A COMPARATIVE STUDY OF BUCCAL MUCOSAL CELLS FOR CYTOLOGICAL CHANGES OCCURRING IN SMOKELESS-TOBACCO EXPOSED POPULATION WITH THE NON-EXPOSED IN THE SAURASHTRA REGION OF GUJARAT STATE

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Abstract:

The study was conducted to detect the impact of consuming smokeless tobacco (in any form) on the buccal mucosal cytology of 100 male subjects (50 exposed and 50 non-exposed) of 18-60 age group. After proper ethical clearance, the samples were collected using sterile wooden spatula, fixed and stained by routine H&E technique. The cells observed for changes of multinucleation, binucleation, karyolysis and chromatin condensation of nuclei. The data obtained was compared with the previous studies done. It was concluded that, the intake of smokeless tobacco leads to an increased tendency of cytological aberrations and the buccal mucosal cytology is an easy, painless and cost effective procedure to detect these changes at an early stage and start preventive measures accordingly.

<u>Keyword</u>: Buccal mucosa, cytology, multinucleated, binucleated, karyolytic, chromatin condensation

Introduction:

Oral cancer is one of the ten most common cancers in the world. Its high frequency in central and Southeast Asian countries like India, Bangladesh, Srilanka, Thailand, Indonesia and Pakistan, has been well documented. *In India:* for the year 2008, with estimated incidences of 9.8 cases per 1 lac population for males and 5.2 cases per 1 lac population for female, oral cancer was found to be a major problem in India. The estimated mortality was about 6.8 per lac in males and 3.6 per lac in females.⁽¹⁾

Despite numerous advances in treatment, the overall long-term survival has remained at less than 50% for the past 50 years. This is due to several factors including the fact that oral cancer is often diagnosed when the disease has already reached an advanced stage. ⁽²⁾

Tobacco in various form of its usage (smoking and chewing) is the major environmental cause of cancer of mouth, lung, larynx, pharynx etc. ⁽³⁾

Approximately 90% of oral cancers in south East Asia are linked to tobacco chewing and tobacco smoking. During 1966-1977, a large epidemiological survey was carried out in different parts of the country. In a follow up study of 10 years on 30,000 individuals in the three districts of Ernakulum (kerela), srikakulum (andhra) and Bhavnagar (Gujarat), the results indicated that:

- a. Oral cancers and precancerous lesions occurred solely among those who smoked or chewed tobacco.
- b. Oral cancer was always preceded by some type of precancerous lesions.
- c. Cancer always occurred on the side of mouth where the tobacco quid was kept and the risk was 36 times higher than for non-chewers if the quid was kept in the mouth during sleep. ⁽¹⁾

Material and method:

After obtaining clearance from the Institutional Ethical Committee of M. P. Shah Government Medical College, Jamnagar , samples were taken from the buccal mucosa of 100 individuals selected from the patients attending at the male - Out Patient Department in the Departments of Medicine, Guru Govind Hospital, Jamnagar. These individuals were from a mixture of urban and rural settings from different areas of Jamnagar districts.

The subjects were divided into 2 groups as follows:

Sr. No.	Type of Addiction	No. of cases
1.	Tobacco Chewer (T)	50
3.	Control (non tobacco chewer and alcohol drinker) (C)	50
Total no. of cases		100

Only males, of 18-60 years of age who were tobacco chewers with normal appearing buccal mucosa and normal jaw mobility were included in the study. After seeking proper consent, personal details and proper history regarding tobacco chewing (mava, gutkha, khaini, jarda etc) was entered in the proforma prepared.

The subjects were asked to rinse their mouth with distilled water. A new wooden spatula was used every time to scrape the of buccal mucosa of each patient, for at least three to four times with firm pressure and the obtained sample was then spread on a clean microscopic slide coded with case number to get the smear. The smear was immediately fixed in 95% ethyl alcohol for 10-15 minutes to assure adequate fixation. Then the smears were stained with routine Haematoxyline and Eosin staining technique in the histology section of Department of Anatomy of MP Shah Govt. Medical College Jamnagar and were visualized under low power and high power of binocular microscope.

The data was summarized and statistically analyzed using SPSS software. Mean values of all parameters were compared by student's't' test to check their statistical significance and P value was obtained from it. If the P value is > 0.05 then the difference is not statistically significant. If the P value is between 0.05 and 0.01 then the difference is statistically significant. If the P value is <0.01 then the difference is statistically highly significant. The observations of the study were tabulated and interpreted for discussion in details.⁽⁴⁾

Result:

- The mean age of subjects in control group was 36.64 ± 13.5 years and in tobacco, exposed group was 39.7 ± 14.8 years.
- The mean number of multinucleated cells in control group was study group was 0.3 ± 0.8 and in tobacco exposed group was 4.0 ± 2.3 and this difference was found to be statistically highly significant (p<.01)
- The mean number of binucleated cells in control group was study group was 0.96 ± 1.7 and in tobacco exposed group was 3.8 ± 1.7 and this difference was found to be statistically highly significant (p<.01)
- mean number of karyolytic cells in control group is 0.5 ± 1.3 , in tobacco exposed group is 4.0 ± 2.3 , and this difference was found to be statistically highly significant (p<.01)
- The mean number of cells with chromatin condensation in control group is 0.2 ± 0.7 , in tobacco exposed group is 0.9 ± 0.9 and this difference was found to be statistically highly significant (p<.01)

Discussion:

The subjects under study were divided in two groups of tobacco exposed (taking only smokeless tobacco without any alcohol intake) and the male subjects with similar age group without any alcohol or tobacco intake were taken as control groups.

The findings thus obtained from the present study were found in accordance with the other coworkers. In the present study, it was found that there was an increase in the mean number of multinucleated cells in tobacco-exposed subjects and it was highly significant as compared to control groups.

These findings are in accordance to those found by Sharma VL et al (2013)⁽⁵⁾, who also found an increase in the incidences of Multinucleation in the subjects exposed to tobacco and the findings were highly significant when compared to controls.

Harikrishna G F (2011)⁽⁶⁾ reported the genotoxic effect of tobacco chewing in vivo and vitro conditions and their conclusions were also similar to present study⁻

Bansal H et al (2012) ⁽⁷⁾also evaluated the occurrence of micronuclei in Punjabi population and concluded the genotoxicity of tobacco chewing over buccal mucosa, which was similar to the present study.

Similar results were obtained by Kashyap B et al (2012)⁽⁸⁾ and Jindal et al (2013)⁽⁹⁾ who detected the alteration in buccal mucosal cells due to effect of tobacco and they concluded that these agents were carcinogenic leading to increase in number of silver stained nucleolar organizer regions and micronuclei in buccal mucosa.

In the present study it was found that there was increase in mean number of binucleated cells in tobacco exposed subjects as compared to control group and this increase was statistically highly significant.

These findings were in accordance with the study of Sharma VL et al (2013) ⁽⁵⁾ who found a significant increase in incidences of binucleated cells in tobacco chewers and it was highly significant as compared to controls.

Increased incidences of Karyolytic cells found in the present study were in accordance with the study of Sharma VL et al (2013)^{(5).} who found that there was a significant increase in incidences of karyolytic cells in both alcohol consumers and tobacco chewers and it was highly significant as compared to controls.

Similar findings were observed by Burzlaff et al (2007)⁽¹⁰⁾, who found increase in number of anucleated cells and the cytological atypia in tobacco and alcohol exposed individuals and it was found to be statistically significant.

Reis et al (2006) ⁽¹¹⁾ in their study found a non-significant increase of karyolytic cells in alcohol exposed subjects. Such differences might be because of population difference or difference in the technique used.

Sharma VL et al (2013) ⁽⁵⁾ also found cells with chromatin condensation in 1% of alcohol and tobacco exposed subjects but did not mentioned about statistical significance. It might be due to some technical error, however in the present study there was a statistically significant increase in cells with chromatin condensation found in the tobacco chewers.

Conclusion:

The increased tendency of nuclear aberrations and statistical analysis suggested that buccal mucosa is susceptible to cancer in those who are consuming smokeless tobacco. The methodology used in this study was simple, rapid and painless. Such a technique is cost effective and can be practised in rural hospitals for the detection of oral cancers at the preliminary stages and in effective patient counselling for prevention.

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INVESTIGATING CATARACT REFERRAL PRACTICES USED BY INDIAN OPTOMETRISTS

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ABSTRACT

Background: Optometrists are primary eye care practitioner and is gaining popularity as the first point of contact with the patients. However, little is known about the cataract surgery referral criteria used by optometrists in India. **Methods:** 2574 Optometrists from India were invited to complete an online survey on cataract referral practice. The survey elicited information on practice demographics of optometrists and cataract referral considerations. **Results:** Total respondents in the present study were 832.Respondents stated visual acuity of $\leq 6/12$ to $\leq 6/18$ as the benchmark for referring the patient for cataract surgery. Considerably lower proportion used glare and contrast sensitivity testing in cataract patients. Patient centred factors such as hobbies, driving, featured in the decision to refer patients sooner. **Conclusion:** Although the reduction in the visual acuity was considered the prime factor affecting the decision making of Indian optometrists for referral for cataract surgery, patient's visual demands also played an important role.

INTRODUCTION

Cataract remains the leading cause of visual impairment in all areas of the world, except for developed countries ^[1]. Visual impairment has been recognized as an important public health problem in India, a country that is now home to a billion inhabitants ^[2-9]. India was the first country in the World to launch a 100% public funded programme for the Control of blindness ^[10]. The National Programme for Control of Blindness has emphasized the need for cataract surgical services and refraction services to be augmented, both in quantity and quality, in order to achieve the goal of eliminating avoidable blindness by 2020 ^[2-9]. A major portion of referral for cataract surgery in India is made by Optometrists. Optometrists are trained in various institutes and some of them have developed their own procedures and protocols for referrals ^[11]. Optometrist play a critical role in the identification and appropriate referral of cataract surgery to ophthalmologists have not been extensively studied in India. The aim of the study was to investigate the referral considerations of cataract surgery by Indian Optometrists. This study gives insight into factors like how socioeconomic factors, remoteness to healthcare, professional experience can impact cataract referral decisions of optometrists practising in India.

METHOD

This was a Cross-sectional Questionnaire survey of Optometrists practicing in India. A preformed questionnaire of Cataract referral practices ^[12] of Australia was considered. It was modified and validated to be used in Indian scenario of practices. An email containing the survey link was distributed to 2574 optometrist in November 2015, explaining the purpose of the

survey and inviting participation. A reminder email was sent to the entire sample six weeks after the initial invitation and the survey closed two week later. No financial incentive was provided for participation.

The survey consisted of total 28 questions. These include questions on patient's Visual acuity, contrast, glare, and symptoms of the patients. Optometrists were asked to rate the level of importance that factors had on their decision of where to refer their patients. Response frequencies were tabulated for all survey questions. The entire filled up questionnaire was entered in Microsoft Office Excel 2007. Mean and standard deviation were computed for Quantitative variable. Odds Ratio was computed for study parameters.

RESULT

The demographic data of 832 respondents are shown in Table 1.

Characteristic	Value	
Туре	Intern	148
	Practitioner	684
Gender	Male	374
	Female	458
Age	Years	26.37 ± 5.43
Years in Practice	<= 5 years	607
	>= 5 years	225
Main Practice Type	Corporate	465
	Independent	367
Professional Scope Of Practice	General primary eyecare	582
	Specialty Practice	250
	Others	0

TABLE 1: Demographic Data

The visual acuity at which the respondents prefer to refer the patients for cataract surgery is shown in figure 1.Visual acuity between $\leq 6/12$ and/or $\leq 6/18$ was a common benchmark for cataract referral with 72.69% of respondents reporting referral at this level of vision. 87% of the respondents prefer not to consider contrast sensitivity testing in decision for cataract referral.

71% of the respondents consider the factor of accidental falls of the patient in the referral for surgery.



Factors affecting decision on "when to refer patients for cataract surgery" is shown in figure 2. A significant proportion of optometrists reported that patient lifestyle and health system influenced their urgency of referral for cataract surgery. Of the life style factors presented, patient's enthusiasm (72.11%) for surgery elicited the highest percentage of respondents who would "refer urgently and refer sooner" followed by patients having high visual demand (56.85%), patient's current employment status (50.84%) and patient's driving dependence (42.78%) factor. There was no influence on factors of continued less vision in the eye after surgery. The factors which had the greatest influence on a decision to delay referral were patients not wanting surgery, patient drives but not dependent, and patients wanting to go on public waiting list for cataract surgery.



Figure 2: Factors influencing urgency for referral



Figure 3: Factors affecting patient referral

Figure 3 shows factors affecting decision on "where to refer patients for cataract surgery". Factors influencing the optometrist's choice of referral location were also examined. Surgeon's skill was ranked 'extremely important' by the highest proportion (61.53%) of optometrists in deciding where they referred patients for cataract surgery. Waiting time for patient seeing the same doctor (If they have received ophthalmic care) was ranked to be "Of little importance" (42.90%).

The opinion of the respondents as to where they chose to refer Cataract patients for surgery is shown in figure 4. Surgical cost was considered for the surgery while referring the patient. Odds ratio with respect to the referrals to private and public hospital is 1.07. This means that the



specific referrals to private hospitals are 1.07 times more than public hospitals.

Figure 4: Choice of referral

DISCUSSION

In our knowledge this was the first study conducted among the Indian optometrist for cataract surgery referrals. The standard guidelines for cataract surgery includes the criteria of Visual acuity, contrast sensitivity and glare testing ^[13]. The patterns of referrals followed by Indian optometrists are mainly based on Visual Acuity and contrast sensitivity. Glare testing is not preferred by most of the optometrist for referral. Emphasis should be equally laid on glare testing as it is affected earlier than visual acuity. Testing for glare and contrast should be performed to rule out the signs of cataract which affect the daily activities of the patient and help to maintain the record for the subsequent follow up visits to detect the changes. The aim of these guidelines is to identify good clinical practice, set standards of patient care and safety and provide a benchmark for outcomes within which high quality cataract surgery can be practiced. They represent the current understanding of the guideline development group.

In the present study the optometrists in India prefer to refer the patients having visual acuity between $\leq 6/18$ to $\leq 6/9$. This criteria is close to the criteria followed by both the optometrists and ophthalmologists of West Midlands ^[14]

In the present study 71% respondents have considered the falling history of the patient in decision making for the referral for cataract surgery. In the study "Prospective study of the rate of falls before and after cataract surgery" by S Brannan, C Dewar et al ^[15] concluded that there was significant reduction in the risk of falls in patients after cataract surgery.

Patient's hobby which has high visual demand is one of the most important factors influencing the urgency of referral for cataract surgery followed by driving dependency of the patient and current employment. Comparing the present study with that of QuangDoV, Li R et al^{[12],} it can be said that the referral criteria is broadly similar in case of patient's hobbies for driving, visual acuity benchmark. According to the present study the specific referrals to private hospitals is 1.07 times more than public hospitals. In the study by QuangDoV, et al ^[12] respondents practicing in more advantaged socioeconomic areas were 2.4 times more likely to refer privately. Surgeon's skill, caring staff are the extremely important factors influencing optometrists' decisions on where to refer for cataract surgery whereas surgeon's personality has minimal importance. Priority is more for private than for public hospitals. Waiting time for surgery is also considered in selecting where to refer the patient. Socio economic status is the only

predictor of whether an optometrist chooses to refer patients publicly or privately for cataract surgeries. Factors affecting the referrals may include the surgical costs at private sector, and the large waiting times at public hospitals prior to the surgery.

The target of NPCB (State wise targets & Achievement for various eye diseases during 2015-16) cataract surgeries is 6600000 out of which 2511867 is achieved. Still 4088133 is the backlog in achieving the goal of the target surgeries in India (As per NPCB data) ^[16]. According to Guidelines for Quality Cataract Services- National Programme for Control of Blindness Health & Family Welfare Department Government of Gujarat 2014^[17], gave the subjective and objective criteria for patient selection for cataract surgery. Practitioners frequently make clinical decisions based on changes in visual acuity. However, acuity is recognized to be only one aspect of visual performance. High frequency contrast sensitivity charts are even more sensitive to refractive blur. Despite of proper refractive correction, some patients will complain of a visual problem but no visual anomalies. This is common with early cataract patients ^[18]. These guidelines are not widely followed and the awareness of these guidelines is very low.

Awareness among the optometrist regarding the referral patterns for all the eye conditions which may require the involvement of an ophthalmologist. Formation of the council to regularize the level of academic activities should be done. Hence, increasing the number of the referrals for the cataract surgery may become helpful to the programme VISION 2020: RIGHT TO SEE to achieve the target. Referrals can be increased by properly screening the patients at various sectors. However the population at the urban area is educated enough to consult an eye care practitioner, but the patients of cataract in rural areas are totally unaware of their condition. Thus, regular eye camps at such places should be conducted and the patients should be referred if needed. Coordination between the ophthalmologist and optometrist also plays an important role. Integrated eye care delivery models typically work by having ophthalmologists and optometrists together in the same practice or institution, where either can see patients at any time. Integrated models do not raise the same legal issues involving referral and postoperative care. This is the stepping stone towards achieving the goal of VISION 2020 to eradicate blindness due to cataract.

The role of optometrists as a first point of contact for patients in the cataract referral pathway places them in a key position to positively influence surgical candidate selection, the subsequent efficiency of surgical processes and the satisfaction of patients with their surgical journey and overall outcomes. By understanding and documenting the current cataract referral practices of optometrists in India, this research provides a foundation for building more effective and efficient management strategies for patients with cataracts and will help to achieve the goal of VISION:2020.

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