International Journal of Applied Research 2015; 1(11): 1115-1118

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2015; 1(11): 1115-1118
www.allresearchjournal.com
Received: 06-08-2015
Accepted: 07-09-2015

Dr. AM Shah
Dept. of Surgery KIMS, Karad, Maharashtra, India

Dr. MB Bagawan
Dept. of Surgery KIMS, Karad, Maharashtra, India

Correspondence
Dr. AM Shah
Dept. of Surgery KIMS, Karad, Maharashtra, India

Toluidine blue staining for detection of malignancy of oral cavity leukoplakia

Dr. AM Shah and Dr. MB Bagawan

Abstract
Aim: Evaluating toluidine blue staining for detection of malignancy of oral cavity leukoplakia.
Material and method: After recording the clinical features and photograph of clinically suspicious lesions, the lesion areas were applied prior with 1% acetic acid with cotton bud for 20 seconds and further rinsed with water. Toludine blue was applied with cotton bud for 10 to 20 seconds Then decolorized with 2% acetic acid using cotton bud for 20-30 seconds and photograph was taken Dark blue was considered as positive for lesions suspicious of malignancy, light blue retention was considered as positive for premalignant lesions unless proved otherwise by biopsy, and lesion without retension consider negative.
Result: In 100 patients of leukoplakia 79(79%) were male patient and 21(21%) were female patient. Out of 100 patients no one was below 20 year age. 6% patient from 21to 30 year, 21% were from 31to 40 years of age, 20% patients from 41 to 50 years of age, 18% patients from 51 to 60 years of age, 25% of patients from 61 to70 years of age, 10% of patients were more than 70 years of age.
Conclusion: As duration of leukoplakia increases its degree of dysplasia or malignant potential increases, so that early detection and diagnosis of leukoplakia is important. Staining should be routinely used as a method to assist in the choice of biopsy site and in the follow up of premalignant lesions.

Keywords: Malignancy of oral cavity, toluidine blue

Introduction
Cancer is among most deadful disease human being suffering. The infection and contagious diseases are sufficiently controlled but cancer is still a major challenge to modern medicine and hence is a major health problem [1, 2].
Cancers involving the different organs and structure of human body have significant marks in morbidity and mortality rates and oral cancers are no exception to it. [3, 4] Although the oral cancer accounts for 3.6% of all malignancy there are approximately 27,000 new cases and 9,000 death each year which would lead to more than 1,00,000 individuals suffering from disease in population in any given year[1]. Less than 40% of oral cancers are diagnosed at early stage as it is difficult to detect either to patients or inexperience [5].
It has been shown that incidence of ultimate malignant changes in oral leukoplakia increases with age approximate 2.4% malignant transformation rate at 10 years which increased to 4% at 20 years. It also showed that, as the age of patient increased, so did the risk of malignant transformation for patient younger than 50 year it was 1%, whereas for those between 70 to 89 years it was 7.5. Kromer *et al* has shown that in Southern England leakoplakia of the floor of mouth and ventral surface of tongue has high incidence of malignant changes, due to pooling of soluble carcinogens in the 'Sump' of the floor mouth [6].
So it is very important for early detection of leukoplakia to prevent malignant changes.

Material and Methods
The present study is done in surgery department, Krishna Institute of Medical Sciences University, Karad during the period of May 2009 to May 2011. All patients of leukoplakia coming in OPD, admitted in words and camp cases are included in study. Study included 100 patients. There are no specific criteria for age, sex etc.
The subjects comfortably seated in the dental chair were examined under artificial illumination. Examination was carried out following the methods described by Kerr DA, Ash

MM, Millard and the relevant data were entered into proforma. Informed consent was obtained from every subjects for carrying out the diagnostic procedure after recording the clinical features and photograph of clinically suspicious lesions, the lesion areas were applied prior with 1% acetic acid with cotton bud for 20 seconds and further rinsed with water. Toludine blue was applied with cotton bud for 10 to 20 seconds Then decolorized with 2% acetic acid using cotton bud for 20-30 seconds and photograph was taken Dark blue was considered as positive for lesions suspicious of malignancy, light blue retention was considered as positive for premalignant lesions unless proved otherwise by biopsy, and lesion without retension consider negative.

Incisional biopsy obtained from the lesion following the methodology of Reichtar PA for the all study subjects and preserved in 10% formalin for histopathological diagnosis. Histopthological grading for Leukoplakia, Speckled Leukoplakia and Erosive as done as per the pathologic features suggested by Axell T, Pindborg, van der Waal I (1996)[31] which were grouped on the basis degree dysplasia into those with no dysplasia, mild dysplasia, moderate dysplasia and severe dysplasia Data are presented by number and percentage, Chi-square test was used to compare the groups and assess the association. Diagnostic validity tests listed below were performed to determine the utility of test results for predicting the various condition of the disease.

Results

Table 1: Male and Female distribution

Sex	No. of Cases	Percentage
Male	79	79%
Female	21	21%

Table 2: Association of duration of symptoms and HPR report

Duration	Benign (%)	Mild (%)	Moderate (%)	Severe (%)	Positive for HPR (%)	Total (%)	χ^2 (p value)
Less than 6 months	12(27.3)	0(0)	1(5.6)	0(0)	0(0)	13	76.054 (0.000)
6months-1 year	15(34.1)	3(13.6)	1(5.6)	0(0)	0(0)	19	
1year-2years	8(18.2)	6(27.3)	1(5.6)	1(11.1)	0(0)	16	
2years-5years	5(11.4)	7(31.8)	3(16.7)	1(11.1)		16	
5years-10year	3(6.8)	3(13.6)	9(50)	1(11.1)	2(28.6)	18	
More than 10 years	1(2.3)	3(13.6)	3(16.7)	6(66.7)	5(71.4)	18	
Total	44(100)	22(100)	18(100)	9(100)	7(100)	100	

The present study comprised of 100 subjects having leukoplakia. The data obtained from the study were entered into master chart. The data from master chart further statically analyzed.

1) Age and sex wise distribution of leukoplakia [table 1&2]

In 100 patients of leukoplakia 79(79%) were male patient and 21(21%) were female patient. Out of 100 patients no one was below 20 year age. 6% patient from 21to 30 year, 21% were from 31to 40 years of age, 20% patients from 41 to 50 years of age, 18% patients from 51 to 60 years of age, 25% of patients from 61 to70 years of age, 10% of patients were more than 70 years of age.

2) Site wise distribution of leukkoplakia

Out of 100 patients most common site was buccal mucosa (58%), 17% patient having lesion on tongue, 10% patient having lesion on gums, 6% patient having lesion on floor of mouth, 9% patient having lesion on lip.

3) Distribution according to duration of symptoms

Out of 100 patients studied 13% patients having symptoms less than 6 month, 19% patient having symptoms between 6 month to 1 year, 16% patient having symptoms between 1 to 2 year,16% patient having between 2 to 5 year, 18% patient having between 5 to 10 year and 18% patient having more than 10 year symptoms

4) Distribution according to type of addiction

All the 100 patient have one or more addiction. Tobacco and its products are major addiction. Out of 100 patients 30% patient have addiction of tobacco of lime, 15% have alcohol as addiction, 7% have pan as addiction, 2% have smoking as addiction and 11% have gutkha as addiction. 3% have both tobacco lime and alcohol as addiction, 13% have tobacco lime and pan as addiction, 3% tobacco lime and smoking as addiction, 5% have tobacco lime and gutkha as addiction, 5% have alcohol and pan as addiction, 6% have smoking and gutkha as addiction.

5) Diagnostic validity of toludine blue

Of 100 patient 36% patient failed to retain the stain, 64% patients retained the stain, staining pattern observed was of uniform light blue with diffuse boundaries.Out of 64 dye positive patient, 56 were dysplastic including malignant and 8 were benign. 36 patient failed to retain the stain, they all were benign. Sensitivity of toluidine blue in determining the dysplastic changes was 100% and specificity was 81.81%. The positive predictive value was 87.5% and negative predictive value was 100%.Diagnostic accuracy of toluidine blue staining in distinguishing early leukoplakia was 92%

6) Degree of dysplasia in leukoplakia

Out of 100 cases of leukoplakia HPR report 44% benign lesion, 22% have mild dysplasia, 18% have moderate dysplasia, 9% have severe dysplasia and 7% have positive for malignancy.

7) Association of symptoms duration and HPR report

Out of 100 cases of leukoplakia more than 50% of benign hpr report has symptom duration upto 1 year. More than 50% of mild dysplasia patient has symptom duration 1 to 5 year. In case of moderate dysplasia 50% of patient has symptom duration 5 to 10 year. In case of severe dysplasia 66.7% has symptom duration more than 10 year. In case of positive for malignancy patient, 28.6% patient has 5 to 10 year symptom duration and 71.4% patient has symptom duration more year. And P value is significant (0.00).Association of symptoms

duration and HPR report s/o as duration of symptoms increases degree of HPR reports also increases.

Discussion

The concept of two-step process of cancer development in the oral mucosa, i.e., the initial presence of a precursor (premalignant or pre-cancerous) lesion subsequently developing into cancer is well established[59] and the early detection of oral mucosal epithelial dysplasia could potentially halt the progression of these lesions into malignant transformation [7-9]. Thus, establishment of useful and objective techniques adjunctive to clinical judgments and microscopic diagnosis have contributed to the control of oral cancers [9].

In vivo staining reveals cytological details that might otherwise not be apparent, however staining can also reveal where certain chemicals or specific chemical reactions are taking place within cells or tissues[24] and thus aid in accelerating biopsies, diagnosis and treatment.

Toluidine blue, an acidophilic metachromatic dye of thiazine group selectively stains acidic tissue components (sulfates, carboxylates and phosphate radicals) thus staining DNA and RNA. It is used as an in vivo stain based on the fact that dysplastic and anaplastic cells may contain quantitatively more nucleic acids than normal tissues. Also malignant epithelium may contain intracellular canals that are wider than normal epithelium, which may facilitate penetration of the dye [10]. The present study of TB staining in premalignant lesions was seen to be highly efficient in the detection of dysplasias, with a sensitivity of 100%. Though the staining techniques used by Mashberg A (1980) was similar to our study the difference in the values could be attributed to interindividual differences in considering the staining pattern, as light blue staining was considered negative by the author. The difference in sensitivity between our study and as reported by Warnakulasuriya KAAS, Johnson NW (1996) can be attributed to the difference in the methodology of the staining techniques as the authors study was carried on with Toluidine blue mouth rinse while our study was carried out with application of Stain to suspicious areas. Inability of the rinse to disclose lesions in the posterior areas might have minimized the sensitivity of TB rinse as compared to our study [11].

In the present study the specificity of Toluidine blue in LEUKOPLAKIA lesions was 81.81%. Our result was differed to the findings of Mashberg A (1980) Warnakulasuriya KAAS who reported the specificity value of 92% in premalignant lesion. Our results of specificity also differed from the findings of Onofre MA, SPOSTO MR, and NOVARRO CM Scully C (1995) Johnson NW (1996) who reported values of 44% and 62% respectively [13].

The difference in the specificity between our study and as reported by Warnakulasuriya KAAS, JOHNSON NW (1996)[50] can be attributed to difference in methodology of staining technique [12]. The authors study was carried on with Toluidine blue mouth rinse while our study comprised of application of the stain to the clinically suspicious areas.In our present study ppv, npv and diagnostic accuracy of toludine blue stain was 87.5%, 100% and 92% respectively which were in accordance to the findings reported by Silverman Jr S, Migliorati C and Barbosa J (1984)[41] who reported values of 90% of PPV, 92% of NPV and 90% of Diagnostic Accuracy. Another study conducted by Epstein JB, Scully C, Spinelli JJ (1992) reported a PPV and DA of 84% and 83% respectively which were in correlation to our study while the NPV showed a significant difference of about 20%. This can be attributed to the false negative results of the stain i.e. failure of dye to retain in dysplastic/ malignant lesions and these results have significantly reduced the NPV in the author's study.

IN our study also male are more affected than females. male are (79%) and female are (21%). In our study also leukoplakia more common in age more than 40 year, approximate 73% of patients are more than 40 year.IN our study most common site of leukoplakia is buccal mucosa (58%) followed by tongue (18%). IN another study by Jerry e. bouquet and Robert j gorlin in American white shows that leukoplakia was the most common of all lesions diagnosed and Americans over 35 years of age and was twise as high for males as for females. Age specific leukoplakia prevalence rates demonstrated a tenfold increase for male from 3rd to 8th decade of life and two fold increase for females from 4th to 7th decade. SITE OF LEUKOPLAKIA in decreasing order of frequency were lip vermilion, buccal mucosa, tongue then floor of mouth.

Conclusion

The diagnostic efficiency of toluidine Blue in detecting the premalignant lesion leukoplakia was assessed. It was also found that Toluidine Blue staining is highly reliable source for the detection of dysplasia and carcinoma. Staining with these stains is an adjunct to clinical judgment and not a substitute. Incidence of leukoplakia increases with age. IT is commonly seen in 40 to 70 years of age. It is four times common in male. As duration of leukoplakia increases its degree of dysplasia or malignant potential increases, so that early detection and diagnosis of leukoplakia is important. Staining should be routinely used as a method to assist in the choice of biopsy site and in the follow up of premalignant lesions.

References

1. Rosenberg D, Cretin S. Use of meta-analysis to evaluate tolonium chloride in oral cancer screening. Oral Surg Oral Med Oral Pathol. 1989; 67:621-627
2. Martin IC, Kerawala CJ, Reed M. The application of toluidine blue as a diagnostic adjunct in the detection of epithelial dysplasia. Oral Surg Oral Med Oral Pathol. 1998; 85:444-446
3. K. Rajgopal Shenoy, Manipal manual of surgery, 2nd EDITION, CBS PUBLICATION: 2006; 206-208.
4. BAILY & LOVE, short practice of surgery edition, Hodder Arnold: 2004, 702-710.
5. Koss GL, Melamed MR. Diagnostic cytology and its histopathologic bases. 5th ed. Philadelphia, Williams and Wilkins, Wolters Kluwer Company; 2006.
6. The world Book encyclopedia. Vol-13. World book, Inc London, 443.
7. Garrison FH. An Introduction to the History of Medicine 4th ed. Philadelphia, W. B.Saunders Company 1967.
8. Major RH. A history of medicine Vol-1. USA Charles c Thomas publishers, 1954.
9. Pispati HP. Concise textbook of physiology for dental students. Oxford press, New Delhi 2001.
10. Chatterjee CC. Human Physiology, 11th ed. Calcutta, Chatterjee AK- Ashutosh Lithographic Co; 1992.
11. Arey LB. Human histology. 4th ed. Khosla Publishing House, Delhi; 1984.

12. Warwick R & Williams PL. Gray's Anatomy 35th ed. Churchill Livingstone, Longman, 1973.
13. Boyd W. Textbook of pathology. 8th ed. Philadelphia, Kempton H Lea & Febiger 1970,