking ≥100 cigarettes and who are current smokers.	History of regularly recurrent episodes of minor oral aphthous ulceration over the preceeding 6 months or longer.
Patients with no history of RAS lesions.	An average of at least two ulcers per month over the previous 6 months.	Patients who are using Nicotine in any other forms.	Patients with a history of smoking ≥100 cigarettes and who are current smokers.

Exclusion criteria

Group -I	Group-II	Group-III	Group-IV
History of any systemic disease in which oral aphthous ulceration may be a feature.	Patients with history of Smoking and any other Tobacco Habits.	History of any systemic disease in which oral aphthous ulceration may be a feature	Patients with no history of smoking habits.
Concurrent medication with systemic Steroids, immunomodulatory or Cytotoxics drugs.	History of any systemic disease in which oral aphthous ulceration may be a feature	Concurrent medication with systemic Steroids, immunomodulatory or Cytotoxics drugs.	Patients with history of any systemic diseases.

Procedure in detail

Individuals satisfying the inclusion and exclusion criteria will be included in the study. Parameters to be analysed are Plasma cotinine, serum ferritin and serum vitamin B_{12}.A total of 3ml blood sample was obtained after informed consent from every willing participant. The sample was centrifuged for 20minutes at 2000 - 3000rpm. Plasma and Serum were separated.1ml of Plasma was used for cotinine estimation, while the remaining 2ml for serum ferritin and serum vitamin B_{12} estimation.

In the lab, the Plasma cotinine assay was performed using a commercial 96 well microplate Cotinine – Specific Competitive immunoassay (fig.1). Ten microlitre of the test samples, control samples or calibration standards were added to the wells of a 96 well coated with immobilized Cotinine specific monoclonal antibody. This was immediately followed by 100microlitre of enzyme conjugate containing horseradish peroxidase labelled cotinine. These were inoculated in the dark for 30min at 21^{o}C.The reaction was then stopped by the addition of 100 microlitre 2.0 N Sulphuric acid. The cotinine concentration in each well was then quantified by measuring the light absorbance at wavelengths of 450 and 630nm and comparing with the standard curve. This assay has a relative sensitivity of 100% and relative specificity of 90.8% and intra assay variability of < 7.5%. Each sample was assayed in duplicate and the mean of the two values used for analysis. (fig.3)

2ml of blood sample is taken and analyzed for serum ferritin and vitamin B_{12} level using Chemiluminescent assay method. (fig.2).

Statistical analysis

The data was expressed in number, percentage, mean and standard deviation. Statistical Package for Social Sciences (SPSS 16.0) version used for analysis. ANOVA (Post hoc) followed by Dunnet t test applied to find the statistical significant between the groups. P value less than 0.05 considered statistically significant at 95% confidence interval.

Results and observation

The present study was undertaken to compare the Plasma cotinine serum ferritin and serum vitamin B_{12} among smokers and nonsmokers with Recurrent Aphthous stomatitis versus controls.

Table -1 depicts the Mean Serum Vitamin B_{12}, Serum Ferritin and Plasma Cotinine values of 4 Groups.Table-2 depicts the Mean Serum Vitamin B_{12}, Serum Ferritin and Plasma Cotinine values of Healthy patients with other Groups. Table-3: Comparison mean vitamin B_{12}, ferritin and plasma cotinine values of Group-II with other

groups. Table-4: Comparison mean vitamin B_{12}, ferritin and plasma cotinine values of Group-III with other groups. Table-5: Comparison mean vitamin B_{12}, ferritin and plasma cotinine values of Group-IV with other groups. Table-6 depicts the Multiple Comparison of mean Serum Vitamin B_{12}, Serum Ferritin and Plasma Cotinine values between each groups with the other group. And showed that P value less than 0.05 significant when compared each group with other groups.

The analysis demonstrated that on comparing the mean Serum Vitamin.B_{12} and Ferritin among 4 groups, the patients with RAS had significantly low levels of Serum Vitamin. B_{12} and Ferritin whereas these parameters are raised in Smokers and also showed significant difference among Smokers with RAS (Graph - I). On Comparing the Plasma levels of Cotinine among 4 groups showed that significant rise in levels of Plasma cotinine among smokers and decreased levels of Plasma cotinine among Smokers with RAS and RAS patients (Graph -2).

Hence, the current study reveals that RAS is predominant among patients with serum ferritin and vitamin B_{12} deficiency. The vital role of plasma cotinine in etiopathogenesis of RAS among smokers was also noted in this study, as its level appeared to be significantly reduced among patients with a history of recent cessation and discontinuity of regular smoking habits along with increased occurrence of RAS in them.

Discussion

Cotinine have protective effect on oral mucosa by causing increased keratinization and this keratin layer act as a mechanical and chemical barrier against trauma or microbes. And it also hypothesized that nicotine may be responsible agent for reduction in RAS prevalence rate in smokers because it modulate local immune responses, as it has been shown to induce T- cells anergy and inhibit the production of Pro - inflammatory cytokines such as interleukins (2,6,8,10)and tumour necrosis factor alpha and also activates the release of adrenocorticotrophic hormone and cortisol, through the hypothalamus –pituitary –adrenal axis and leading to further suppression of inflammatory pathways.[5,6,8]

Number of studies have been reported that, pure nicotine is a safe component, although it depends on the dose. Until date, tobacco has had only a prophylactic role against aphthous ulcers, (fig.4) therefore the role of submerged nicotine in tobacco as a therapeutic factor should be taken in to account. [18-20] Hence, these interesting factors leads us to undertake this study to found out the comparison of this Plasma Cotinine, Serum Ferritin and Vitmin.B_{12} levels among smokers(fig.5) and nonsmokers with Recurrent Aphhous Stomatitis versus controls.

In the present study we found out that patients who are smokers have significantly high Plasma Cotinine, it was mainly due to the increased levels of cigarettes smoking, on basis of number of package and duration, when compared with smokers with RAS (fig.6). And this study results were consistent with the epidemiological study done by Atkin et al in 2002 to compare the blood cotinine levels between a group of patients with minor recurrent aphthous stomatitis and a group representative of the general population. Result of his study showed that the mean cotinine level among smokers in the RAS group was significantly lower than in smokers. [8]

And also our present study showed decreased prevalence rate of Recurrent Aphthous Stomatitis among smokers when compared to nonsmokers, due to protective effect of nicotine by causing keratinizaion of oral mucosa among smokers. This result was found to be consistent with the case control study done by Mohamed S and Janaki ram C in 2014. [22]

In the present study, on comparing the Serum VitaminB_{12} and Ferritin levels among 4 group. The patients with RAS had significantly low levels of Serum VitaminB_{12} and Ferritin whereas these parameters are raised in Smokers and also showed significant difference among Smokers with RAS. This study results were consistent with study done by Sumathi K et al in 2014 among, 50 positive cases of oral ulcers and 25 normal healthy controls. From this study, it was concluded that Screening of RAS patients by doing serum ferritin estimations is mandatory. [23]

In the present study we found that optimal cut off values for distinguishing smoker from non-smoker was serum 10ng/ml, cotinine content in serum depends mainly upon the duration of smoking before the time of questionnaire about the smoking habits, which was also consistent, with the study done by Duques et al in 2017 among smokers and periodontitis patients. [24]

On analysing the Serum Ferritin level among smokers and nonsmokers, the level was found to be increased in smokers in our study. The study results is also found to be similar to the cross-sectional study done by Shivasekar et al in 2018 between smokers and nonsmokers comprised of 200 subjects out of which 100 were smokers and rest were nonsmokers. [25]

From this study, on comparing the plasma cotinine levels between smokers and Smokers with RAS, it was found that Plasma Cotinine levels were significantly reduced among smokers with RAS. It was well evident that Cotinine plays one of the etiological factor for RAS and also Cotinine substitutes can be supplemented for this patient for prevention of further recurrence.

Hence the present study sheds light on the importance of investigating Serum Ferritin and Vit.B_{12} in patients with RAS and also shows the need for close supervision regarding the dietary intake of Iron and nutritional supplements, especially Vitamin B_{12} in such patients. The study also reveals the fact that the prevalence of RAS is high among patients with recent cessation of smoking habits, therefore Nicotine Replacement Therapy can be suggested for these patients.

Conclusion

Knowledge from the study suggested that, pure nicotine (not the other components of tobacco) is a safe component, although it depends on the dose. Until date, tobacco has had only a prophylactic role against aphthous ulcers, therefore the role of submerged nicotine in tobacco as a beneficial factor should be taken in to account. Among smokers and nonsmokers, the prevalence of RAS is high among patients with recent discontinuation of smoking habits, therefore Nicotine Replacement Therapy can be suggested for this patient. It is also vital to analyse the Serum Ferritin and Vit.B_{12} levels as a prior investigative procedure as well as close supervision regarding the dietary intake of Iron and nutritional supplements especially Vitamin B_{12} while managing patients with Recurrent Aphthous Stomatitis.

Reference

1. Compilato DO, Carroccio AN, Calvino FR, Di Fede G, Campisi G. Haematological deficiencies in patients with recurrent aphthosis. J Eur Acad Dermatol Venereol 2010;24(6):667-73.

2. Marchini L, Campos MS, Silva AM, Paulino LC, Nobregon FG. Bacterial diversity in aphthous ulcers. Oral Microbiol Immunol 2007; 22:225-311.

3. Healy CM, Williams DM, Thornhill MH. Haematinic deficiency in recurrent aphthous stomatitis: its prevalence and response to treatment. Oral Biosic Med 2004; 1:259-66.

4. Kumar A, Ananthakrishnan V, Goturu J. Etiology and pathophysiology of recurrent aphthous stomatitis: a review. Int J Cur Res Rev 2014; 6(10):16-22.

5. Shapiro S, Olson DL, Chellami SJ. The association between smoking and aphthous ulcers. Oral Surg Oral Med Oral Pathol 1970; 30:624-34.

6. Axell T, Henricsson V. Association between recurrent aphthous ulcers and tobacco habis. Scand J Dent Res 1985; 93:239-242.

7. Tuzun B, Wolf R, Tuzun Y, Serdaroglu S. Recurrent Aphthous stomatitis and smoking. Int J Dermatol 2000; 39:358-60.

8. Atkin PA, Xu X, Thornhill MH. Minor recurrent aphthous stomatitis and smoking: an epidemiological study measuring Plasma Cotinine. Oral Dis 2002; 8:173-6.

9. Ussher M, West R, Steptoe A, McEwen A. Increase in common cold symptoms and mouth ulcers following smoking cessation. Tob Control 2003;12(1):86-8.

10. Mc Robbie H, Hajek P, Gillison F. The relationship between smoking cessation and mouth ulcers. Nicotine Tob Res 2004; 6:655-9.

11. Marakogu K, Sezer RK, Toker HC, Marakoglu I. The recurrent aphthous stomatitis frequency in the smoking cessation people. Clin Oral Investig 2007; 11:149-53.

12. Dorsey C. More observation on relief of aphthous somatitis on resumption of Cigarettes Smoking: A report of three cases. Calif Med 1964; 101:377-8.

13. Dolcini MM, Adler NF, Lee P, Bauman KL. An assessment of the validity of adolescent self-reported smoking using three biological indicators. Nicotine Tob Res 2003; 5:473-83.

14. Patrick DL, Cheadle A, Thomson DC, Diehr P, Koepsell T, Kinnes S. The validity of self-reported smoking: A review and meta-analysis. Am J Public Health 1994; 84(7):1086-93.

15. Eskenazi B, Prehn AW, Christianson RE. Passive and active maternal smoking as measured by serum Cotinine: The effect on birth weight. Am J Public health 1995;85(3):395-8.

16. God tfred son NS, Prescott E, Vestbo J, Osler M. Smoking reduction and biomarkers in two longitudinal studies. Addiction 2006; 101(10):1516-22.

17. Simoni M, Baldacci S, Punctoni R, Pistelli F, Farchi S, Presti EL et al. Plasma, Salivary and urinary cotinine in nonsmoker Italian women exposed and unexposed to environmental tobacco smoking. Clin Chem Lab Med 2006; 44(5):632-8.

18. Joseph AM, Norman SM, Ferry LH, Prochazka AV, Westman EC et al. The Safety of transdermal nicotine as an aid to smoking cessation in patients with cardiac disease. N Engl J Med 1996;335(24):1792-8.

19. Motamedi MRK, Golestannejad Z. Use of pure nicotine for the treatment of aphthous ulcers. Dent Res J 2015;122(2):197-8.

20. Bittoun R. Recurrent aphthous ucers and nicotine. Med J Aust 1991;154(7):471-2.

21. Schoenborn CA, Vickerie JL, Barnes PM. Cigarettes Smoking Behaviour of Adults: United States. CDC 2003; 331:1-28.

22. Mohamed S, Janakiram C. Recurrent Aphthous Ulcers among Tobacco Users- Hospital Based Study. J Clin Diag Res 2014;8(11): ZC64-ZC66.

23. Sumathi K, Shanthi B, Palaneeswari MS, Manjuladevi AJ. Significance of Ferritin in Recurrent Oral Ulceration. J Clin Diag Res 2014;8(3):14-5.

24. Duque A, Martínez PJ, Giraldo A, Gualtero FD, Ardila MC, Contreras A, et al. Accuracy of cotinine serum test to detect the smoking habit and its association with periodontal disease in a multicenter study. Med Oral Patol Oral Cir Bucal 2017;22 (4): e425-31.

25. Shivasekar M, Vinothini VM, Kumar RY. Study of serum ferritin in smokers. Asian J Pharm Clin Res 2018; 11(1):374-5.

Results and observation

Table 1: Mean serum vitamin B12, ferritin and plasma cotinine values of different groups.

Groups	Vitamin, B12(pg/ml) (MEAN±SD)	Ferritin(ng/ml) (MEAN±SD)	Plasma cotinine (ng/ml) (MEAN±SD)
Group-I	345.80±1.94	44.21±2.70	0.0099±0.10
Group-II	330.20±1.18	27.95±2.76	0.0034±0.01
Group-III	427.30±1.94	158.14±9.79	0.0100±0.01
Group-IV	382.50±1.91	118.29±7.50	0.0013±0.01

Table 2: Comparison mean vitamin B12, ferritin and plasma cotinine values of Group-I with other groups.

Groups	Vitamin B12 (pg/ml) (MEAN±SD)	p value	Ferritin (ng/ml) (MEAN±SD)	p value	Plasma cotinine (ng/ml) (MEAN±SD)	p value
Group-I	345.80±1.94		44.21±2.70		0.0099±0.10	
Group-II	330.20±1.18*	0.04	27.95±2.76*	0.001	0.0034±0.01*	0.01
Group-III	427.30±1.94*	0.001	158.14±9.79*	0.001	0.0100±0.01*	0.01
Group-IV	382.50±1.91*	0.001	118.29±7.50*	0.001	0.0013±0.01*	0.01

(*p<0.05 significant compared Group-I with other groups)

Table 3: Comparison mean vitamin B12, ferritin and plasma cotinine values of Group-II with other groups.

Groups	Vitamin B12 (pg/ml) (MEAN±SD)	p value	Ferritin (ng/ml) (MEAN±SD)	p value	Plasma cotinine (ng/ml) (MEAN±SD)	p value
Group-I	345.80±1.94*	0.04	44.21±2.70*	0.001	0.0099±0.10*	0.01
Group-II	330.20±1.18		27.95±2.76		0.0034±0.01	
Group-III	427.30±1.94*	0.001	158.14±9.79*	0.001	0.0100±0.01*	0.01
Group-IV	382.50±1.91*	0.001	118.29±7.50*	0.001	0.0013±0.01*	0.01

(*p<0.05 significant compared Group-II with other groups)

Table 4: Comparison mean vitamin B12, ferritin and plasma cotinine values of Group-III with other groups

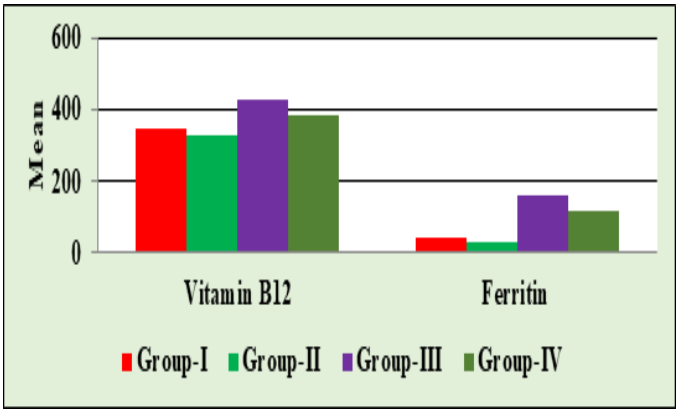
Groups	Vitamin B12 (pg/ml) (MEAN±SD)	p value	Ferritin (ng/ml) (MEAN±SD)	p value	Plasma cotinine (ng/ml) (MEAN±SD)	p value
Group-I	345.80±1.94*	0.001	44.21±2.70*	0.001	0.0099±0.10*	0.01
Group-II	330.20±1.18*	0.001	27.95±2.76*	0.001	0.0034±0.01*	0.01
Group-III	427.30±1.94		158.14±9.79		0.0100±0.01	
Group-IV	382.50±1.91*	0.001	118.29±7.50*	0.001	0.0013±0.01*	0.01

Table 5: Comparison mean vitamin B12, ferritin and plasma cotinine values of Group-IV with other groups.

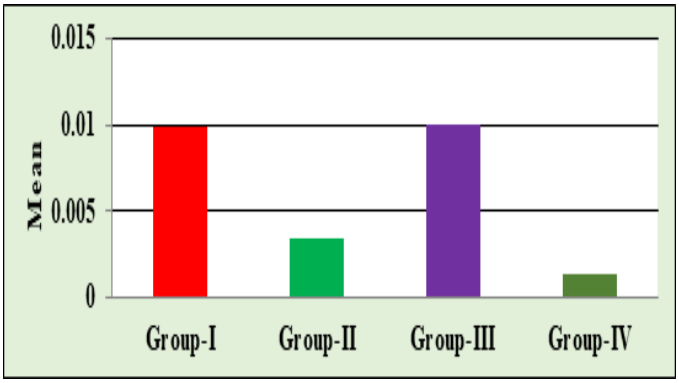
Groups	Vitamin B12 (pg/ml) (MEAN±SD)	p value	Ferritin (ng/ml) (MEAN±SD)	p value	Plasma cotinine (ng/ml) (MEAN±SD)	p value
Group-I	345.80±1.94*	0.001	44.21±2.70*	0.001	0.0099±0.10*	0.01
Group-II	330.20±1.18*	0.001	27.95±2.76*	0.001	0.0034±0.01*	0.01
Group-III	427.30±1.94*	0.001	158.14±9.79*	0.001	0.0100±0.01*	0.01
Group-IV	382.50±1.91		118.29±7.50		0.0013±0.01	

Table 6: Multiple comparison mean vitamin B12, ferritin and plasma cotinine values between the groups.

Groups	Vitamin B12 (pg/ml) (MEAN±SD)	Ferritin (ng/ml) (MEAN±SD)	Plasma cotinine (ng/ml) (MEAN±SD)
Group-I	345.80±1.94	44.21±2.70	0.0099±0.10
Group-II	330.20±1.18*	27.95±2.76*	0.0034±0.01*
Group-III	427.30±1.94*,#	158.14±9.79*,#	0.0100±0.01*,#



Graph 1: Mean serum vitamin B12, ferritin values of different groups.



Graph 2: Mean plasma cotinine values of different groups.

Figure 1: Human plasma Cotinine Elisa Kit.

Figure 2: Estimation of serum vitamin b12 and ferritin byclia method.

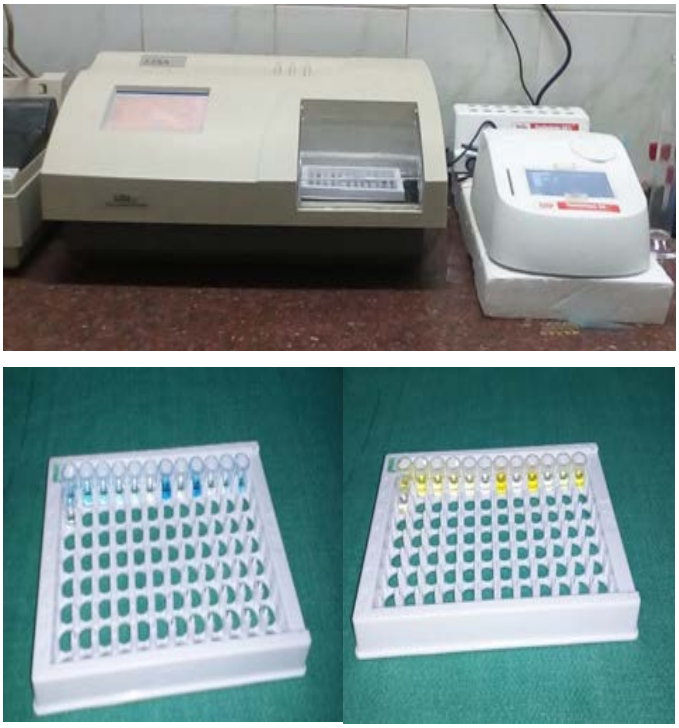


Figure 3 : Estimation of plasma cotinine and Elisa reader.

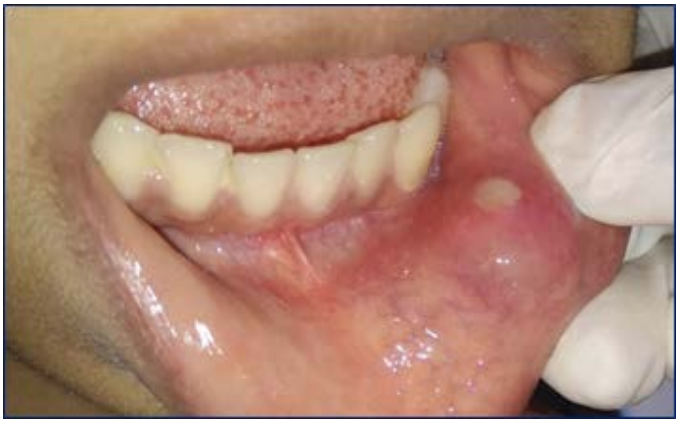


Figure 4: Recurrent aphthous stomatitis.

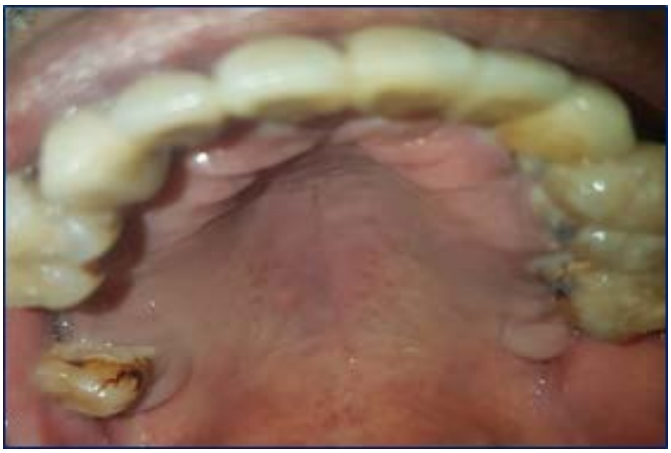


Figure 5: Smoker without RAS

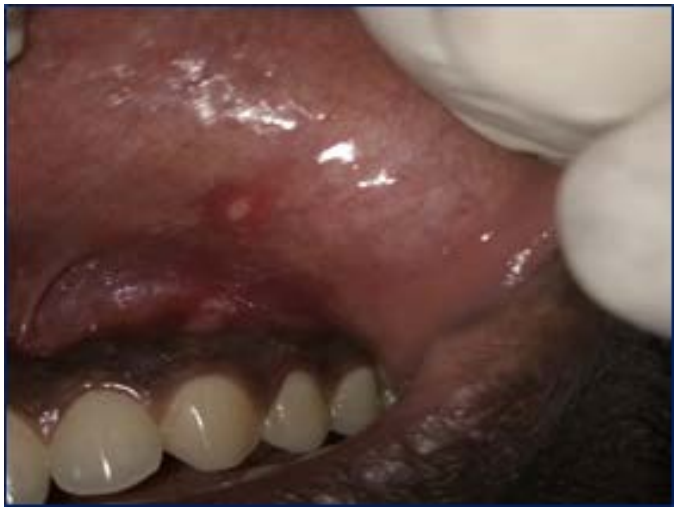


Figure 6: Smoker with RAS.