

## RESEARCH ARTICLE

# Comparison of Different Screening Methods in Estimating the Prevalence of Precancer and Cancer Amongst Male Inmates of a Jail in Maharashtra, India

Amit Chaudhari<sup>1\*</sup>, Sahana Hegde-Shetiya<sup>2</sup>, Ravi Shirahatti<sup>3</sup>, Deepti Agrawal<sup>2</sup>

### Abstract

**Aim:** To compare the effectiveness of self-examination, clinical examination and screening methods using 1% toluidine blue and Lugol's iodine in estimating the prevalence of lesions at risk of malignancy and oral malignant disease amongst the male inmates of Yerwada Central Jail, Pune. **Material and Methods:** Study was carried out on male inmates in two phases. In the first phase self-examination and clinical examination was carried out on 2,257 male inmates. 164 suspicious cases were subjected to phase II of the study out of whom, 82 participants were screened with 1% toluidine blue and 2% Lugol's iodine followed by biopsy procedure. **Results:** Sensitivity and specificity for self-examination with clinical examination was 92.2% and 96.6% respectively. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) and positive (+LR) and negative likelihood ratio (-LR) for Toluidine Blue were 88.1%, 66.6%, 97.1%, 30%, 2.63 and 0.17 respectively while for Lugol's Iodine they were 94.7%, 83.8%, 98.6%, 55.5%, 5.67 and 0.06 respectively. **Conclusions:** Prevalence of lesions at risk of malignancy and oral malignant disease by self-examination was 7.8% and by clinical examination was 6.3%. Self-examination is an effective tool in early detection of oral cancer. Use of Lugol's iodine as a screening tool for oral lesions is highly effective in inmate populations.

**Keywords:** Lesions at risk of malignancy - oral cancer - mouth self-examination - toluidine blue - Lugol's iodine

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### Introduction

Cancer is one of the major threats to public health in the developed world and increasingly so in the developing world. According to the World Health Report 2004, cancer accounted for 8 million deaths in 2003 and it is estimated that the overall number of new cases will rise by 50% in the next 20 years. The prevalence of oral cancer is particularly high among men, the eighth most common cancer worldwide (Kunjan et al., 2005; Peterson, 2005).

Consumption of tobacco is a major risk factor for mortality. Recent shifts in global tobacco consumption indicate that an estimated 930 million of the world's 1.1 billion smokers live in developing countries, with 182 million in India alone (Subramanian et al., 2004). By 2020 tobacco consumption has been projected to account for 13% of all deaths in India (Subramanian., 2004). Its high risk in the Indian subcontinent is related to popularity of tobacco chewing (a combination of betel leaf, lime, areca nut, and sun cured tobacco) (Kuruvilla, 2004; Sankaranarayanan et al., 2005).

Unfortunately, most oral cancers lack early signs and despite improvements in diagnostic and therapeutic

modalities, the prognosis of patients with oral malignancies has remained poor (Kunjan et al., 2005). Conventional oral exploration (visual and palpation examination) constitutes the gold standard screening study for oral precancer and cancer; the relevant study for the detection of cases is the biopsy and histopathological diagnosis (Lestón et al., 2010). There are also a number of techniques that may contribute to the diagnosis of oral cancer such as toluidine blue (Mashberg, 1980) exfoliative cytology or brush biopsy (Bhoopathi et al., 2009), Chemiluminescence (Oh et al., 2007), Lugol's iodine (Epstein et al., 1992).

Regular self examination is known to be effective in decreasing mortality and incidence of breast and cervical cancers (Kunjan et al., 2009). Apart from a few studies in India and Sri Lanka and some oral cancer screening programme in Cuba, very few investigations have been undertaken to evaluate the role of self-examination in early detection of oral cancer (Mathew et al., 1995).

The use of toluidine blue (TB) has been extensively studied, overall, the sensitivity of TB staining for the detection of oral cancer varies between 78% and 100% but the specificity between 31% and 100% (Lingen et al., 2008). Lugol's iodine has been used extensively in

<sup>1</sup>Department of Public Health Dentistry, Kothiwad Dental College, Moradabad, <sup>2</sup>Department of Public Health Dentistry, Dr. D. Y. Patil Vidyapeeth's Dr. D.Y. Patil Dental College, Pimpri, <sup>3</sup>Department of Public Health Dentistry, Sinhgad Dental College, Pune, India \*For correspondence: e-mail [dr.amit.chaudhari@gmail.com](mailto:dr.amit.chaudhari@gmail.com)

the detection of early carcinoma of the cervix (Epstein et al., 1992), mucosal carcinoma of esophagus (Dawsay et al., 1998). Sensitivity and specificity of lugol's iodine in detecting mucosal carcinoma varies between 91-100% and 72-95% respectively (Dawsay et al., 1998).

The literature on oral health and tobacco consumption amongst prisoners is limited. There are more than 1,100 prisons in India with a total estimated capacity of 277,304 prisoners. In spite of the high prevalence of tobacco consumption in the Indian population and its status as an oral cancer risk factor there is no information available about tobacco use in Indian prisoners and their awareness of oral cancer (Dahiya et al., 2010). Providing screening and treatment for oral precancer and cancer in prison presents a number of challenges like free availability of tobacco products in the jail and various security concerns.

Hence the need was felt to conduct a study among male inmates of Yerwada Jail in Pune with an aim to estimate the prevalence of lesions at risk of malignancy and oral malignant disease and comparison of self-examination, clinical examination and two different screening methods.

## Materials and Methods

The study was carried out in Yerwada central jail amongst male inmates near Pune city. Smokeless tobacco and tobacco in the form of 'Bidi' is readily available in the jail premises. Almost all male inmates are consuming tobacco and tobacco related product due to which they are at a high risk for potentially malignant oral lesions.

Prior permission for carrying out the study in Yerwada jail was obtained from the jail authority. Ethical clearance for the study was obtained from the Institution Ethics Committee of Dr. D.Y.Patil University. The study objectives and procedure was explained in detail to the participants. Informed and written consent was obtained from study participants prior to undertaking the study. All the male inmates consenting for self-examination, clinical examination and screening test were included in the study. Inmates below 18 years of age, those who were uncooperative, participants with debilitating medical conditions, participants to whom permission was not granted by Jail authority were excluded from the study.

Pilot study was carried out before commencing the main study on 50 participants to evaluate feasibility of self-examination and clinical examination as well as methodology. After pilot study, case history proforma was modified and demographic variables such as income, occupation and diet were deleted. Training and calibration of the investigator (AC) in examination process was carried in the Department of Oral medicine and Radiology of the institution.

### *Study was carried out in two phases*

#### Phase I – Self-examination and clinical examination:

For self-examination, participants were educated regarding tobacco and its ill effects on the oral cavity with the help of series of photographs depicting precancerous lesions and conditions. They were asked to identify similar lesions in their oral cavity using face mirror under natural light. Their responses were noted down under the following categories

as Abnormality Suspected/Not Present/Not Sure/Unable to Examine by recording assistant and demographic details were obtained. The clinical examination was carried out by the chief investigator (AC), using ADA type III examination under natural illumination. The findings of clinical examination recorded by the chief investigator was noted down in following categories as normal or non-referable lesions, referable lesions that were suggestive of precancerous lesions, lesions suggestive of cancer (Sankaranarayanan et al., 2005).

Study population consisted of 4,000 male inmates of Yerwada central jail. 2,572 male inmates were recruited for phase I of the study i.e. self-examination and clinical examination. The reasons for 1,428 not consenting for phase I of the study were, absent on the day of examination, unwilling to participate in the study, unable to examine due to strict security reasons.

Out of 2,572 study participants, 175 were suspected of having lesions at risk of malignancy (precancerous lesions and conditions) and malignant lesions. Due to security reasons photography of lesions were not allowed in the jail premises. Hence duplicate examination was carried out with the help of an expert examiner (DH) from the Department of Oral Medicine and Radiology. 250 (175 positive cases+75 non-positive cases) participants for duplicate examination were randomly selected using Microsoft Excel Spreadsheets (2007) by independent operator (RS). A high level of agreement (0.79) between the examiners showed good inter-examiner consistency.

Phase II: Of the study all the cases detected positive by chief investigator and expert examiner were considered i.e. 164. Considering the feasibility of biopsy procedure and dropout rates, sample size was decided to be 77 (Proportion -0.85, precision 8%, confidence interval- 95%) (nMaster version 1.0 software, Department of Biostatistics, CMC, Vellore, India). Considering the dropouts, sample size was increased by 10% and fixed at 85 participants. However, 82 participants consented for staining procedure and biopsy procedure.

Before application of vital stain all participants were asked to stop the tobacco habit, they underwent oral prophylaxis and were asked to apply antifungal medication and vitamin A supplements were given to reduce false positive cases (Epstein et al., 1997; Mashberg 1981; 1983).

### *Staining procedure*

After recording the clinical features of clinically suspicious lesions, 1% Toluidine blue and 2% Lugol's iodine were applied as illustrated by Epstein JB10. Out of 82 participants, 41 participants were first screened with toluidine blue using above procedure and interpretation. After application of toluidine blue the 41 participants underwent Lugol's iodine application after 24 hours of washout period. All the procedure was interchanged for 41 participants i.e. 41 participants were first screened with Lugol's iodine and subsequently with Toluidine Blue. Lugol's Iodine was applied on the same region 24 hrs after Toluidine Blue application and subjected for biopsy. After taking biopsy, specimen was immediately stored in 10% Formalin solution and subjected to histopathological analysis. For study purpose histopathological interpretation

was limited to the dysplasia present (positive) or absent (negative). All histopathological interpretations were carried out by the Oral Pathologist (SN).

The biopsy reports were collected and all participants were informed about the result. Tobacco cessation was reinforced. All the positive cases were advised to undergo treatment and were referred to Government hospital, Pune along with participants who were diagnosed positive with clinical examination and were not included in phase II of the study. While all negative cases were kept under observation of visiting Dental Surgeon.

## Results

### Sample characteristic

Out of 4,000 male inmates, 2,572 prisoners were included in the study (self-examination and clinical examination). Hence participation rate of prisoners was 64.3%. Refer to flowchart (Figure 1). Mean age of the participants was 34.98 years (SD=12.65, Range 19, 69 years). Out of 2,572 participants, 353 (13.7%) smoked mostly Bidis, 702 (27.3%) were tobacco chewers who chewed the tobacco in the form of Gutka or raw tobacco with lime. While 1,415 (55.01%) were both smokers as well as tobacco chewers (exposure rates 96.04%). However, 102 (3.96%) participants out of 2,572 never had tobacco in either smoking or smokeless form. Mean year of exposure to tobacco product among participants was 7.63 years (SD=5.23).

### Mouth self-examination

Out of 2572 participants, 201 (7.82%) participants identified their oral cavity with one of the photographs shown, while 1382 (53.7%) participants reported as lesion not present. However, 611 participants were not sure about the lesions and 378 participants were unable to examine their oral cavity (Table 1).

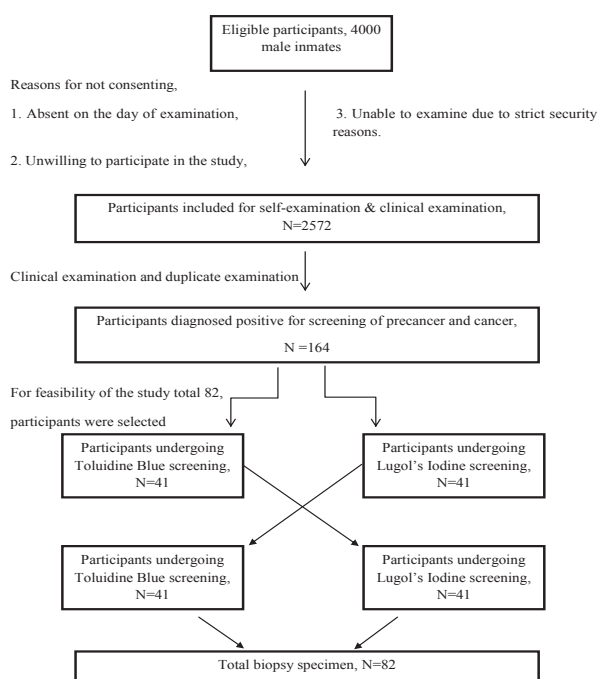


Figure 1. Flow of Participants Through Phases

Table 1. Self-examination vs. Clinical-examination

Self-examination	Clinical Examination		Total
	Present	Absent	
Abnormality suspected	154	47	201
Not present	13	1369	1382
Not sure	5	606	611
Unable to examine	3	375	378
Total	175	2397	2572

\*Sensitivity: 92.2%; Specificity: 96.6%; Positive predictive value: 76.6%; Negative predictive value: 98.5%

Table 2. Diagnostic Accuracy of Toluidine Blue and Lugol's Iodine

	Histopathological Examination		Histopathological Examination		
	+	-	+	-	
Toluidine +	67	2	Lugol's +	72	1
Blue -	9	4	Iodine -	4	5

\*Toluidine blue; Sensitivity=88.1%; Specificity=66.60%; Positive predictive value=97.1%; Negative predictive value=30%; Positive likelihood ratio=2.63; Negative likelihood ratio=0.17, \*\*Ugol's Iodine; Specificity=83.8%; Positive predictive value=98.6%; Negative predictive value=55.5%; Positive likelihood ratio=5.67; Negative likelihood ratio=0.06

Table 3. Diagnostic Accuracy when Either or Both the Stain Positive

	Histopathological Examination		Histopathological Examination		
	+	-	+	-	
Either tissue +	74	2	Both the stain +	65	1
Stain -	2	4	Stain -	9	5

\*Either tissue Stain; Sensitivity=97.3%; Specificity=66.60%; Positive predictive value=97.3%; Negative predictive value=66.6%; Positive likelihood ratio=2.92; Negative likelihood ratio=0.03, \*\*Both the stain; Specificity=83.3%; Positive predictive value=98.4%; Negative predictive value=31.2%; Positive likelihood ratio=5.27; Negative likelihood ratio=0.14

### Clinical examination

Out of 2572 participants, 2397 (93.1%) participants had no precancerous lesions or condition or early signs of cancer. 176 (6.4%) participants had precancerous lesions or condition while 8 (0.3%) were diagnosed as participants with lesions suggestive of cancer (Table 1).

Sensitivity, specificity, positive predictive value, negative predictive value was calculated for self-examination with clinical examination (gold standard) considering only 'Abnormality Suspected' and 'Not Present' cases (Table 1).

For phase II of the study, examiners showing overall agreement were selected (n=164). 82 participants were subjected to screening method using vital staining and histopathological examination using biopsy.

The results of the tissue staining with Lugol's iodine and toluidine blue are shown in Tables 2 and 3.

## Discussion

Many studies on prisoners have been conducted in countries like North America, United Kingdom, Australia and New Zealand. But there is scarcity of data regarding use of tobacco and its ill effect amongst prisoners especially in the Indian scenario (Dahiya et al., 2010). This is an extensive study carried out in the Prison setting

in which various parameters such as Self-examination, Clinical examination, and two Screening methods (1% Toluidine blue and 2% Lugol's iodine) were used.

Self-examination of one's own body is not a novel concept. It has been proved that Breast self-examination has been most effective way to prevent breast cancer in young women (Evans et al., 2005; Hacıhasanoğlu et al., 2008). However studies related to self-examination of oral cavity are very few. One of such study was carried out by Mathews et al. (1995) in Kerala, India (Mathew et al., 1995). In this particular study they gave information brochure and asked people to check their oral cavity. In this study setting, rate of illiteracy was high, hence instead of supplying a brochure we opted for health education through series of photographs.

In the study carried out by Mathew et al. (1995) response rate was 36% (8,028) from which 3.0% (237) identified their oral cavity on having suspicious precancerous lesions. In the present study, 2,572 responded for self-examination from which 7.8% (201) reported themselves with suspicious precancerous lesions. This could be due to high prevalence of tobacco use (96.04%) in the study setting and the methodology used i.e. we asked the responses immediately once photographs were shown.

Out of 201 participants, 154 (76.6%) were diagnosed with precancerous lesions by clinical examination, while 47 (23.3%) participants had normal mucosa or variation. These findings are in accordance with finding of Mathew et al. (1995) where they found 51 (21%) normal variation out of 247 subjects. However, during phase II of the study, 71 (98.6%) participants had dysplastic changes amongst the 72 participants who were abnormally suspected as having cancerous lesion.

Sensitivity, specificity, PPV and NPV for self-examination as compared with clinical examination were 92.2%, 96.6%, 76.6% and 98.5% respectively. The study findings were contrasting with findings of Scott et al (2010) who reported sensitivity, specificity, positive predictive value and negative predictive value to be 33%, 54%, 17% and 73%. Such difference in finding could be attributed to the small sample size in the study conducted by Scott et al. (2010) where 53 participants were included while present study has a sample size of 2,572. Another difference could be the methodology as Scott et al. (2010) provided leaflet with the information which might have been insufficient. This emphasizes the importance of proper channel of education to educate illiterate people in high risk group which is essential. If people in high risk groups are sensitized about ill-effects of tobacco, early detection of oral lesions can be made possible.

This shows self-examination could be beneficial in early detection of precancerous or cancerous lesions. However, self-examination strategies need health education, which is one of the approaches in cancer control that can be carried out using mass media. Oral cancer is predominant in low socioeconomic groups. Hence, considering demographic and literacy rates, the method of implementing health education should be carefully determined.

Clinical examination is an important step in early

detection of oral precancerous lesions or oral cancer. In studies of oral cancer, oral lesions are usually photographed for future reference. Due to strict security in the Jail it was not possible to take photographs, however duplicate examination was carried out with the help of an expert examiner (DH).

In western countries many studies have been conducted on prison population. But most of the studies are related to dental caries using DMFT/DMFS, periodontal status, drug abuse or quality of life (Walsh et al., 2008). However study on oral precancerous lesions or cancers are few. One of such study was carried out by Mathew et al. (2005) in prison population on cancer epidemiology in US (Mathew et al., 2005). In this particular study the prevalence of oral cancer was 6.6%. These findings were similar to the finding of our study where prevalence of oral cancer was observed to be 6.3%. However, prevalence of oral precancerous lesions and oral cancer is much higher than national prevalence which is 0.4%. This could be due to the fact that the study population is a high risk group which has easy access to tobacco products. This finding is also similar to the study carried out by Mathew et al. (2005).

Toluidine blue is an acidophilic metachromatic dye of the thiazine group which selectively stains acidic tissue components (sulfates, carboxylates and phosphate radicals), thus staining DNA and RNA, It's use in vivo is based on the fact that dysplastic and anaplastic cells may contain quantitatively more nucleic acid than normal tissues<sup>10</sup>.

The first use of Lugol's iodine staining to detect mucosal abnormalities was done by Schiller, who used this technique to highlight squamous lesions in the cervix (Epstein et al., 1992; Dawsay et al., 1998). Similar staining was used in the esophagus by many other researchers such as Voegeli, Brodmerke, Northmann et al. and Toriie et al. (Dawsay et al., 1998). The basis of the Lugol's iodine staining technique is that iodine reversibly stains glycogen into brown. In normal mucosa, the superficial epithelium contains abundant glycogen, so the mucosa stains dark brown, but in abnormal mucosa, including areas of atrophy, keratinization, squamous dysplasia, and squamous carcinoma, the superficial epithelium often loses much of its glycogen, and remains partially or totally unstained (Shizaki et al., 1990). However Epstein et al. (1992) first time used Lugol's Iodine as a screening tool for oral cancer.

In this study, the sensitivity of Toluidine blue in detecting premalignant or malignant lesions was 88.1% and our results were in accordance with finding of Warnakulasuriya et al. (1996), Mashberg (1980) and Vahidy et al. (1972), in which sensitivity for toluidine blue was found to be 86%, 90% and 86% respectively. However, Epstein et al. (1997) showed sensitivity to be 100%. This may be due to different study methodology. As Epstein et al. (1997) evaluated the utility of toluidine blue application in aiding the recognition and diagnosis of clinically evident lesions in patients previously treated for oral cancer and undergoing post monitoring. In this study, the specificity of the toluidine blue was found to be 66.6% and our results were in accordance with the finding of Warnakulasuriya et al. (1996), Epstein et al.

(1997), Onofre et al. (1995), Nagaraju et al. (2010) in which specificity was found out to be 62%, 63%, 65% and 60% respectively. However, results of the present study was different from Niebel and Chomet (1964), Myers (1970) and Mashberg (1981) who observed specificity to be much higher i.e. 100%, 100% and 95% respectively. Such difference between specificity could be attributed to the study setting, as all these studies were carried out in specialized institutions by experienced clinicians for underlining tumor border or squamous cell carcinoma. In this study PPV of Toluidine Blue stain was 97.1% which was in accordance to the finding reported by Silverman et al. (1984) who reported PPV to be 90% and somewhat close to Epstein et al. (1992) who reported a PPV to be 84%. However, NPV in this study was 30%, much less than Silverman et al. (1984) who reported NPV to be 92% and Epstein et al. (1992) reported it to be 80%. The predictive values of a test in clinical practice depend critically on the prevalence of the abnormality in the patients being tested; this may well differ from the prevalence in a published study assessing the usefulness of the test (Oliver et al., 2004). In the present study, LR+ and LR- were found to be 2.63 and 0.17 respectively which were in accordance with Epstein et al. (1992), Silverman et al. (1984), Onofre et al. (2001) who found LR+ to be 2.513, 2.08 and 2.33 and LR- to be 0.11, 0.15 and 0.34 respectively.

In this study, the sensitivity of Lugol's Iodine in detecting premalignant or malignant lesions was found to be 94.7%. Result of this study were in accordance with Epstein (1992) and Nagaraju et al. (2010) who showed sensitivity to be 87.5% and 92.7% respectively. Specificity in present study was 83.8%, which was in accordance with Epstein et al. (1992) where specificity was 84.2% while Nagaraju et al. (2010) found specificity to be 60% which was less than findings of present study. This can be attributed to reduction in false positive cases after application of Lugol's Iodine. Positive predictive value i.e. 98.6% of this study was in accordance with study conducted by Nagaraju et al. (2010) and Epstein et al. (1992) who found it to be 96% and 92.1% respectively. However, Negative predictive value was 55.5% which was somewhat close to Nagaraju et al. (2010) i.e. 43% and lesser than Epstein et al. (1992) who found it to be 76.1%. This difference can be contributed to difference in biopsy report as both the authors had biopsy report for benign and malignant lesions while in present study biopsy report accounted for dysplasia present or absent. Positive likelihood ratio and negative likelihood ratio were 5.67 and 0.063 respectively which were in accordance with Epstein et al. (1992) in which it was found out to be 5.53 and 0.14 respectively.

In the present study when either of the stains were retained the sensitivity was 97.3% while the specificity was 66.6%. The PPV, NPV, LR+ and LR- were 97.3%, 66.6%, 2.921, and 0.039 respectively. The results of this study regarding the sensitivity, specificity, PPV, LR+ and LR- were in accordance with the findings of Epstein JB et al (1992) while results regarding the NPV of our study (66.6%) and their study (84.6%) was not in accordance to each other. This can be contributed to the difference in prevalence of precancerous lesions in study sample.

In the present study, when both the stains were retained in premalignant and malignant lesions the sensitivity was 87.8% while the specificity was 83.3%. The PPV, NPV, LR+ and LR- were 98.4%, 31.2%, 5.27 and 0.146% respectively. The results of the study regarding the sensitivity, specificity, PPV, LR+ and LR- were in accordance with the findings of Epstein JB et al (1992) while results regarding the NPV of this study (31.4%) and their study (73.9%) was not in accordance to each other and the variations in the NPV can be attributed to the selection criteria of the cases. True negatives (5 out of 16) in this study were less as compared to Epstein et al. (1992) (17 out of 23).

In the present study we combined the use of toluidine blue and Lugol's iodine to assess the value of using both stains in assessment of oral lesions. Results showed that when either of the stains were positive sensitivity was greatest (97.3%), but specificity was reduced (66.6%), The positive predictive value remained high (97.3%), as did the negative predictive value (66.6%), When the results were reviewed with the requirement that both stains be positive, the sensitivity was reduced (87.8%), but the specificity was increased (88.3%), The positive predictive value was also enhanced (98.4%), and the negative predictive value was reduced to (31.2%). Hence, it can be concluded that combined use (either tissue stain and both the stains) of Toluidine blue and Lugol's Iodine can have better results. The result of the study is in accordance with the study conducted by Epstein et al. (1992). However, in this study Lugol's Iodine has shown high sensitivity and specificity. Hence instead of using both the stains only use of Lugol's iodine is sufficient to screen lesions at risk of malignancy and malignant lesions especially in high risk group.

However, the study has some inherent limitations. During clinical examination, only lesions which were suggestive of precancerous lesions or of oral cancer were selected, while all borderline cases were not considered. Hence prevalence of oral premalignant lesions could be underestimated. After duplicate examination, there was disagreement in 9 cases, which were excluded from the phase II of the study. However, participants with questionable lesions were warned about their oral health status and referred. Taking biopsy for all suspicious lesions was not feasible hence, sample was selected which could have introduced sampling error.

Generalizability of this study has to be made with caution, as this study has been carried out in a high risk group, in which exposure to tobacco product was high. However, finding of this study can be useful in high risk group where exposure to tobacco and prevalence of oral precancer and cancer is high.

In conclusion, the opportunities for oral cancer control, in view of the known risk factors, long natural history, identifying by oral examination and acceptable as well as effective therapy for early lesions, are considerable. Oral self-examination may be used as an effective tool to improve awareness of oral cancer and for early detection of lesions in a high-risk population. However, clinical examination of oral cavity for precancer and cancer is an inevitable part in early detection. In the present study prevalence of lesions at risk of malignancy and oral

malignant disease by self-examination was 7.8% and by clinical examination was 6.3%. Hence in high risk group self-examination does play an important role in early detection of oral precancerous lesion and oral cancer.

Use of vital staining such as toluidine blue and Lugol's iodine can definitely aid in detecting positive cases. In the present study, sensitivity (94.7%) and specificity (83.8%) of Lugol's iodine has been found to be superior to sensitivity (88.1%) and specificity (66.6%) of Toluidine blue. However, toluidine blue has been extensively researched as a screening tool, and Lugol's Iodine has promising results. It appears that the use of Lugol's iodine in diagnosis of precancerous lesions and oral cancer could be considered.

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