

ASSOCIATION OF CLINICAL VARIANTS OF LEUKOPLAKIA WITH DIFFERENT TYPES OF TOBACCO - AN INSTITUTION BASED RETROSPECTIVE STUDY

¹Gayathri Karthikeyan, ²Hannah R, ³Jaiganesh Ramamurthy

¹Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077, Tamilnadu, India.

²Senior lecturer, Department of oral Pathology and Microbiology, Saveetha Dental college & Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077 Tamilnadu, India.

³Professor and Head, Department of periodontics, Saveetha Dental college & Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077 Tamilnadu, India.

151601039.sdc@saveetha.com

hannahr.sdc@saveetha.com

jaiganeshr@saveetha.com

ABSTRACT

The aim of this study is to assess the prevalence of leukoplakia in different types of tobacco users. Tobacco utilization in India is increasing but there are considerable changes in the types and methods by which it is used. Oral cancer affects as many as 274,000 people worldwide annually 20. In this study The data collection was carried out during the period between July 2019 to march 2020. After searching through 86,000 patient records the Sample size for the study was taken as 93 patients diagnosed with leukoplakia. The data that was collected was tabulated in excel and then imported into SPSS software. Smoking form of tobacco was found to be used by 72.92% of patients. Smokeless form of tobacco use was noticed among 25% of the patients and a combination of both smoking and smokeless form of tobacco used was noticed among 2.083% of the patients . Homogenous types of leukoplakia were found among 76.04% of the patients followed by the non-homogenous form which included about 23.96% of the patients. Males were the most common gender comprising about 96.88% of the patients and females comprised about 3.13% . It was also noted that the most common type of tobacco used was cigarettes. Chi-square test was done to associate the type of tobacco used and clinical variants of leukoplakia, it was not statistical significant (p=0.596). Within the limitations of the present study we were able to conclude that there was no association between the habit of tobacco use and the clinical variant of leukoplakia . Multicentric study with a larger sample size will give a more definitive outcome.

KEYWORDS: Cancer , Leukoplakia , Smoking , Tobacco, Potentially malignant disorder

INTRODUCTION:

Tobacco utilization in India is increasing but there are considerable changes in the types and methods by which it is used. The World Health Organization has predicted that death caused due to tobacco use in India may exceed 1.5 million annually by 2020. According to WHO 194 million men and 45 million women use tobacco in smoked or smokeless form in India (World Health Organization, 2003). Persistent use of tobacco use will have detrimental effects on the body sometimes even leading to cancer. Oral cancer affects as many as 274,000 people worldwide annually (Garg, Raj and Chandra, 2013). 20 per

100000 population are affected by oral cancer which accounts for about 30% of all types of cancer (Sankaranarayanan et al., 2005). Over 5 people in India die every hour everyday because of oral cancer and the same number of people die from other cancers too (Gupta, Ariyawardana and Johnson, 2013). Oral Potentially malignant disorders (OPMD) have been considered to be a heterogeneous group of lesions associated with a risk of malignant transformation (MT) to form invasive cancer either at the time of diagnosis or at a later stage. Oral squamous cell carcinoma (OSCC) is the major form of oral cancer and the sixth common malignancy in the world (Sridharan, Ramani and Patankar, 2017) and the most common in India with an incidence rate as high as 30- 40% (G. Jayaraj et al., 2015). These lesions have some characteristic features. These are dysplastic features, dysplasia does not follow a predictable sequential progression from mild to moderate to severe dysplasia and in rare cases may revert to normal. It is not uncommon for a mild dysplasia to rapidly progress to an invasive carcinoma; however, not all epithelial dysplasia develops into carcinoma, while they transform these characteristic features are referred to as precancerous lesions (Gifrina Jayaraj et al., 2015). Documentation of these lesions with photographs helps with registration of clinical information in the oral cavity. To improve the learning, communication (Hannah et al., 2018). Recent molecular and genetic studies have provided us with evidence that the most of, if not all, head and neck squamous cell carcinomas (HNSCCs) generally develop within a field of a preneoplastic lesion or condition and hence have a tendency to spread rapidly (Gupta and Ramani, 2016). It has also been noticed that viruses also play a role in the carcinogenesis process of the oral squamous cell carcinoma.

There are several types of Oral premalignant lesions existent, The most common are oral leukoplakia, oral submucous fibrosis (OSMF), and oral erythroplakia (Yardimci, 2014) OPMD have the transformation rate ranging from 0.6%-18% (Viveka et al., 2016). Leukoplakia has been defined as “A white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer (van der Waal, 2009). Oral premalignant lesions will exhibit mild dysplasia and it is not uncommon for a mild dysplasia to rapidly progress to an invasive carcinoma. Numerous researches have been conducted on the prevalence of leukoplakia on different types of tobacco users, A Study conducted by Roed-Petersen and Pindborg in a Danish sample of 450 leukoplakias found that 32% were exclusively male snuff users (Roed-Petersen and Pindborg, 1973). Another study conducted by Mehta et al surveyed 50,915 Indian villagers and found that prevalence of oral leukoplakia was between 0.2 and 4.9 with involvement of both habits (Mehta et al., 1969)

Like any other dental procedure which requires early detection like the impaction of 3rd molar (Sivaramakrishnan and Ramani, 2015) Early detection of premalignant lesions and oral cancer is also very important. With the advancement in technology various new methods have been discovered for the early detection, one such technique is the use of Microarrays, it is a tool for genomic scale profiling of gene expression, and a potentially valuable means of understanding the complex interactions and networks in the development of several diseases including cancer (Thangaraj et al., 2016). Recent studies have also shown that saliva can also be used in the diagnosis of oral cancer (Shree et al., 2019) A range of salivary metabolites were significantly altered in oral leukoplakia and oral squamous cell carcinoma (Sridharan et al., 2019)

The need for this type of research is to fulfill the gap present between the clinical practitioners and the researchers. It helps formulate better and more efficient methods for early detection that can be used by clinical practitioners while diagnosing patients. The aim of this study is to assess the prevalence of leukoplakia among different types of tobacco users.

MATERIALS AND METHOD:

The study was conducted in a university setting. The advantage of conducting this study in a university was the easy access to large amounts of data. Considering the disadvantage the main one would be the

data collected not being location specific. Ethical approval was obtained from institutional ethical committee (ethical approval number: SDC/SIHEC/2020/DIASDATA/0619-0320) .The number of people involved in the study included two researches and the data and results were reviewed by two reviewers. The data collected was carried out during the period between July 2019 to march 2020 .After searching through 86,000 patient records the Sample size for the study was taken as 93 patients diagnosed with leukoplakia. The sampling bias for the study was minimized by including all the data that was obtained. Internal and external validity for the study was present.

The data that was collected was tabulated in excel and then imported into SPSS software. Incomplete or censored data was excluded from the study. The Statistical test that was run was the chi sequence test using the statistical software SPSS by IBM. the Independent variables that were included in the study was age, sex , and type of tobacco consumption. The Dependent variables included the form of tobacco used type. Correlation of type of tobacco with leukoplakia was done using Chi-square test.

RESULT AND DISCUSSION:

Based on the habit of tobacco it was found that the smoking habit of tobacco was used by 72.92% of patients. Smokeless form of tobacco was used by 25% of the patients and the combination of both smoking and smokeless form of tobacco was noticed among 2.083% of the patients (Figure 1). Based on the type of clinical variant of leukoplakia among tobacco users homogenous types of leukoplakia was found among 76.04% of the patients followed by the non homogenous form which included about 23.96%% of the patient (Figure 2) . Based on the gender distribution among patients , males were the most common gender comprising about 96.88% of the patients and females comprised about 3.13% (Figure 3). Based on the distribution of type of tobacco use he most common type of tobacco use noted was cigarette which included about 61.46% of the population followed by pan which was 18.75% of the population, bedi was 8.333% , bedi or cigarette was 4.167% , gutkha was 3.125%(3) and haans and pan or cigarette was 2.083% .There was no statistical significance present when correlation was made between habit or type of tobacco use ($p=0.596$) and the clinical variants of leukoplakia ($p=0.615$) (Figure 5) (Figure 6) after running the chi-square test.

Knowledge about the prevalence of tobacco and the prevalence of leukoplakia is not enough , the cause of such a change should also be known. The chemical constituents of tobacco and its combustion end products as tars and resins are irritating substances capable of causing leukoplakia (Mondal *et al.*, 2017). Nicotine, one of the principal constituents, exerts a negative reaction at the cellular level by altering metabolism and invading the contacts between tissues (Aggarwal, 2018). Nicotine also decreases the salivary pH by the accumulation of calcium and phosphorus ions, and its addiction is a critical factor in the progression of oral potentially malignant disorders such as leukoplakia . The combination of use of tobacco and alcohol will aggravate the process even further (Negi *et al.*, 2019). Consumption of alcohol will lead to other complications as well like hepatocellular carcinoma Hepatocellular carcinoma is the second most common cause of cancer death in the world and its incidence has dramatically increased worldwide in the past two decades (Gheena and Ezhilarasan, 2019)

Oral cancer still remains a major health problem, however early detection has been seen to reduce this burden (Negi *et al.*, 2019). . The treatment of oral cancer is a very painful process and sometimes can even lead to other complications. One such complication is following surgical treatment of cancer of the floor of the mouth where reconstruction by pedicled or free flaps is done. A rare complication that can be encountered following such treatment is the attachment of the tongue to the floor of the mouth (Boffano *et al.*, 2019) which is termed as ankyloglossia (Jangid *et al.*, 2015) . Another surgical compilation that can occur is the formation of pulp stones, Pulp stones are discrete calcifications found in the coronal or radicular pulp some etiological factors like aging, caries, operative procedures, and periodontal disease is claimed to pre-dispose pulp stone formation (Swathy, Gheena and Varsha, 2015). Cancer cells of the oral

cavity have also been shown to exhibit Cellular cannibalism which is also seen as giant cells . The presence of various types of giant cells depends on the lesions in which they are present (Urs, Yaming and Malhotra, 2018) (Kumar et al., 2015) Oral cancer can be prevented by action against risk factors, especially tobacco which is the key factor , the prominent inclusion of strong pictorial warnings in existing written warnings on the labels of tobacco and alcohol products, The enforcement of laws on youth access to tobacco and alcohol; the prohibition of all advertising and promotional activities by the tobacco industry. The role of HPV should be tackled in culturally acceptable health programmes promoting safe sexual practices(Elango et al., 2006) .

The results of this study showed that there was no statistical significance present between clinical variant and habit of tobacco. Not many studies were conducted obtaining this type of results. Studies conducted by Marija Bokor et al (Bokor-Bratic and Vuckovic, 2002) and J. J. Pindborg et al (Pindborg et al., 1967) had contradicting results. The reason could be the inconsistency in the use of tobacco between the different types of study populations . The results obtained from the study also showed that there was no significant correlation between the clinical variant of leukoplakia and type of tobacco , this was similar to the study conducted by lima et al. (Lima et al., 2012).

The study also showed that the most prevalent gender of patients diagnosed with leukoplakia who consumed tobacco were males which was similar to previous studies (Waldron and Shafer, 1975). The reason could be the higher prevalence of smoking and pan masala chewing habits among them . In this study the most prevalent form of tobacco used among patients diagnosed with tobacco was found to be smoking which was similar to other studies (Waldron and Shafer, 1975) . The homogenous type of leukoplakia was the most common type of leukoplakia that was observed among patients consuming tobacco which was similar to other studies (Bisht et al., 2013). It was also noted that the most common type of tobacco used the cigarette.

The limitations of the study conducted includes the reduced sample size , Unequal distribution of cases and the Follow up results were not possible to obtain. The future scope of this study will include a Large sample size present including different ethnicities to obtain better results ,and also long term assessment of treatment can be recorded.

CONCLUSION:

Based on the results of the present study we were able to conclude that there was no association between the type of tobacco used and the clinical variants of leukoplakia. Non homogeneous leukoplakia are known to have a higher potential for malignant transformation. Hence, awareness needs to be created regarding cessation of habit. Multicentric study with a larger sample size will give a more definitive outcome.

AUTHOR CONTRIBUTION:

The study was designed and coordinated by Gayathri Karthikeyan, Dr.Hannah.R and Dr. Jaiganesh. Gayathri Karthikeyan collected the data, performed the statistical analysis and drafted the manuscript. Dr. Hannah and Dr. Jaiganesh participated in its design and coordination and helped in the revision of the manuscript, they also provided conceptual and methodological guidance. All authors read and approved the final manuscript .

CONFLICT OF INTEREST:

The authors declare no potential conflict of interest

REFERENCES:

- [1] Aggarwal, N. (2018) “Leukoplakia- Potentially Malignant Disorder of Oral Cavity -a Review”, Biomedical Journal of Scientific & Technical Research. doi: 10.26717/bjstr.2018.04.0001126.
- [2] Bisht, R. S. et al. (2013) ‘Study over the clinical picture and histopathology of leukoplakia and to establish the correlation between causative factors in the patients of Garhwal hill region’, National journal of maxillofacial surgery, 4(2), pp. 177–180.
- [3] Boffano, P. et al. (2019) ‘Mobilisation second surgery of the tongue following oral floor cancer surgery’, Oral Surgery, pp. 45–47. doi: 10.1111/ors.12363.
- [4] Bokor-Bratic, M. and Vuckovic, N. (2002) ‘Cigarette smoking as a risk factor associated with oral leukoplakia’, Archive of oncology, pp. 67–70. doi: 10.2298/aoo0202067b.
- [5] Elango, J. K. et al. (2006) ‘Trends of head and neck cancers in urban and rural India’, Asian Pacific journal of cancer prevention: APJCP, 7(1), pp. 108–112.
- [6] Garg, K. N., Raj, V. and Chandra, S. (2013) ‘Trends in frequency and duration of tobacco habit in relation to potentially malignant lesion: A 3 years retrospective study’, Journal of oral and maxillofacial pathology: JOMFP, 17(2), pp. 201–206.
- [7] Gheena, S. and Ezhilarasan, D. (2019) ‘Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells’, Human & Experimental Toxicology, pp. 694–702. doi: 10.1177/0960327119839173.
- [8] Gupta, B., Ariyawardana, A. and Johnson, N. W. (2013) ‘Oral cancer in India continues in epidemic proportions: evidence base and policy initiatives’, International dental journal, 63(1), pp. 12–25.
- [9] Gupta, V. and Ramani, P. (2016) ‘Histologic and immunohistochemical evaluation of mirror image biopsies in oral squamous cell carcinoma’, Journal of oral biology and craniofacial research, 6(3), pp. 194–197.
- [10] Hannah, R. et al. (2018) ‘Awareness about the use, Ethics and Scope of Dental Photography among Undergraduate Dental Students Dentist Behind the lens’, Research Journal of Pharmacy and Technology, p. 1012. doi: 10.5958/0974-360x.2018.00189.0.
- [11] Jangid, K. et al. (2015) ‘Ankyloglossia with cleft lip: A rare case report’, Journal of Indian Society of Periodontology, p. 690. doi: 10.4103/0972-124x.162207.
- [12] Jayaraj, G. et al. (2015) ‘Inter-observer agreement in grading oral epithelial dysplasia – A systematic review’, Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology, pp. 112–116. doi: 10.1016/j.ajoms.2014.01.006.
- [13] Jayaraj, G. et al. (2015) ‘Stromal myofibroblasts in oral squamous cell carcinoma and potentially malignant disorders’, Indian journal of cancer, 52(1), pp. 87–92.
- [14] Kumar, A. et al. (2015) ‘Expression of CD 68, CD 45 and human leukocyte antigen-DR in central and peripheral giant cell granuloma, giant cell tumor of long bones, and tuberculous granuloma: An immunohistochemical study’, Indian journal of dental research: official publication of Indian Society for Dental Research, 26(3), pp. 295–303.
- [15] Lima, J. S. et al. (2012) ‘Oral leukoplakia manifests differently in smokers and non-smokers’, Brazilian Oral Research, pp. 543–549. doi: 10.1590/s1806-83242012005000024.
- [16] Mehta, F. S. et al. (1969) ‘Epidemiologic and histologic study of oral cancer and leukoplakia among 50,915 villagers in India’, Cancer, 24(4), pp. 832–849.
- [17] Mondal, K. et al. (2017) ‘An inter-correlative study on clinico-pathological profile and different predisposing factors of oral leukoplakia among the ethnics of Darjeeling, India’, Journal of orofacial sciences. Medknow Publications and Media Pvt. Ltd., 9(1), p. 34.
- [18] Negi, M. et al. (2019) ‘Prevalence of oral parameters in smokeless tobacco-associated precancer’, Journal of family medicine and primary care, 8(12), pp. 3956–3961.

- [19] Pindborg, J. J. et al. (1967) ‘Studies in oral leukoplakias. Prevalence of leukoplakia among 10,000 persons in Lucknow, India, with special reference to use of tobacco and betel nut’, *Bulletin of the World Health Organization*, 37(1), pp. 109–116.
- [20] Roed-Petersen, B. and Pindborg, J. J. (1973) ‘A study of Danish snuff-induced oral leukoplakias’, *Journal of oral pathology*, 2(6), pp. 301–313.
- [21] Sankaranarayanan, R. et al. (2005) ‘Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial’, *The Lancet*, pp. 1927–1933. doi: 10.1016/s0140-6736(05)66658-5.
- [22] Shree, K. H. et al. (2019) ‘Saliva as a Diagnostic Tool in Oral Squamous Cell Carcinoma – a Systematic Review with Meta Analysis’, *Pathology & Oncology Research*, pp. 447–453. doi: 10.1007/s12253-019-00588-2.
- [23] Sivaramakrishnan, S. M. and Ramani, P. (2015) ‘Study on the Prevalence of Eruption Status of Third Molars in South Indian Population’, *Biology and Medicine*. doi: 10.4172/0974-8369.1000245.
- [24] Sridharan, G. et al. (2019) ‘Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma’, *Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, 48(4), pp. 299–306.
- [25] Sridharan, G., Ramani, P. and Patankar, S. (2017) ‘Serum metabolomics in oral leukoplakia and oral squamous cell carcinoma’, *Journal of Cancer Research and Therapeutics*, p. 0. doi: 10.4103/jcrt.jcrt_1233_16.
- [26] Swathy, S., Gheena, S. and Varsha, S. L. (2015) ‘Prevalence of pulp stones in patients with history of cardiac diseases’, *Research Journal of Pharmacy and Technology*, p. 1625. doi: 10.5958/0974-360x.2015.00291.7.
- [27] Thangaraj, S. V. et al. (2016) ‘Molecular Portrait of Oral Tongue Squamous Cell Carcinoma Shown by Integrative Meta-Analysis of Expression Profiles with Validations’, *PloS one*, 11(6), p. e0156582.
- [28] Urs, A. B., Yaming, P. and Malhotra, R. (2018) ‘An insight into the cannibalistic behavior of giant cell granulomas of the jaws’, *Journal of oral and maxillofacial pathology: JOMFP*, 22(3), p. 449.
- [29] Viveka, T. S. et al. (2016) ‘p53 Expression Helps Identify High Risk Oral Tongue Premalignant Lesions and Correlates with Patterns of Invasive Tumour Front and Tumour Depth in Oral Tongue Squamous Cell Carcinoma Cases’, *Asian Pacific Journal of Cancer Prevention*, pp. 189–195. doi: 10.7314/apjcp.2016.17.1.189.
- [30] van der Waal, I. (2009) ‘Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management’, *Oral oncology*, 45(4-5), pp. 317–323.
- [31] Waldron, C. A. and Shafer, W. G. (1975) ‘Oral carcinoma in situ’, *Oral surgery, oral medicine, and oral pathology*, 39(2), pp. 227–238.
- [32] World Health Organization (2003) *Diet, Nutrition, and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation*. World Health Organization.
- [33] Yardimci, G. (2014) ‘Precancerous lesions of oral mucosa’, *World Journal of Clinical Cases*, p. 866. doi: 10.12998/wjcc.v2.i12.866.

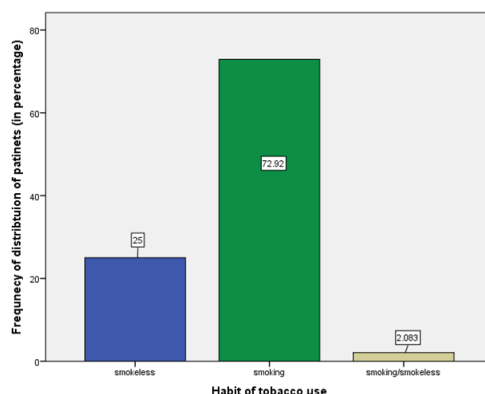


Figure 1 : This Bar graph shows the distribution of tobacco use among patients with leukoplakia (x- axis depicting the habit and depicting percentage, y-axis: frequency of distribution in percentage) where Blue indicates smokeless form of tobacco use , green indicates smoking habit of tobacco use and grey indicates both smoking and smokeless habit of tobacco use. It was noticed that about 72.29% (70) were patients who had a smoking habit of tobacco use , 25%(24) had smokeless habit of tobacco use and about 2.083% (2) had both smoking and smokeless habit of tobacco use. The most common habit of tobacco use noticed was smoking

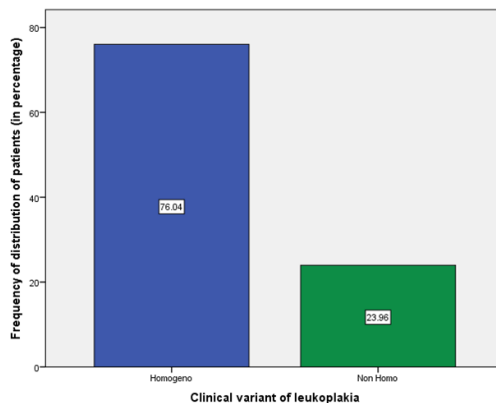


Figure 2 : This Bar graph shows the distribution of the clinical variants of leukoplakia (x- axis: clinical variant of leukoplakia, y-axis: frequency of distribution of patients in percentage) where blue indicates homozygous types of leukoplakia and green indicates non homogenous types of leukoplakia. It was noticed that about 76.04%(73) had homogenous types of leukoplakia and 23.96% had non-homogenous type of leukoplakia. The most common type of clinical variant of leukoplakia was homogenous.

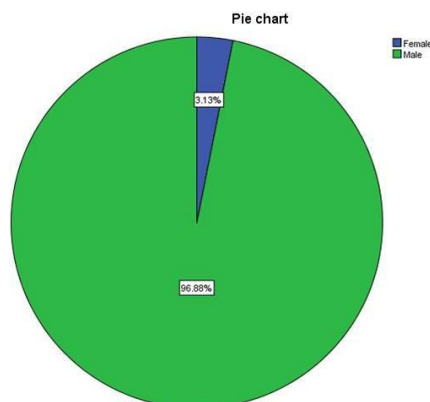


Figure 3: This Pie chart shows the distribution of gender among tobacco consuming patients with leukoplakia where green indicates males and blue indicates females . The chart shows that 96.88% of the population were males and 3.13% of the population were females. The most common gender noticed in patients diagnosed with leukoplakia was males compared to females.

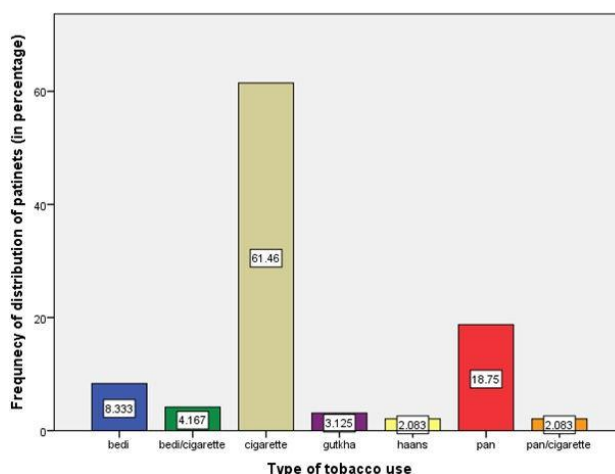


Figure 4: This Bar graph shows the distribution of patients with leukoplakia based on the type of tobacco used. X axis represents the type of tobacco used and the y-axis depicts the percentage of leukoplakia cases. where blue indicates bidi, green indicates bidi or cigarette, grey indicates cigarette, purple indicates gutkha, yellow indicates haans, red indicates pan and orange indicates pan or cigarette use. The graph shows that 61.46% (59) of patients used cigarettes, 18.75% (18) used pan 8.333% (8) used bidi ,4.167% (4) used bidi and cigarette, 3.125% (3) used gutkha and 2.083% (2) used haans and 2,083 % used pan and cigarette . The most common type of tobacco used was cigarette

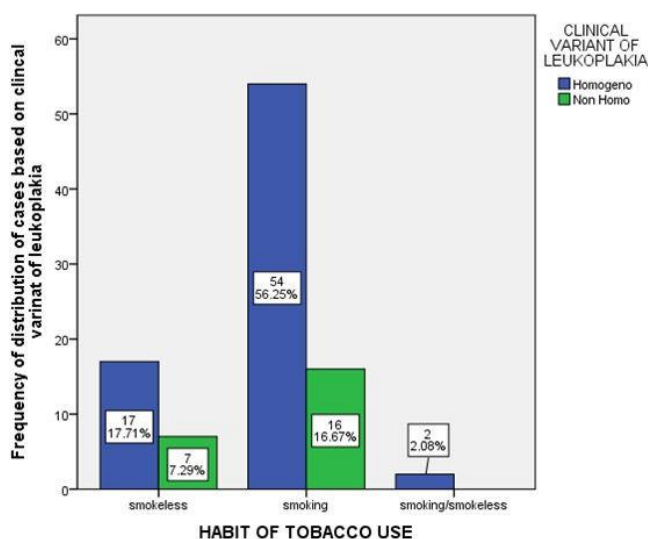


Figure 5: Bar graph shows the association between habit and clinical variants of leukoplakia.(x- axis represents the habit of tobacco use and y-axis represents frequency of distribution of cases based on clinical variants of leukoplakia) where blue indicates homogenous leukoplakia and green indicates non homogenous leukoplakia. The graph shows that the most common habit of tobacco use was smoking and 56.25% was associated with homogenous leukoplakia. Chi-square test was done and the association was not found to be statistically significant. Peason’s value:1.034, DF:2, P value: 0.596(>0.05) hence statistically not significant , proirving that there is no association present between the habit of tobacco use and clinical variant of leukoplakia.

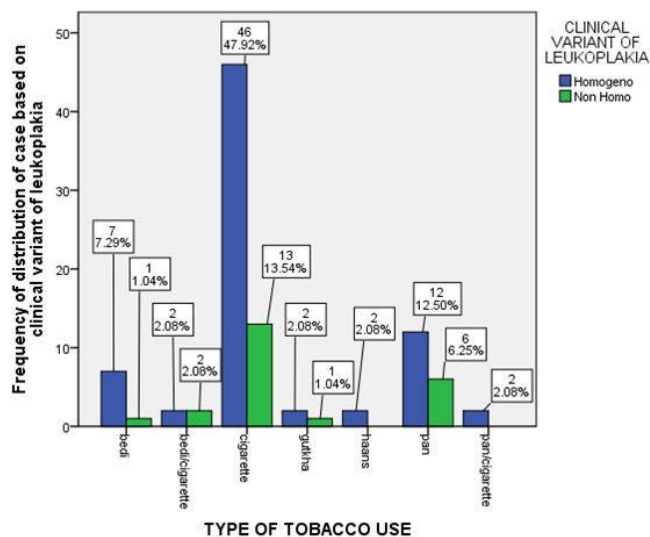


Figure 6: This Bar graph shows the association between the type of tobacco used and clinical variants of leukoplakia. x-axis represents the type of tobacco used, y-axis represents the frequency of cases based on the clinical variants of leukoplakia. where blue indicates homogenous leukoplakia and green indicates non homogenous leukoplakia. The graph shows that the most common type of tobacco used was cigarette and 47.92% was associated with homogenous type of leukoplakia. Chi-square test was done and the association was found to be statistically not significant. Peason’s value:4.459, DF:6, P value: 0.615(>0.05) hence statistically not significant , proving that there is no association present between type of tobacco use and clinical variant of leukoplakia.