DOI: 10.7860/JCDR/2021/49720.15679

Original Article

Pathology Section

Association of Oral Melanosis with Soft Tissue Pathologies: A Hospital-based Observational Study

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ABSTRACT

Introduction: Oral pigmentation can be physiological/pathological, exogenous/endogenous. Melanin is the most important pigment responsible for normal pigmentation. Recent increasing evidence shows that melanosis occurs in many oral soft tissue pathologies.

Aim: To assess the pigmentation in various oral soft tissue lesions with that of normal oral mucosa.

Materials and Methods: An observational study was conducted at Maulana Azad Institute of Dental Sciences, New Delhi, India, for a period of six months. A total of 805 study participants were included in the study population. Among these, 605 study participants with soft tissue pathologies with or without tobacco habits were selected and 200 study participants without any pathology and habit comprised were taken as controls. Assessment of oral melanosis was done at various sites within the oral cavity. Prevalence of melanosis with oral lesions and habits was estimated using Statistical Package for the Social Sciences (SPSS) statistical software (version 16.0).

Results: Out of 605, 280 cases (46.28%), and out of 200, 54 controls (27%) showed melanosis which was statistically significant. Melanosis was most prevalent in men, in the age group of 21-40 years predominantly involving the buccal mucosa. Maximum cases presenting melanosis were amongst smoked tobacco users and with both tobacco habits were found in leukoplakia (37.7% and 28.3%, respectively). However, 74.2% smokeless tobacco users with melanosis showed Oral Submucous Fibrosis (OSMF) which was statistically significant.

Conclusion: Various forms of tobacco consumption commonly induce intra mucosal pigmentation which could be due to high chemical and mechanical irritation by tobacco. The study attempts to assess the overall prevalence of melanosis in study participants with pathologies and habits to understand the implications of melanosis in pathologies in the general population.

Keywords: Leukoplakia, Oral lichen planus, Oral submucous fibrosis, Pigmentation, Tobacco habits

INTRODUCTION

Oral pigmentation is the process of deposition of different pigments in the oral tissues. Four types of pigments contribute to the normal colour of the skin and mucosa namely, melanin, carotenoids, reduced haemoglobin, and oxygenated haemoglobin of which melanin is the most important. It is an endogenous non haematogenous pigment, produced by melanocytes in the basal layer of the epithelium and is transferred to adjacent 30-40 keratinocytes via membrane-bound organelles called melanosomes. Melanin is also synthesised by nevus cells, found in the oral mucosa and skin which are neural crest derivatives. Depending on the location and amount of melanin in the tissues, melanin induced pigmentation can be black, grey, blue, or brown in colour [1]. Melanin is derived from the metabolism of the amino acid L-tyrosine or L-phenylalanine and is of different types of melanin namely, Eumelanin, Pheomelanin, Mixed type melanin, Neuromelanin and Oxymelanin. Tyrosinase activity leads to the eumelanin production, whereas downregulated tyrosinase activity leads to the default production of pheomelanin. Individuals with richly pigmented skin have a higher eumelanin to pheomelanin ratio [1,2].

Melanosis is a form of hyperpigmentation associated with increased melanin production in the basal layer of the epithelium. Physiological pigmentation increases with age, and its intensity may be influenced by tobacco, hormones, and systemic treatments. The attached gingiva is the most common site, but physiological pigmentation can be noted at any place in the oral cavity, including the tips of the fungiform papillae on the dorsal surface of the tongue [3]. There is increasing evidence now-a-days to show that melanosis is occurring in many oral soft tissue pathologies. Some of these are associated with habits like tobacco, areca nut etc., Tobacco use could be defined as any habitual consumption of the tobacco plant leaf and its products. The major use of tobacco is by smoke inhalation of cigarettes, cigars, and pipes. Smokeless tobacco represents a range of tobacco products that are either sniffed, sucked or chewed [4]. Probable reasons for melanosis due to tobacco consumption could be the ability of nicotine to produce melanocyte, binding of melanin to noxious substance and high chemical and mechanical irritation by smokeless tobacco.

The current study aimed to assess the pigmentation in various oral soft tissue lesions with that of normal oral mucosa with the objective to compare various grades of melanosis in oral soft tissue lesions in study participants with or without tobacco habits.

MATERIALS AND METHODS

The hospital based observational study was undertaken on 875 study participants who attended the Outpatient Department (OPD), at Maulana Azad Institute of Dental Sciences, New Delhi, India, from 1st May 2019 to 31st October 2019. The study was certified by the Institutional Ethical Committee (Ref. no.: MAIDS/Ethical committee/2016/1400). An informed consent was taken by all the study participants. A detailed history was recorded along with thorough clinical examination to assess the melanosis. Study participants were assessed as they came in OPD within the specified period of the study.

Sample size calculation: Sample size was calculated using the formula: $n=\{Z^2 \times P \times (1-P)\}/e^2$ where Z=value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI), P is expected true proportion and e is desired precision (half desired CI width).

Inclusion criteria: Study participants showing features of clinically diagnosed oral soft tissue pathology with or without tobacco habits which were designated as cases (605) and those having no pathology and tobacco habit were taken as controls (200).

Exclusion criteria: Study participants with drug history and any systemic disease history were excluded from the study (70).

Study Procedure

Clinically diagnosed oral soft tissue pathologies studied were classified as OSMF, leukoplakia, Oral Lichen Planus (OLP), Oral Squamous Cell Carcinoma (OSCC) and miscellaneous which included candidiasis, inflammatory fibrous hyperplasia, pyogenic granuloma etc. Based on the habit, patients were categorised as using smoked tobacco, smokeless tobacco, both and without any habit. Study participants were grouped based on the different parameters like age and sex. Age distribution among the study groups were divided into four subgroups as 0-20 years, 21-40 years, 41-60 years, and 61-80 years. Assessment of oral melanosis appearing as brown-black hyperpigmentation of the oral mucosa was done at various sites in oral cavity like buccal mucosa, tongue, palate and gingiva [5]. Further, these clinical findings were compared with various forms of tobacco consumption.

STATISTICAL ANALYSIS

Prevalence of melanosis with oral lesions and habits was estimated using SPSS statistical software version 16.0. Chi-square test was used to estimate the effect of different variables on oral lesions.

RESULTS

Among 805 patients enrolled in the study, 605 study participants showed soft tissue pathology and 200 study participants were without any soft tissue pathology which served as controls. Out of the 605 cases, 280 cases exhibited melanosis whereas out of 200 controls, only 54 showed melanosis with a significant p-value [Table/Fig-1].

Variables	Cases	Controls	Total	p-value (Chi-square test)
With melanosis	280	54	334	0.0001* (significant)
Without melanosis	325	146	471	
Total	605	200	805	
Prevalence	46.28%	27%		

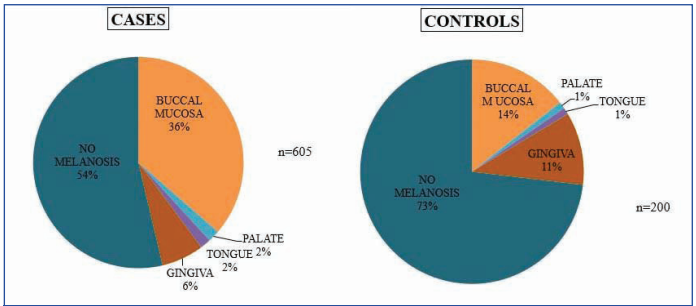
[Table/Fig-1]: Distribution of melanosis in cases and controls.
*Statistically significant

Comparison of age of study participants showing melanosis between cases and controls was done by using Chi-square test and the results were not significant [Table/Fig-2].

Parameter		Cases (n=280)	Controls (n=54)	Total	p-value
Age (years)	<20	22 (7.9%)	4 (7.4%)	26 (7.8%)	0.780
	21-40	135 (48.2%)	24 (44.4%)	159 (47.6%)	
	41-60	99 (35.4%)	19 (35.2%)	118 (35.3%)	
	>61	24 (8.6%)	7 (13%)	31 (9.3%)	
Sex	Males	184 (65.7%)	36 (66.7%)	220 (65.9%)	0.892
	Females	96 (34.3%)	18 (33.3%)	114 (34.1%)	
Distribution of melanosis at various sites	Buccal mucosa	220 (78.6%)	29 (53.7%)	249 (74.5%)	0.0001* (significant)
	Palate	11 (3.9%)	2 (3.7%)	13 (3.9%)	
	Gingiva	39 (13.9%)	21 (38.9%)	60 (18%)	
	Tongue	10 (3.6%)	2 (3.7%)	12 (3.6%)	

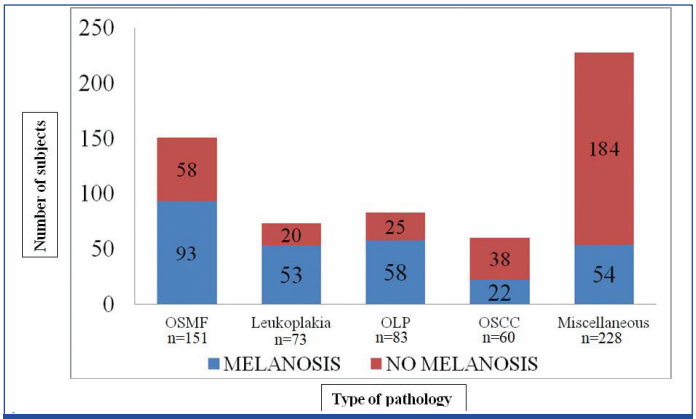
[Table/Fig-2]: Various parameters between cases and controls using Pearson's Chi-square test.
n=334; *Statistically significant

Among 280 cases showing melanosis, 184 were males and 96 were females. Out of 54 controls showing melanosis, 36 were males and 18 were females. On comparison between the two groups using chi-square test, p-value was not significant (0.892) [Table/Fig-2]. Melanosis in the oral mucosa was assessed at four different sites in the oral cavity namely, buccal mucosa, palate, tongue, and gingiva. Among 605 cases, 36% (220) showed melanosis on buccal mucosa, 2% (11) on palate, 6% (39) and 2% (10) on gingiva and tongue, respectively. Similarly, among 200 controls, 14% (29) showed melanosis on buccal mucosa, 1% (2) on palate, 11% (21) and 1% (2) on gingiva and tongue, respectively. These results had a highly significant p-value of 0.0001 on application of chi-square test [Table/Fig-2,3].



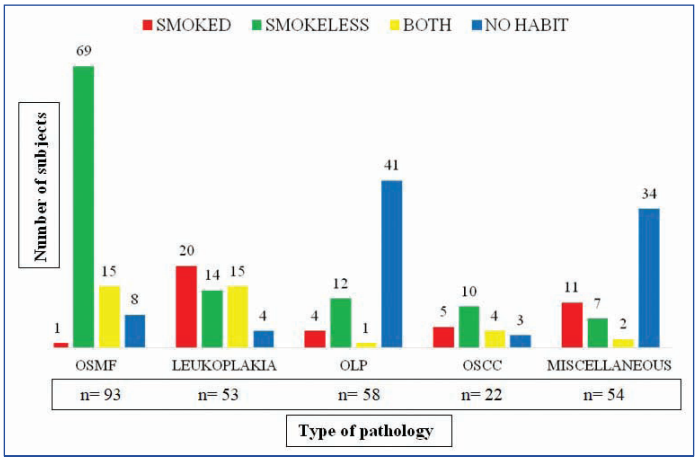
[Table/Fig-3]: Pie-chart showing distribution of melanosis in cases and controls.

Mild melanosis was most common among both cases and controls, 66.5% (222), followed by moderate, 24.8% (83), and severe, 8.7% (29). Melanosis was assessed among five different subgroups of cases depending on the pathology. Among 151 cases of OSMF, 61.6% (93) showed melanosis. Among 73 leukoplakia patients, 72.6% (53) showed melanosis. Out of 83 OLP patients, 70% (58) showed melanosis. Out of 60 OSCC patients, 36.7% (22) showed melanosis and those of 238 miscellaneous cases only 22.6% (54) cases showed melanosis [Table/Fig-4].



[Table/Fig-4]: Prevalence of melanosis among different pathologies in cases.

Maximum cases presenting melanosis amongst smoked tobacco users as well as with both tobacco habits were leukoplakia, 37.7% (20) and 28.3% (15), respectively. However, maximum cases showing melanosis amongst smokeless tobacco users were OSMF, 74.2% (69). Most cases showing melanosis without any tobacco habits were OLP, 70.7% (41). These results were highly significant on using Pearson's chi-square test with a p-value of 0.0001 [Table/Fig-5,6].



[Table/Fig-5]: Comparison between various forms of habits among different pathologies having melanosis.

Parameters	Value	df	Asymptotic significance (2-sided)
Pearson's Chi-square	1.714E2[a]	12	0.0001*(significant)
Likelihood ratio	177.615	12	<0.001
Linear-by-linear association	45.438	1	<0.001
No. of valid cases	280		

[Table/Fig-6]: Comparison between various forms of habits among different pathologies having melanosis by Pearson's Chi-Square test.
*Statistically significant; a: 2 cells (10.0%) have expected count less than 5. The minimum expected count is 2.91

DISCUSSION

Melanosis of the oral cavity can either be physiological or pathological. Multiple aetiologies are associated with the occurrence of pathologic melanin pigmentation of the oral mucosa. It could be either biological, physical, or chemical factors but they are interrelated which could possibly influence the size, distribution, or intensity of areas of pigmentation [1]. In recent times, melanosis has been found to be associated with various forms of tobacco consumption and soft tissue pathologies. The present study was a hospital based observational study conducted to estimate the prevalence of melanin pigmentation in both cases and controls. The overall prevalence of oral melanin pigmentation was 41.49% which was higher compared to previous studies in other populations like Swedish (9.9%), Caucasians (12.9-14.9%) and white Londoners (5%). Jews and Australian aborigines show significantly marked prevalence of 68% and 100%, respectively. This variation can be attributed to cultural differences, their own habits, education level and even genetic differences [6-11]. In the present study, prevalence of oral melanosis was higher in cases 46.28% (280/605) as compared to controls which were found to be statistically significant (p-value=0.0001). Frequency of oral melanin pigmentation varies with age. The pigmentation was most prevalent in the age group of 21-40 years and decreased thereafter, most distinctly between the age group of 45-80 years. This finding agrees with previous studies reported for Swedish and Indian population [6,12]. This could probably be due to the younger age group practising deleterious habits. Among all study participants examined, pigmentation was more prevalent in men over women and this finding was in accordance with previous studies conducted by Saraswathi TR et al., Hedin CA and Axell T and Ray JG et al., [12-14]. This could be attributed to males having easier access to tobacco products which cause melanosis. Contrary to present study findings, female predominance was seen in study conducted by Naveen-Kumar B et al., who found that reverse smoking was more predominant in females, as this habit was more prevalent in Bhimavaram, India [15].

In this study, buccal mucosa was found to be the most common site of pigmentation (36%). The association between tobacco users and high frequency of melanin pigmentation was found to be statistically significant (p-value=0.001). This result agrees with previous findings published in Nigerian and Thailand population [13,16]. To the best of our knowledge this was the first study to examine the association of melanosis with various soft tissue pathologies in a north Indian population. The prevalence of melanosis was higher in OSMF (61.6%) and OLP (69.8%) study participants as compared to other soft tissue pathologies. In the present study, authors found significantly increased smokeless tobacco induced melanosis in OSMF (74.2%). However, both smoked (37.7%) and smokeless tobacco (26.4%) induced melanosis was evident in leukoplakia. The present finding was similar to other studies conducted by Saraswathi TR et al., Naveen-Kumar B et al., Behrua SS et al., and Hashibe M et al., [12,15,17,18]. Significant risk factors for leukoplakia are smoking and smokeless tobacco. According to Alvarez Gomez GJ et al., effect of nicotine from smoked form of tobacco on the melanocytes which are located beside the basal cells could possibly result in basilar melanosis with varying amount of melanin incontinence [19]. It has been proposed that the melanin formed in response to tobacco smoke is a protective reaction, causing detoxification of nicotine, polyaromatics, and benzopyrene [4]. Also, it is hypothesised that the mechanical and chemical irritation from smokeless tobacco may induce melanin pigmentation. According to Larsson BS, the reason behind the lesions developing in the surrounding tissues is long term exposure of injurious chemicals leading to high levels being stored in melanocytes resulting in their degeneration [20]. There are two possible hypothesis associated with smoker's melanosis. The first being ability of nicotine to produce melanocyte and secondly, binding of melanin to noxious substance [7,21-23].

Melanosis in OLP was not associated with any tobacco habit. According to Chitturi RT et al., post inflammatory pigmentation is the possible cause of melanosis in OLP which can be described by two processes. The first process suggests the role of inflammatory mediators directly stimulating the melanocytes and second being abnormal distribution of melanin pigment. Further, melanin laden macrophages in the superficial connective tissue phagocytose the melanin pigment released from apoptotic keratinocytes leading to post inflammatory pigmentation [24]. The current study has found a very high association of melanosis with OSMF. The reason for this high prevalence in OSMF patients could be high chemical and mechanical irritation by smokeless tobacco. However, it is not well documented in the literature and further research is needed to ascertain the association between smokeless forms of tobacco with oral melanosis in OSMF.

Limitation(s)

In the current study, the diagnosis of melanosis was not correlated with the histopathological findings of the soft tissue pathologies.

CONCLUSION(S)

The present study provides epidemiological data on oral melanosis associated with various soft tissue pathologies in relation to tobacco habits. Oral melanosis was mainly evident in men in the age group of 21-40 years with a high prevalence of melanosis in OSMF associated with smokeless tobacco. Since the clinical diagnosis of melanosis was not correlated with the histopathological findings, further studies may be taken up in this direction to elicit a relationship between melanosis and histopathological findings. Hence, efforts should be taken to educate individuals and masses regarding the risks of tobacco usage through different tobacco cessation programs to increase the awareness of its hazards especially towards the smokeless form of tobacco.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Apr 03, 2021
- Manual Googling: Jun 25, 2021
- iThenticate Software: Oct 29, 2021 (17%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Apr 01, 2021**
Date of Peer Review: **May 20, 2021**
Date of Acceptance: **Jun 30, 2021**
Date of Publishing: **Nov 01, 2021**