

**Study of palatal lesions by scrape cytology in reverse smokers among the tribal population of Paderu, an agency area in Visakhapatnam district, Andhra Pradesh.**

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**Abstract**

**Background:** Reverse smoking is smoking with the lightened end of the cigarette inside the mouth. It is associated with oral leukoplakia, smoker’s melanosis, nicotinic palate - smoker’s palate, black hairy tongue and squamous cell carcinoma.

**Aim:** This study highlights the oral palatal lesions by scrape cytology in reverse smokers among the tribal population of Paderu, an agency area in Visakhapatnam district, Andhra Pradesh.

**Materials and Methods:** A cross sectional study was done in the Department of Pathology, a private medical

college in Visakhapatnam, Andhra Pradesh, from 01-08-2019 to 23-10-2019. A total of 352 cases were included.

**Results:** In our study, age distribution included from 20 years to 80 years. Majority of patients were among 51-60 years i.e, 45.4 %. Females (59.6%) outnumbered as compared to males (40.3%). 26.9% were relatively normal on clinical diagnosis followed by leukoplakia i.e., about 17%. On cytology, 41.1 % were reported as normal and 22.7 % as non-neoplastic lesions and 18.4% as low-grade dysplasia.

We are introducing the RISE protocol score that may help to identify patients who need immediate attention

and further investigations like biopsy and IHC for grading and prognostic assessment.

Conclusion: Reverse smoking is one of the endemic habits still practiced in the Rural Andhra. Palate undergoes a series of precancerous changes with reverse smoking. In this study, an attempt was made to highlight the changes of the palatal mucosa on scrape cytology.

**Keywords:** Cigarette smoking, reverse smokers, scrape cytology.

### **Introduction**

Tobacco smoking is one of the most common causes of morbidity and mortality in developed and developing countries now [1]. Cigarettes contain over 4000 constituents of chemicals and free radicals like nicotine, ammonia, acrolein, phenols, acetaldehyde, benzopyrene, nitric oxide, carbon monoxide, polonium, radium & thorium that can cause cellular damage [2, 3]. Cigarette smoking is associated with oral leukoplakia, smoker's melanosis, nicotinic stomatitis or smoker's palate, black hairy tongue and squamous cell carcinoma [4, 5]. Reverse smoking is smoking with the lightened end of the cigarette inside the mouth [6]. The highest internal temperature of the cigarette can reach upto 760°C and the internal air can be heated up to 120°C. This temperature and the products of combustion increase the frequency of lesions inside the mouth compared to conventional smokers [7]. The clinical aspect of oral mucosa in patients with the habit of reverse smoking differs from that of conventional smokers in that the most commonly affected areas are palate and tongue [8]. Previously, these changes were called nicotinic stomatitis [9] and although there is not a consensus about the proper terminology yet, the term "Palatal changes related to reverse smoking" or "Palatal keratosis associated with reverse smoking" [10] (FIG – 7) is mostly accepted, since now it is well known that nicotine is not the sole etiological factor but also

other components of tobacco and cigarette. This is a peculiar habit mostly prevalent among adult fishermen and women of coastal rural Andhra Pradesh, India. The frequency of reverse smoking is 6.23 times higher in females than in males [11].

Cytology can play a very important role in identifying leukoplastic lesions of oral cavity and help in mass screening programmes especially in rural areas as it is affordable and the report can be obtained very fast and enables early detection of developing malignant transformations [12]. Many studies have been conducted in the past to evaluate the role of cytology in the diagnosis of tumors. These studies have concluded that cytology has the advantage of being much less time consuming, easy to adopt, reliable and does not require special instruments [13]. The present study is carried out to record the clinical changes and to evaluate the utility of scrape cytology in studying the cytological changes of palate in individuals habituated to reverse smoking and for the rapid diagnosis of oral premalignant and malignant lesions.

### **Aims And Objectives**

Our study aims to record clinical and morphological changes in palatal lesions by scrape cytology in reverse smokers among the tribal population of Paderu, an agency area in Visakhapatnam district, Andhra Pradesh.

### **Materials And Methods**

Ethical institution permission was taken. Informed consent was taken from all the patients included in the study.

**Sample size:** 363 patients were selected randomly, but on cytology 11 samples were not included due to inadequate sampling. Hence data is prepared on 352 patients.

**Study design:** Descriptive study, A cross sectional study

**Place of study:** Study was conducted in the Department of Pathology, a private medical college in Visakhapatnam, Andhra Pradesh. Study population - Tribal population habituated to reverse smoking in Paderu village, Visakhapatnam district, Andhra Pradesh.

**Duration of study:** (From 1 - 6 -2019 to 1 - 10 - 19) – 4 months

**Inclusion criteria**

Both males and females aged 12-80 years with reverse smoking habit. Among tribal population and patients willing to participate in the study

**Exclusion criteria**

Patients not willing to participate in the study and those with habit to use other forms of tobacco consumption, already diagnosed cases of oral lesions, Pregnant women were not included in the study.

**Materials/Instruments:** Normal saline, toothbrush-medium bristle, Slides, Mouth wash, Cover slips, Papanicolaou stain and H&E stain, Microscope and Camera

**Data Collection Method:** A case study form was used to collect data from study subjects, which contains relevant details like type of smoking habit, duration and frequency of smoking habit, type of tobacco used and clinical features of the palatal lesions. All the individuals are informed about the study and only those who gave written consent were included in the study. The study design was approved by the Ethics Committee or the study on human subjects.

**Quality Control:** Thorough cleaning of oral mucosa to prevent bacterial flora from overlapping the morphology of smears was the preferred method. After which adequate sampling was done. Care was taken to be gentle and not to injure or spread the existing lesion. Care was taken not to injure or spread the existing lesion. Precautions were taken to avoid smudging of smears.

Two smears were prepared for each lesion. Discarding of overstained and understained slides was done.

**Data management and Statistical analysis:** Data will be entered in MS Excel and analyzed in SPSS V22. Descriptive statistics are represented with Percentages and other relevant statistical tests were applied based on nature of the distribution.

**Proposed Intervention:** Clinical examination and recording of oral mucosa especially palate was done.

**Scrape Cytology:** Cytological smear was prepared as per the protocol given below.

All the patients were asked to rinse their mouth thrice with mouth wash. Dry lesions were moistened with normal saline. By using a sterile toothbrush, the lesions were gently scraped and the material thus obtained is smeared on glass slides. The glass slides were kept in coplin jars filled with Isopropyl alcohol. Staining of smears was done by Papanicolaou, Hematoxylin and eosin stain and were screened under microscope to identify the pathology.

**Reverse smoking individualistic scoring exercise- Rise protocol:**

We introduced the RISE protocol score that may help to identify patients who need immediate attention to proceed for further investigations like biopsy and IHC for grading and prognostic assessment. Its follow up proposals may give an approximate understanding of the care that needs to be taken for the patient.

Table 1: RISE Protocol score

RISE Grades are given by adding a+b+c+d

Score	Reverse smoking since No. of years(a)	No. of cigars per day (b)	Clinical Examination(c)	Scrape cytology Impression(d)
0	-	-	Normal	Normal
1	0-1	<5	Nonneoplastic	Nonneoplastic
2	2-5	6-10	Premalignant	Low Grade dysplasia
3	6-9	11-19	Suspicious of malignancy	High Grade Dysplasia
4	>10	>20	Malignancy	Malignancy

RISE Grade 1: 4-7

RISE Grade 2: 8-11

RISE Grade 3: 12-15

RISE score 4 by scrape cytology requires biopsy for confirmation irrespective of other scores and belongs to grade 3 requiring immediate attention.

RISE grade 1 requires follow up every 5-6 months.

RISE grade 2 requires follow up every 8-12 months.

### Implications

Health education and screening camps along with oral clinical examination together with cytological examination for screening early signs of palatal premalignant lesions thereby preventing malignancy.

Help the tribal population to understand the potentially harmful effects of reverse smoking habit and motivate them to quit smoking.

### Results And Observation

A total of 363 cases were selected randomly, all cases underwent scrape cytology, but only 352 patients were included as 11 patients were excluded because of inadequate samples. In our study age group distribution (Table I) included from 20 years to 80 years. Majority of patients were among 51-60 years i.e., 45.4 %, next common age group was among 41-50 years i.e., 31.2%. Females 59.6% (Table II) outnumbered as compared to males 40.3%. In our study, we observed 91.4% (322/352)

of patients have history of having 1-5 cigarettes per day (Table III).

In our study, 37.7 % (133/352) of patients have smoking history of about 11-20 years followed by 14.7% (52/352) have about 21-30 years (Table IV). A new attempt to introduce RISE grades (Table VII) was done, 22 cases needed follow up, RISE score 4 by scrape cytology requires biopsy for confirmation irrespective of other scores and belongs to grade 3 requiring immediate attention. (12 cases- grade 3) RISE grade 1 (3 cases) may require follow up every 5-6 months RISE grade 2 (7 cases) require follow up every 8-12 months.

Table 2: Age Distribution

Age Distribution	No. of cases	%
20-30 years	08	2.2
31-40 years	32	9.0
41-50 years	110	31.2
51-60 years	160	45.4
61-70 years	28	7.9
71-80 years	14	3.9
Total	352	99.9

Table 3: Gender Distribution

Gender Distribution	No. of cases	%
Males	142	40.3
Females	210	59.6
Total	352	99.9

In our study, 37.7 % (133/352) of patients have smoking history of about 11-20 years followed by 14.7% (52/352) have about 21-30 years (Table IV). A new attempt to introduce RISE grades (Table VII) was done, 22 cases needed follow up, RISE score 4 by scrape cytology requires biopsy for confirmation irrespective of other scores and belongs to grade 3 requiring immediate attention. (12 cases- grade 3) RISE grade 1 (3 cases)



may require follow up every 5-6 months RISE grade 2 (7 cases) require follow up every 8-12 months.

Table 4: No. of cigarettes per day distribution

No. of cigarettes per day Distribution	No. of cases	%
1-5 cigarettes per day	322	91.4
6-10 cigarettes per day	15	4.2
11-15 cigarettes per day	05	1.4
16-20 cigarettes per day	04	1.1
21-25 cigarettes per day	06	1.7
Total	352	99.8

Table 5: Duration of smoking history

Duration of smoking history	No. of cases	%
5-10 years	46	13.0
11-20 years	133	37.7
21-30 years	52	14.7
31-40 years	60	17
41-50 years	44	12.5
51-60 years	09	2.5
61-70 years	08	2.2
Total	352	99.6

Usefulness of RISE scoring is time tested. 26.9% were relatively normal on clinical diagnosis followed by leukoplakia (FIG – 8) i.e., about 17% (Table V). On cytology, 41.1% reported as normal and 22.7 % as non-neoplastic and 18.4% as low-grade dysplasia (Table VI). 34 cases which were suspicious of malignancy on clinical examination were diagnosed as 1 case of low-grade dysplasia (FIG- 2), 30 cases of high-grade dysplasia (FIG - 3) and 3 cases of malignancy (FIG -1) on scrape cytology. Malignant cases were further subjected to biopsy for confirmation.

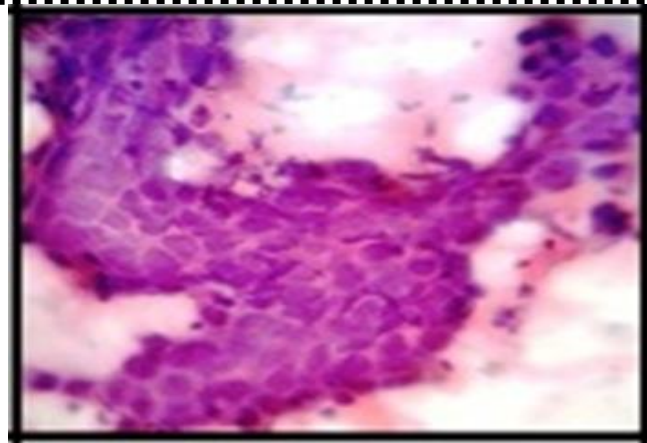


Figure 1: Scrape Cytology smear – Squamous cell carcinoma – H and E Stain (X 400)

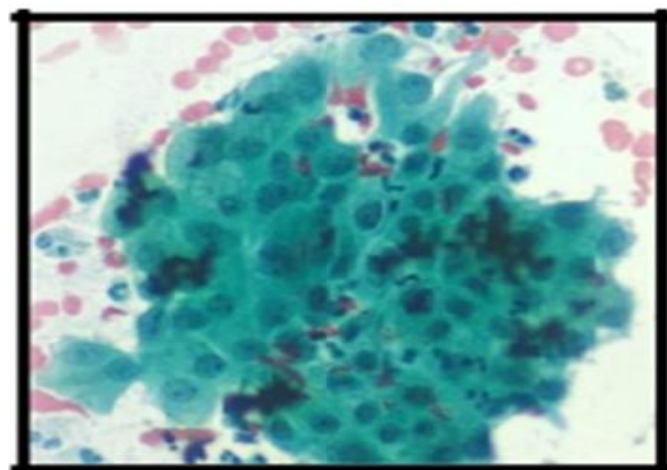


Figure 2: Low grade Dysplasia (Pap stainX400)

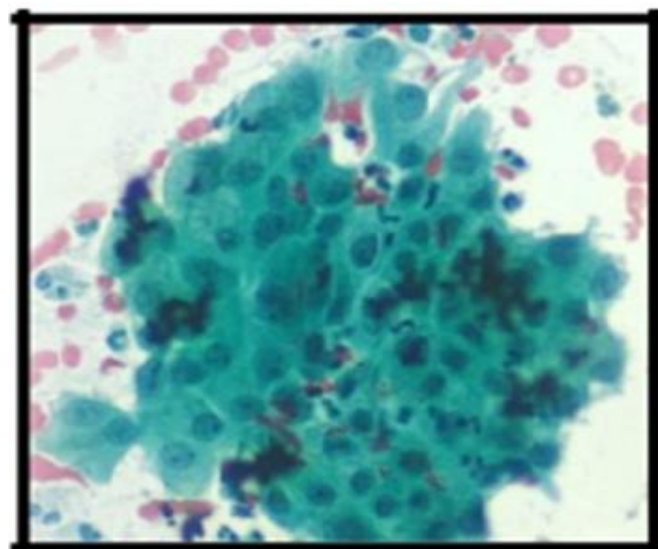


Figure 3: High grade dysplasia suspicious of carcinoma (Pap stainX400s)



Figure 4: Leukoplakia

Table 6: Clinical diagnosis

Clinical diagnosis	No. of cases	%
Relatively Normal	95	26.9
Suspicious	34	9.6
Leukoplakic	60	17
Papule less than 5mm	38	10.7
Hyperpigmentation	29	8.2
Nicotinic palatani	54	15.3
Erythroplakia	08	2.2
Elevation with umbilication	20	5.6
Leukoplakia with palatani	03	0.8
Leukoplakia with Hyperpigmentation	04	1.1

### Discussion

“Smoking is injurious to health” a major anti-tobacco campaign however vigorously done, still smoking practices exist and are a major concern as they cause many nonneoplastic and neoplastic muscosal lesions.

**Comparative studies based on Sample size:** In the present study about 352 patients were included in the study where as in Mrunali Dubal, et al study <sup>[14]</sup> 50 individuals were chosen above 20 years of age who practiced smoking. In Gloria J. Alvarez Gómez et al study<sup>[15]</sup> 46 persons were clinically studied and in Ortiz GM et al study<sup>[8]</sup> 91 volunteer women smokers (61

reverse and 30 conventional) were examined clinically and photographically. Smears were also taken from three areas and the palate to investigate the cytology of palatal mucosal epithelium.

### Comparative studies based on Age distribution:

Present study showed majority of patients among 51-60 years i.e., 45.4 %, next common age group were among 41-50 years i.e., 31.2%. Where as in Gloria J. Alvarez Gómez et al study <sup>[15]</sup> from 46 persons clinically studied, average age was 59.3 years old (range 29 -85), In J. J. Pindborg et al study <sup>[6]</sup> the peak prevalence for females appears in the 55–64-year age group, 75 % of all women in this group being reverse smokers. The results of our study are almost correlating with those of other studies.

**Comparative studies based on Gender distribution:** In our study, female preponderance was noted 59.6% as compared to males 40.3%. This was correlating with other studies including Gloria J. Alvarez Gómez et al <sup>[15]</sup> study where, 42 (91.3%) were females and 4 (8.6%) were males it was similar with J. J.

Pindborg et al <sup>[6]</sup> study as was seen that females indulge in reverse smoking to a greater extent than males, the female: male ratio for all age groups combined being 1-7: 1. A study by Ayesha, noted that, in comparison with males, females were predominantly involved reverse smoking. <sup>[16]</sup> and Gavarasana y Susarla study, <sup>[11]</sup>in India observed similar findings that reverse smoking habit was 6.23 times more frequent in females than in males by the sixth decade of life.

### Comparative studies based on History of smoking:

In our study 37.7 % of patients have smoking history of about 11-20 years followed by 14.7% have about 21-30 years. and observed 91.4% of patients have history of 1-5 cigarettes per day followed by 4.2% have 6-10 cigarettes per day. In Alvarez Gómez et al study <sup>[15]</sup> The average duration of the smoking habits in their observations was

30 years while the average frequency per day of smoking noted as 2.29% with the range of 1-7 times/ day.



Figure 5: Hyperpigmentation with papules

**Comparative studies based on Clinical diagnosis:** In our study, 26.9% were relatively normal on clinical diagnosis .9.6 % were suspicious, leukoplakia was seen in about 17% .10.7 % papule less than 5mm ,8.2% as hyperpigmentation , 15.3 % as nicotinic palatni , 2.2 % erythroplakia(FIG.5) ,5.6 % as Elevation with umbilication(FIG. 6), 0.8 % as Leukoplakia with palatani .1.1 % as Leukoplakia with Hyperpigmentation and Nicotinic palatani with Suspicious, 0.8 % as Leukoplakia with Nicotinic palatani with Suspicious(FIG 7)



Figure 6: Erythroplakia



Figure 7: Elevation with umbilication



Figure 8: Palatal nicotinic stomatitis

In Gloria J. Alvarez Gómez et al study <sup>[15]</sup> moderate lesions tongue presented the higher frequency (80%), followed by severe lesions on palate (74%). Alvarez Gómez et al<sup>[15]</sup> studied reverse smoking in Colombia and found that palatal changes were the second most common manifestations, whereas in Mrunali Dubal, et al <sup>[14]</sup> study, an analysis of the type of smoking habit revealed cigarette smoking manifested smoker's palate more often than the beedi smokers.

The analysis of patients on the basis of the grade of the lesions showed higher proportion of patients manifested severe (II or III) grades of the lesion. Ortiz GM et al study <sup>[8]</sup> showed that subjects grouped into three categories: Group A subjects showed pigmentation and some erythema only; Group B subjects included those with ulceration, marked erythema and non-descript



mucosal roughening; Group C subjects (comprising the majority of reverse smokers) exhibited various combination of leukoplakia, fissuring, thickening and pigmentation of the palatal mucosa. Another study by Sreenivasa Bharath showed palatal changes with presence of 87.77% hyperpigmented areas, 64.44% depigmented areas, 51.66% excrescences, 32.22% potentially malignant lesions and 9.72% frank ulcerations.<sup>[17]</sup>

In our study, 18.4% as low-grade dysplasia, 12.5% were reported to have high grade dysplasia and 1.2% were frank malignancy (FIG 4)

In a study by Reddy,<sup>[18]</sup> risk of developing hard palate carcinoma increased for females 132 times with reverse smoking of chuttas. He stated that reverse smoking allows particulate material (including carcinogens) from the smoke to enter glad openings of the hard palate which do not empty as effectively as do those of the soft palate. In a study by Ayesha<sup>[16]</sup>, out of 20 reverse smokers, 2 subjects (10%) found to have carcinomatous transformation of palatal lesion, much higher than our study. This is because of the chemicals released during reverse smoking induces hyperplasia and hypertrophy initially both in the ductal epithelium and surface epithelium. As a consequence, the ductal lining undergoes dysplastic changes from which micro invasive carcinoma and then invasive carcinoma may arise<sup>[19]</sup>.

Van Der EB et al. in 1993 stated that reverse chutta smoking was a major determinant of palatal cancer as all the cases diagnosed as palatal carcinoma were observed within the group of reverse smokers<sup>[20]</sup>.



Figure 9: Carcinoma

### Conclusion

Reverse smoking is one of the endemic habit still practiced in the Rural Andhra Pradesh, INDIA. And this habit is noted more in females as compared to males. Palate undergoes series of precancerous changes with reverse smoking. In view of low awareness in tribal population and absence of widely available sensitive and specific diagnostic tests, these lesions are difficult to diagnose. There is paucity of compendious and succinct evidence-based data about the protean manifestations, optimum duration of follow up, and long-term outcomes related to palatal changes in the reverse smokers. In this study, an attempt was made to highlight the changes of the palatal mucosa on scrape cytology. Scrape cytology is the preliminary, cost-effective tool for screening of large population at a time to identify the cases that need immediate attention and diagnose malignancy. RISE protocol that we have introduced in this study needs further observation and follow up and we strongly believe that these protocols would help in timely screening and early diagnosis. This study helps to contribute our experiences in early diagnosis and to enhance the knowledge regarding palatal lesions of reverse smoking for future references.



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