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Original Article

Utility of Tolonium Chloride Rinse in the Diagnosis of Oral Cancer

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Abstract

Background: *Oral cancer burden in developing countries is huge. In Bangladesh and India incidence were 10.6% and 10.4% respectively on 2018. At the same time worldwide incident was only 2%. Majority patients present at advanced stage and their survival rate is poor. Diagnostic delay from patient side and health delivery system side is significantly longer and it is more in developing countries. Like other malignancy early diagnosis can save a lot of these patient.*

Methods: *This prospective study was carried out in Khulna Medical College Hospital from July 2017 to July 2019. Patients who will meet the criteria for this prospective study and capable and willing to give informed consent were enrolled. 20 second rinse/gargle with 10ml of 1% Tolonium chloride solution was done. Biopsy were taken from stained lesion, biopsy report were compared with TC stained lesions.*

Results: *Out of 1650 patients in our outdoor department for various problem and only 30 cases were selected for this study according to selection criteria. Of 30 patients, 18 (60%) were male and 12 (40%) were female. The male-to-female ratio was 3:2. It was found that the sensitivity of 1% Tolonium Chloride rinse for oral precancer and cancer detection was 83.33%, whereas the specificity was 84.21%.*

Conclusion: *Tolonium chloride rinse is a good screening test for oral cancer diagnosis with sensitivity 83.33% and specificity 84.21%.*

Keywords: *Oral cancer, Tolonium Chloride, Oral cancer screening;*

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Introduction:

There were 18078957 new cases of cancer and 9555027 deaths worldwide in 2018, of which male and female are 9456418 and 8622539 respectively.[1] In 2018 total population of Bangladesh was 166 million, number of new cancer case were 150781 and death due to cancer were 108137. Among this oral and lip cancer were 8895(10.6%) and it is almost similar to India (10.4%). But worldwide incidence rate is only 2%, that indicates oral cancer burden is higher in developing countries. The American Cancer Society estimated that 34,360 men and women (24,180 men and 10,180 women) would be diagnosed with and

7,550 men and women would die of cancer in oral cavity and pharynx in 2007.[2] Although definite figure about oral cancer is not available in Bangladesh, it has become a serious issue for Bangladesh. Source based on 21,238 cancer (Male 14,222 and female 7,076) treated at the Radiotherapy Department of Dhaka Medical College Hospital during the period of 1985-92, relative percentage of oral cancer was 12% and 13% among male and female respectively.[3] In India and Pakistan when compared with overall incidence of all body cancers Oral cancer stands second to bronchogenic carcinoma in males and breast carcinoma in female.[4]Oral cancer is a significant health threat with one of the lowest survival rates(fewer than 50% patients surviving more than 5 years).[5] Early diagnosis of oral cancer is of critical importance because of the impact of stage of cancer in prognosis for cure. The 5 year prognosis for oral cancer is reduced by approximately half if tumor has spread regionally to lymph nodes or bone and morbidity and cost of treatment increase with stage of the disease.[6] The stage distribution based on historic stage shows that 33% of oral cavity and pharynx cancer cases are diagnosed while the cancer is still confined to the primary site (localized stage); 52% are diagnosed after the cancer has spread to regional lymphnodes or directly beyond the primary site; 10% are diagnosed after the cancer has already metastasized (distant stage) and for the remaining 5% the staging information was unknown. The corresponding 5-year relative survival rates were 81.8% for localized; 52.1% for regional; 26.5% for distant; and 46.2% for unstaged.[2] The median total delay of diagnosis is 4 months of which two-thirds is patient delay.[7] Both patients and professionals were responsible for the delay in diagnosis. This delay was longer for tumors in early stages. Multiple logistic regression analysis indicated that the professional delay was the most associated variable to the stage of tumor ($P = 0.03$).[8] Despite this late presentation, oral cancer occurs in known risk groups and might be seen with detectable mucosal changes, including leukoplakia, erythroplakia, or mixed red and white lesion earlier in the course of the disease.[6] Screening for oral cancer might be useful, because of the easily detectable precancerous lesions, early invasive cancers. [9] Visual inspection of oral cavity with Tolonium Chloride (TC) rinse is simple procedure of oral cancer screening. TC used in this study is a component of toluidine blue, a metachromatic, basic aniline dye that was first used approximately 50 years ago as an anti-heparin agent in certain types of bleeding disorders.[20] The safety of the topical application of TC in the oral cavity has been demonstrated in a number of studies. In the early 1960s, initial reports were published suggesting the clinical utility of topically applied TC as an aid in the diagnosis of carcinoma of the cervix and oral mucosa and delimitation of the area to be treated. Subsequent studies suggested that TC staining might be useful in identifying the presence and dimensions of bronchogenic, esophageal, and gastric carcinomas during endoscopic examination. Since the early 1970s, numerous clinical studies have been conducted in which the sensitivity and specificity of TC in the diagnosis of oral carcinoma were evaluated. Prior studies support the use of TC as an adjunct in the detection and clinical evaluation of oral lesions suspicious for cancer.[10-15] A study conducted in Sri Lanka assessed 102 patients in whom 18 oral/oropharyngeal Ca were found and of these 7 were detected only on TC assisted examination.

It is strange to think that, at present, pelvic examination and pap smears appear more

acceptable than looking in the mouth. We have to remember that screening for oral cancer is simple non-invasive procedure, which need lighting, gauze, and gloves, whereas the detection of most solid malignancies in their early asymptomatic stages almost always require special , costly, and often invasive techniques.

Methods:

This prospective study was carried out in Khulna Medical College Hospital from July 2017 to July 2019. Patients who will meet the criteria for this prospective study and capable and willing to give informed consent were enrolled. Patient of age 18 years or older and having clinically suspicious or pre-malignant lesion in oral cavity was included in this study.

Patients treated for oral cancer in the 3 months before entry, Pregnant and breast feeding women, Known medical condition with oral manifestations and Medical condition that would prevent study participation were excluded from this study.

During first visit after informed consent demographic and medical data will be recorded, oral cavity was examined. Clinically suspicious or premalignant lesions in the oral cavity was rinsed with Tolonium Chloride. Following procedure will be adopted for staining:

a. Patient's oral cavity rinse with 30 ml 1% acetic acid solution for 20 seconds. This solution remove excess saliva and provides a consistent oral environment.
b. Patient rinse with water for 20 seconds and expectorate.
c. 20 second rinse/gargle with 10ml of 1% Tolonium chloride solution.
d. Patient then rinse with 30ml 1% acetic acid solution for 20 seconds and this step is repeated.
e. Final water rinse and it is repeated.

After TC staining, any lesions believed to require biopsy or that stained with TC were biopsied. Biopsy specimens was placed in 10% formalin and sent to the local pathology laboratory. Lesions were categorized as benign (keratosis, hyperkeratosis, hyperplasia), inflammatory (mild, moderate, severe), dysplasia (mild, moderate, severe), CIS (carcinoma in situ), Ca, or other. Dysplasia was characterized as cellular abnormalities, which might include variation in cell size, morphology, or orientation. When dysplasia is seen in the full thickness of the epithelium in any one microscopic field, the diagnosis is CIS, whereas squamous cell carcinoma (SCC) involve disruption of the basement membrane in the presence of dysplastic cells.

Statistical analysis:

Sensitivity was calculated using the following lesion definitions. Lesions were considered clinically suspicious (CS+) and TC positive (TC+) if they stained. Exceptions to this rule include biopsied lesions that were (1) CS-/TC+, (2) CS-/TC- (3) CS+/TC- and 4) CS+/TC+. Continuous and categorical variables were characterized by means/standard deviations and proportions, respectively. In general, groups were compared using Student's *t* test for continuous variables and Fisher's exact test when the variables were categorical.

Result:

In this study we examined 1650 patients in our outdoor department for various problem and only 30 cases were selected for this study according to selection criteria. Data was collected by interview of the patients, visual screening and investigation. Of 30 patients, 18 (60%) were male and 12 (40%) were female. The male-to-female ratio was 3:2.

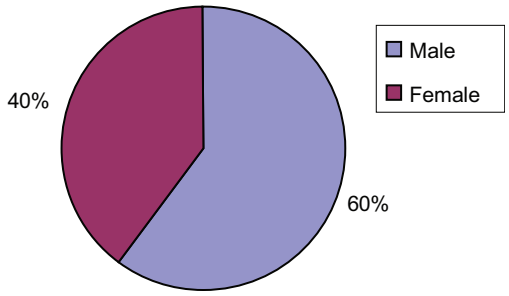

Fig. 1: *Showing the sex distribution of the participants*

The age range was 39 to 77 with an average age of 60.50±10.88 years. Duration of the lesions ranged from 1 to 24 months with the average duration of 6.13±6.80months. Locations of lesions were as follows: floor of the mouth, 8 (24.2%); lateral tongue, 8 (24.2%); buccal mucosa, 7 (21.2%); lower lip, 4 (12.1%); hard palate, 2 (6.1%); soft palate, 2 (6.1%); and alveolar ridge, 2 (6.1%).

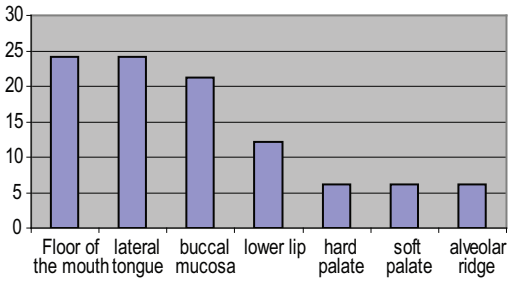

Fig. 2: *Distribution of oral lesion according to site*

Seventy-seven percent of our patients smoked, 25% consumed alcohol, and 67% chewed betel nut. When the patients were separated into 2 groups by sex, it was found that 100% and 25% of male patients smoked and consumed alcohol. On the other hand, only 16.7% of female patients smoked and 0% consumed alcohol, while 96.7% chewed betel nut.

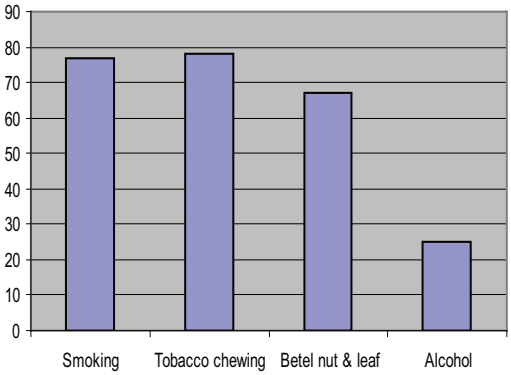

Fig. 3: *Showing the percentage of Tobacco and Betel leaf & nut user*

The results from 1% Tolonium Chloride rinse and histopathological diagnosis are shown in Table I.

Table I :

The results from Tolonium chloride application and the histopathological diagnosis

	Histopathological diagnosis		
Tolonium chloride rinse	Disease +[1]	Disease -[2]	Total
+ Result (%)	30(90.9)[3]	3(9.1)	33
- Result (%)	6(27.3)	16(72.7) [4]	22
Total	36	19	55

1- Disease- are dysplasia, carcinoma in situ and squamous cell carcinoma.
2- Disease- are hyperplasia, inflammation and normal mucosa
3- Sensitivity- 30/36 (83.33%)
4- Specificity- 16/19 (84.21%)

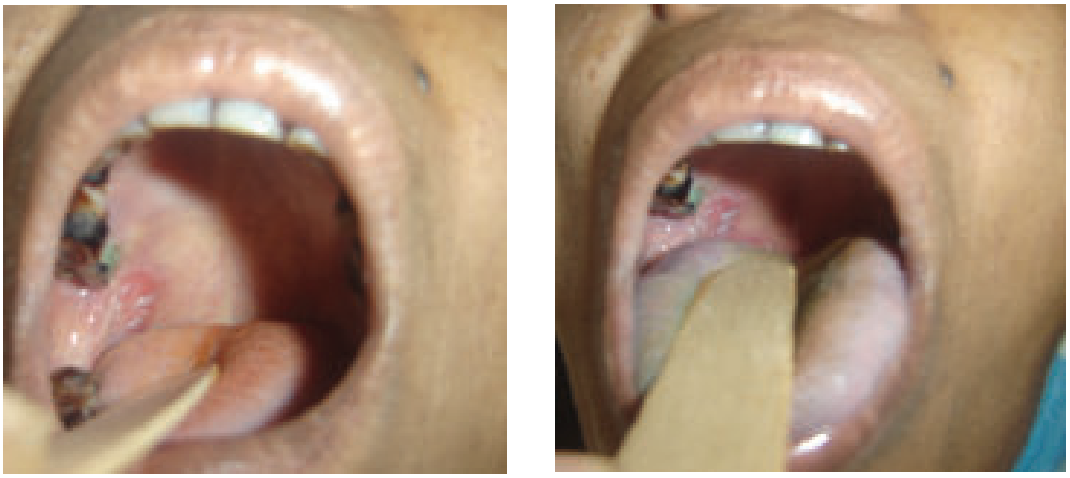

Fig. 4: *Showing Blue coloration of mucosal lesion in oral cavity.*

It was found that the sensitivity of 1% Tolonium Chloride rinse for oral precancer and cancer detection was 83.33%, whereas the specificity was 84.21%. In addition, the positive predictive value of the use of 1% Tolonium Chloride rinse for oral cancer examination was 90.91% and the negative predictive value was 72.73%. The relationship between the results of oral cancer examination using 1% Tolonium Chloride rinse and the results of histopathological diagnoses revealed significant association (P =.001).

Discussion:

The average age of our patients was 60.50± 10.88 with a male-to-female ratio of 3:2. These findings are consistent with age and gender of oral cancer patients reported by other studies.[18,19] However, the proportion of female patients in our study is slightly higher than that of other studies. This is probably because betel nut is still used by our elder female population. The distribution of lesions, from 24.2% at the lateral tongue and floor of mouth, to 6.1% at the hard palate, soft palate, and alveolar ridge is also comparable to the distribution of oral cancer lesions obtained from other studies.[1,19,20]

We, however, observed a higher occurrence of oral cancer on the buccal mucosa (21.2%) than that reported by others. This could stem from the use of betel nut as well.

It is widely accepted that smoking, alcohol consumption, and betel nut chewing are leading risk factors for the development of oral cancer. We found that 66.7% of our patients smoked, 25% drank alcohol, and 67% chewed betel nut. This confirms the danger of those risk factors. The association between sex and the risk factors indicates that sociological habits associated with gender determine the risk factors for oral cancer development.

This study aimed to examine the sensitivity, specificity, and accuracy of 1% Tolonium Chloride rinse in the detection of early oral cancer, an area in which no researcher has explored the possibilities. When we compared the sensitivity and specificity of 1% Tolonium Chloride rinse (83.33% and 84.21%, respectively) from this study to the reported sensitivity (77% to 100%) and specificity (44% to 93%) of toluidine blue from 9 studies.[20-26]

The significant relationship found between clinical examination using 1% Tolonium Chloride rinse and histopathological diagnoses confirmed that 1% Tolonium Chloride rinse reacted better with tissues that had turned dysplastic or malignant than with normal tissues and warranted the use of Tolonium Chloride rinse in oral cancer examination.

We stated earlier that clinical changes due to 1% Tolonium Chloride rinse in oral cancer examination and the results of histopathological diagnoses are significantly correlated. Regarding patients' tolerance after the application of 1% Tolonium Chloride rinse, we found that most patients had no complaint over the use of 1% Tolonium Chloride rinse. Four patients had records of bleeding and 3 patients had burning sensation. These were patients with ulcerations of at least 1 cm in diameter.

This is a preliminary study investigating the use of 1% Tolonium Chloride rinse in oral cancer detection. Thus, any patients with lesions suspected of having oral squamous cell carcinoma were included in the study. The added values of 1% Tolonium Chloride rinse can be summarized as follows:

(1) In 14 patients we also biopsied the sites that did not change to blue but were close to the lesions. We found that all those specimens were normal mucosa, chronic inflammation or epithelial hyperplasia. Thus, 1% Tolonium Chloride rinse helps demarcate dysplastic areas from nonmalignant tissue. Interestingly, specimens from a lesion that we biopsied in 3 areas were histopathologically identified as squamous cell carcinoma, lichen planus, and normal mucosa, respectively.

(2) There were 3 patients in whom we also biopsied normal areas that had turned blue but were not part of the lesions of primary interest. We found that those areas were dysplastic.

This study, however, has few limitations. First, only a single investigator assessed patients with clinically known cancer or dysplastic lesions. Thus, the results of the 1% Tolonium Chloride rinse examination were influenced by the clinical examination. Second, only patients with lesions were included in this study; therefore, we recommend further study and evaluation of 1% Tolonium Chloride rinse used for oral cancer screening in rural communities because of its acceptable sensitivity, specificity, and accuracy.

Conclusion:

Tolonium Chloride rinse showed promising sensitivity, specificity, and accuracy for oral cancer examination. The results of clinical examination using 1% Tolonium Chloride rinse also correlate with the histopathology. 1% Tolonium Chloride rinse is suitable to be used for oral cancer examination, especially in developing countries.

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