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PREVALENCE OF OSMF AND COORELATION OF MICRONUCLEI COUNT WITH HABITS IN OSMF PATIENTS IN UDAIPUR : A HOSPITAL BASED STUDY

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ABSTRACT:

Background: Oral Submucous Fibrosis (OSMF) is well recognized for its malignant potential rate of 7.6% and is particularly associated with use of areca nut in various forms with significant duration and frequency of chewing habits. Oral exfoliate cytology is valuable for mass screening purposes.

Objectives: To evaluate the prevalence of OSMF and to evaluate and correlate the mean micronuclei (MN) count in normal healthy individuals and Oral Submucous Fibrosis patients. Also to correlate the mean MN count with various variables like type, duration and frequency of habit, clinical staging and final cytological diagnosis of OSMF.

Methodology: OSMF cases reporting to the institution from June 2014 to June 2015 were included in the study. A total of 197 subjects were divided into two as study (167 patients clinically diagnosed with OSMF) and control group (30 healthy individuals).Relevant detailed history was recorded and buccal smears were prepared and stained for evaluating cytological class and screening micronuclei.

Results:A prevalence rate of 0.24% was observed for Oral Submucous Fibrosis. Statistically significant result was obtained for MN count in Oral Submucous Fibrosis patients as compared to control group. The count was higher in patients having a combination of gutkha chewing and tobacco smoking habit and increased as the duration, frequency of habit, clinical staging and final cytological diagnosis increased.

Conclusion: The disease is precancerous with a high relative risk for malignant transformation. To evaluate the genotoxic risks related to OSMF, micronucleus test is the best indicator of mitotic interference and chromosomal mutations or breakages.

Keywords: Prevalence, Oral Submucous Fibrosis, Micronuclei, Biomarker, Habits

INTRODUCTION:

It has been suggested that ingestion of arecanut and chilies, genetic susceptibility, nutritional deficiencies, altered salivary constituents, and autoimmunity and collagen disorders may be involved in the pathogenesis of Oral Submucous Fibrosis (OSMF).

The condition is well recognized for its malignant potential rate of 7.6% and is particularly associated with use of areca nut in various forms with significant duration and frequency of tobacco chewing habits. Most examples of the disease are found in India, especially in the southern states No relationship to any community or religious group has been suggested, but an ethnic basis is indicated because OSMF is found mostly in Asians or Asians settled in other countries.[1]

Micronuclei are induced in oral exfoliated cells by a variety of

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substances, including genotoxic agents and carcinogenic compound in tobacco, betel nut, and alcohol.[2] Oral exfoliative cytology is valuable for mass screening purposes.

So the present study was aimed to evaluate the prevalence of Oral Submucous Fibrosis. Also the mean Micronuclei count was evaluated for healthy controls and OSMF subjects and a correlation between the mean Micronuclei count and various variables like type, duration and frequency of habit, clinical staging and final cytological diagnosis of OSMF was carried out.

MATERIALS AND METHODS:

The present study was carried out from the year June 2014 to June 2015. A total of 67,649 patients reported to the outpatient department of Pacific Dental College and Hospital, Udaipur during this period and amongst these 167 were clinically diagnosed as Oral Submucous Fibrosis (OSMF). The study protocol was reviewed by the Ethical Committee and was granted ethical clearance.

Patients clinically diagnosed with Oral Submucous Fibrosis were included as study group and age and sex matched normal healthy individuals were included as controls. Patients with any other oral and systemic diseases were excluded.

Prior to sampling, all the participants were asked to rinse their mouth with water so as to remove residual food particles, cells were collected by scraping the buccal mucosa with moderate pressure using Cytobrush. The cells were then smeared onto a pre cleaned microscopic slide. Two slides were prepared from each individual and the smears were allowed to air-dry, and then fixed in 95% ethyl alcohol. Each fixed smear was stained using Papanicolaou stain (RAPID-PAP KIT) and evaluated for cytological changes and micronuclei.

Total of 1000 cells were evaluated in zig zag method for screening of MN from the buccal smear of each individual. Cells with intact nuclei and cell boundaries were counted. The mean number of micronuclei (MN) was calculated and the data was statistically analyzed using student t test and ANOVA test.

RESULTS:

In the present study a prevalence rate of 0.24% was observed for OSMF. It was more prevalent in 21-40 years age group. Males (89.82%) had a higher gender predilection than females (10.17%).With respect to the socioeconomic status, 59.28 %(n=99) belonged to lower class and 40.71 %(n=68) to middle class. Majority of the individuals were literate. The average micronuclei count of the age and sex matched controls was found to be 1.63+1.629 while that of study group was 17.30+10.47.Statistically significant difference was obtained with p value being 0.000 (Table 1).

Gutkha chewing (GC) was found to be the most common habit in OSMF patients. This was followed by combination of gutkha chewing and

tobacco smoking (TS), was further followed by arecanut chewing (AC), tobacco smoking, combination of arecanut chewing, gutkha chewing, tobacco smoking and alcohol with snuff dipping. The correlation between the type of habit in OSMF and mean MN count revealed statistically non-significant results with a p value of 0.625(Table 2).

The mean MN count increased with increased duration of habit. The correlation between the duration of habit in OSMF and mean MN count revealed statistically significant result with a p value of 0.006(Table 3).

The mean MN count increased with the increase in the frequency of habit. The correlation between the frequency of habit in OSMF and mean MN count revealed statistically non-significant result with a p value of 0.586. (Table 4)

The mean MN count increased as the clinical stage advanced from Stage 1 to Stage 3. The correlation between clinical staging of OSMF and mean MN count revealed a statistically non-significant result with a p value of 0.491. (Table 5)

The mean MN count increased with the increase in the cytological class. The correlation between final cytological diagnosis of OSMF and mean MN count revealed a statistically non-significant result with a p value of 0.718. (Table 6)

DISCUSSION:

Deleterious habits like tobacco chewing, smoking and alcohol consumption are becoming regular practices among the public, due to which there has been an increase in oral lesions associated with them. In India, the prevalence of OSMF have increased over the past four decades from 0.03% to 6.42% (Nigam et al, 2014.[3] The condition carries a high relative risk for malignant transformation (7.6%) [4] It is therefore important to identify markers that could help us to ascertain those lesions that have high potential for malignant conversion and treat them aggressively.

In the present study a prevalence rate of 0.24% was observed for OSMF from the period June 2014 to June 2015. The results were in accordance with the studies conducted by Pindborg et al (1968)[5] who reported a prevalence of 0.04% in Andhra Pradesh to 0.4% in Kerala. Gupta et al(1980)[6] found prevalence to be 0.36% in Kerala, 0.04% in Andhra Pradesh and 0.16% in Gujarat. Kumar et al (2014)[7] reported a prevalence rate of 0.41% in Bariely. However the prevalence rate was found to be high in studies conducted by Sharma et al (2012)[8] where the prevalence of OSMF in Jaipur was found to be 3.39% and Nigam et al(2013)[3] where the prevalence rate in Moradabad of OSMF in habitual gutkha and arecanut chewers was found to be 6.63%.The difference in prevalence rate could be attributed to difference in availability of commercially prepared products.

OSMF was more prevalent in 21-40 years age group with a prevalence rate of 66.46% (n=111). The result was in accordance with the studies conducted by Sinor et al(1990)[9], Shah et al(1998)[10], Haider SM et al (2000)[11], Sami MA et al (2006)[12], Anuradha P and Gaurav Mishra (2011)[13], Sharma et al (2012)[8] and Smita Jyoti et al(2013)[14] where OSMF was more prevalent in 25 to 34 and 35 to 44 years age group. The advent of attractive, conveniently packed sachets and mass and media advertisements, consuming of gutkha and panmasala by younger people has increased. The other reason might be easy availability of gutkha and panmasala in every corner as well as a social status evil.

Males had a higher gender predilection than females. The results were consistent with the studies conducted by Afroz N et al (2006)[15], Sami MA et al (2006)[12] , Pandya et al(2009)[16], Anuradha P and Gaurav Mishra (2011) [13] , Sharma et al (2012) [18] and Nigam et al(2013)[3]where a male predominance was observed. This may be because of easy availability and access of males to the commercially prepared products in all the places where as females being more conscious about their health and esthetic value, probably felt uncomfortable to ask the vendors in getting the gutkha products. This is one of the reasons, which may be responsible for a high male to female ratio.

With respect to the socioeconomic status, majority of the individuals belonged to lower class (n=99). The results were in accordance with the studies conducted by Shiau and Kwa (1979)[17] observed OSMF mostly in farmers belonging to low socioeconomic class. Ramanathan(1981)[18] also found most of the OSMF cases from India were also of low socioeconomic group. McGurk and Crag(1984)[19]also observed that the OSMF patients of Asian community settled in United Kingdom were from low or middle-income group. The reason for OSMF cases coming from low socioeconomic group might be due to poor quality of food, low vitamins particularly in iron deficiency and use of more spices and chillies to make the food tasty, coupled with lack of health consciousness.

Comparison of micronuclei in study and control groups revealed a statistically significant result. In the literature, very few studies have been carried out for evaluation of micronuclei in OSMF.The results were consistent with the studies conducted by Desai et al(1996)[20], Anila et al(2011)[21],Smita Jyoti et al(2013)[14] and Shah N et al (2015)[22] where a significant increase in the micronuclei frequency of OSMF patients as compared to normal individuals was observed. The results suggested that micronuclei test can be used as an early indicator of genotoxicity in OSMF as buccal mucosa is seen to be the most affected. It is most accessible site in oral cavity and its epithelium is non keratinized. In addition, more surface area of the mucosa is exposed to the insults in the

oral cavity making it more vulnerable to changes.

Gutkha chewing was found to be the most common habit amongst OSMF patients. This was in accordance with the studies conducted by Sami MA et al (2006)[12] , Anila et al(2011)[21], Sharma et al(2012)[18] and Bansal et al (2012)[23]where majority of individuals chewed gutkha .The mean MN count was also higher in gutkha chewers than arecanut chewers. This may be due to dry contents of gutkha, which has comparatively more areca nut and tobacco. Dry tobacco absorbed by the mucosa in more amounts produces addiction to the patients.

The individuals who chewed gutkha and smoked tobacco revealed the highest mean micronuclei count. The results were consistent with the studies conducted by Stich et al(1982)[24], Sellappa et al. (2009)[25], Kausar et al. (2009)[26] and Anila et al(2011)[21] where a combination of smoking and gutkha chewing showed a higher mean micronuclei count. A slight lower frequency of micronuclei in exfoliated oral epithelial cells of gutkha chewing group was observed as compared to chewing along with smoking group. This could be because of synergistic effect of both gutkha chewing and tobacco smoking.

The intragroup comparison for the mean MN count with the type of habit in OSMF patients was found to be statistically non-significant with a p value of 0.625.The reason for non-significant result between above group could be due to variation in the number of individuals in each group. In the literature none of the studies have been carried out for the intra group comparison of micronuclei in OSMF patients with different type of habits.

The mean MN count according to duration of habit in OSMF patients increased with the increase in duration. The maximum no of mean MN count was observed in patients who had a longer duration of exposure i.e. more than 10 years. A higher mean MN count may be conclusive that patients who were more exposed to various habits had more genotoxic accumulation and damage. These observations indicate genetic damage, which correlates with precancerous nature of OSMF predisposing to malignant transformation. The result was in accordance with study conducted by Singla et al(2014)[27] where the mean MN count increased as the duration of habit increased.

The intragroup comparison for the mean MN count according to the duration of habit was found to be statistically significant with a p value of 0.006. In the literature none of the studies have been carried out for the intra group comparison of micronuclei in OSMF patients with duration of habit.

The mean MN count according to the frequency of habit in OSMF patients increased with the increase in the

frequency of habit. The highest mean MN count was observed in the individuals with the frequency of habit of more than 10 packets/day. The result was in accordance with the study conducted by Singla et al(2014)[27] where the mean MN count increased with the increase in the frequency of habit increased. It was seen that as the frequency and duration of habit increased, the number of mean MN count also increased, thus establishing a positive correlation between the frequency and duration of habit and genotoxic effect.

Reddy et al(2011)[28] and Mukram Ali et al(2013)[29]conducted studies to evaluate and correlate the effect of frequency, duration, clinical grading and type of areca nut products on the incidence and severity of OSMF and concluded that habitual chewing of gutkha and other areca nut products played a major role in the etiology of OSMF. The duration of habit of more than 10 years and a frequency of more than 10 packets/ day increased the severity of the disease with maximum number of cases observed in grade II and grade III OSMF.

The intragroup comparison for the frequency of habit was found to be statistically non-significant with a p value of 0.586.The non-significant result may be due to variation in the number of individuals in each group.There were no literature reports that revealed any similar study conducted to correlate the effect of frequency and duration of tobacco related habits to genotoxicity observed as MN.

The mean MN count increased as the clinical stage advanced from Stage 1 to Stage 3.The results were consistent with the study conducted by Anila et al (2011)[21] where a significant increase in the mean MN count was observed from Stage 1 to Stage 4 in OSMF patients. Our study could be second study in the literature although many studies with comparison of clinical staging in squamous cell carcinoma with micronuclei are available.

The intragroup comparison of the mean MN count for the clinical staging of OSMF revealed a statistically non-significant result of p value 0.491. The non-significant result may be due to unequal distribution of study participants in each group.

The mean MN count increased with the increase in the final cytological diagnosis. This could probably be explained by the fact that development of MN is a cytological feature under regulation of the nucleus. The effect of genotoxic agents such as tobacco are cumulative and DNA damage is passed on to the daughter cells. The effects of these damages reflected by MN may lead to the development of pre-cancer and cancer.

In the present study a 45 year old male patient with a history of gutkha chewing of 1 packet/day since 25 years and mouth opening of 22 mm had a clinical staging of Stage 3 OSMF.On cytological

evaluation the features were suggestive of Class III . On biopsy it was diagnosed as well differentiated squamous cell carcinoma. This reflects the high rate of malignant transformation of OSMF into oral squamous cell carcinoma (OSCC).Thus, MN assessment and a high index would presuppose predisposition of an individual to the development of these changes. This knowledge can be used to monitor, prognosticate and educate the individuals into cessation of the habits and thus prove to be an educational and screening tool. Longitudinal studies need to be carried out to quantify and support this contention.

Intragroup comparison of the mean micronuclei count of final cytological diagnosis of OSMF revealed a statistically non-significant result of p value 0.718. The non-significant result may be due to unequal distribution of study participants in each group.

From the present study it becomes clear that the type of habit, duration of habit, frequency of habit, clinical staging and final cytological diagnosis have direct significant relation with the severity of OSMF. Variation in the mean MN count explains their cytogenic and mutagenic effect on oral mucosa when compared with respect to type of habit, duration of habit, frequency of habit, clinical staging and final cytological diagnosis.

CONCLUSION:

Epidemiologic studies have demonstrated a wide variety in prevalence rates in Oral Submucous Fibrosis in different population due to various habits. The disease is precancerous and carries a high relative risk for malignant transformation.

It is generally accepted that oral carcinogenesis is a multi-step process of accumulated genetic damage leading to cell dysregulation with disruption in cell signaling, DNA-repair and cell cycle which are fundamental to homeostasis.To evaluate the genotoxic risks, DNA damage can be assessed by cytogenetic markers like chromosomal aberrations, sister chromatid exchanges and micronuclei. Out of all these, micronucleus test is preferable as it does not require tedious procedures like cell culture and metaphase preparation. To further add, as it is applicable on interphase cells only, it is the best indicator of mitotic interference and chromosomal mutations or breakages. It is a non-invasive and very economical procedure.

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TABLES:

TABLE 1: Comparison of the Mean Micronuclei(MN) Count between the Study and Control Group

GROUP	N	MEAN± SD	p VALUE
OSMF	167	17.30±10.473	0.000(S)
CONTROL	30	1.63±1.629	

Student t test, p value <0.05 significant

TABLE 2: Correlation between the Type of Habit in OSMF patients and Mean MN Count

TYPE OF HABIT	N	PREVALENCE RATE (%)	MEAN ± SD	p VALUE
GC	122	73.05	17.53±9.91	0.625
GC+TS	12	7.18	20±10.45	
AC	9	5.38	14.56±12.48	
TS	8	4.79	16.25±9.70	
AC+GC+TS	8	4.79	19.75±9.13	
ALCOHOL+ SNUFF DIPPING	8	4.79	13.25±2.89	

ANOVA test, p value <0.05 significant

TABLE 3: Correlation between the Duration of Habit in OSMF patients and Mean MN Count

DURATION OF HABIT	N	PREVALENCE RATE (%)	MEAN ±SD	p VALUE
<5 years	47	28.14	14.36±10.33	0.006(S)
5 to 10 years	85	50.89	19.82±9.39	
More than 10 years	35	20.95	19.83±10.20	

ANOVA test, p value <0.05 significant

TABLE 4: Correlation between the Frequency of Habit in OSMF patients and Mean MN Count

FREQUENCY OF HABIT	N	PREVALENCE RATE (%)	MEAN ± SD	p VALUE
<5 packets/day	128	75.44	16.17± 10.98	0.586
5 to 10 packets/day	16	50.29	16.70±10.01	
More than 10 packets/day	23	25.74	18.33±10.25	

ANOVA test, p value <0.05 significant

TABLE 5: Correlation between Clinical Staging in OSMF patients and Mean MN Count

CLINICAL STAGING	N	PREVALENCE RATE (%)	MEAN ± SD	p VALUE
Stage 1	1	0.59	17.50±.707	0.491
Stage 2	152	91.01	18.44±.826	
Stage 3	14	8.38	21.86±3.328	

ANOVA test, p value <0.05 significant

TABLE 6: Correlation between Final Cytological Diagnosis in OSMF patients and Mean MN Count

FINAL CYTOLOGICAL DIAGNOSIS	N	PREVALENCE RATE (%)	MEAN ±SD	p VALUE
Class I	15	8.98	16.67±7.965	0.718
Class II	133	79.64	17.23±0.904	
Class III	18	10.77	20.53±2.376	
Class IV	1	0.59	22.50±1.50	

ANOVA test, p value <0.05 significant