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COMPARISON OF EFFICACIES OF TOLUDINE BLUE STAIN & 5%ACETIC ACID IN DETECTION OF OSCC & THEIR CORRELATION WITH TUMOUR MARKER p53.

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Abstract

Introduction: *Oral squamous cell carcinoma (OSCC) is the most common cancer in the oral cavity. It accounts for more than 90% of all oral cancer. Tobacco use is a major risk factor for many chronic diseases including cancers, Cardiovascular diseses, lung diseases and stroke.* ***AIM:*** *This study was conducted to compare the efficacy of Toluidine blue and Acetic acid in detection of Oral Squamous Cell Carcinoma and to evaluate the association of these two tests with expression of tumor marker p53.* ***Materials & Methods:*** *A total of 50 patients were included in study 25 patients underwent acetic acid test while other 25 underwent toluidine blue test. All the 50 patients underwent histopathological and immunohistochemical investigation of lesion for p53.*

Results: *In Toluidine Blue group 20 (80%) patients were male while 5 (20%) patients were female. Similarly in Acetic Acid group there were 20 (80%) male and 5 (20%) were females. Sensitivity of Toluidine Blue (100%) was found to be higher than that of Acetic Acid (83.3%) in detection of OSCC A significant association was observed between Toluidine blue test and p53 immunohistochemistry. ($p < 0.05$ i.e. 0.010) (χ^2=6.618).*

Conclusion: *There is a significant association of between Toluidine Blue test and immunohistochemistry for tumor marker p53.*

Key words: Toluidine blue, Acetic acid, P53, Oral cancer, OSCC.

Introduction

Cancer is one among the most dreadful disease human race is suffering since ages. Cancer of different organs and structures of human body has become a matter of prime concern in the field of medicine due to high rate of morbidity and mortality.[1] **Oral squamous cell carcinoma** (OSCC) is the most common cancer in the oral cavity. It accounts for more than 90% of all oral cancer. Tobacco use is a major risk factor for many chronic diseases including cancers, Cardiovascular diseses, Lung diseases and stroke. It is a major cause of death and disease in India and accounts for nearly 1.35 million deaths every year.[2]

Nearly 267 million adults (15 years and above) in India (29% of all adults) are users of tobacco according to the Global adult Tobacco Survey (GATS) India 2016-17. The most prevalent form of tobacco use in India is smokeless tobacco and commonly used products are khaini, gutkha, betel quid with tobacco and zarda. Smoking forms of tobacco used are bidi, cigerette and hookah.[2]

Oral cancer is usually first diagnosed when it becomes symptomatic. By this stage approximately two thirds of patients will have already developed advanced disease with regional metastasis and have a consequently diminished prognosis. Subsequent treatment frequently requires surgery and adjuvant radiotherapy with its attendant high rate of morbidity and mortality. If patients could receive treatment for disease that was less advanced or (ideally) for premalignant lesions, then both survival rates and quality of life should be improved. Early diagnosis is therefore of paramount importance. A number of techniques have been developed to supplement clinical examination and thus improve the diagnosis of early oral malignancy.[3]

Many in-vivo staining methods like **Toluidine Blue, Lugol's iodine, Acetic acid** etc have been used since decades in chairside detection of malignant changes in oral lesions. Toluidine blue is a basic metachromatic stain has affinity for the perinuclear cisternae of DNA and RNA. By virtue of the fact that cancer cells contain quantitatively more DNA and RNA than normal epithelial cells, toluidine blue delineates areas of malignancy. There is another staining method which has been used in gynecology for detection of malignant change of the cervix i.e. **Acetic acid (3-5%) staining**. Acetic acid dissolves mucous and accentuates atypical areas by causing cellular dehydration and coagulation of cellular protein[4]. As dysplastic cells contain more protein they are stained more whitish then the normal surrounding area. Visual inspection with acetic acid (VIA), also known as direct visual inspection, involves naked eye inspection of the cervix after the application of a 3% to 5% solution of acetic acid[5]. Recently a study was done to assess the use of 5% acetic acid in the detection of oral squamous cell carcinoma. Results of the same study had shown high sensitivity and specificity[1].

Multiple tumor markers also have role to play in pathogenesis of oral cancer. One of them is **p53,** a transcription factor which regulates cell proliferation and apoptosis to prevent division of potentially malignant cells. Using immunohistochemistry, p53 protein has been reported in 34-100% of head and neck and oral cancers, with mutation detected in 40-50% of these tumours, suggesting that loss of function of p53 is important in the pathogenesis of these cancers[6].

This study was conducted to compare the efficacy of Toluidine blue and Acetic acid in detection of Oral Squamous Cell Carcinoma and to evaluate the association of these two tests with expression of tumor marker p53.

Materials and methods

The present study was conducted in the Department of Oral Medicine and Radiology, Vokkaligara Sangha Dental College and Hospital, Bengaluru, Karnataka and study subjects were taken from its Out-patient department. 50 patients were included in study. 25 patients underwent acetic acid test while other 25 underwent toluidine blue test. All the 50 patients underwent histopathological and immunohistochemical investigation of lesion for p53.

Individuals with Leukoplakic (white patch), Erythroplakic (red patch), Erythroleukoplakic (mixed area of white and red) or Chronic Ulcers persisting for more than 4-6 weeks were included in the study. Patients who were undergoing treatment for Oral Squamous Cell Carcinoma or previously diagnosed as Oral Squamous Cell Carcinoma were excluded from the study.

A list was prepared in which serial numbers 1 to 50 were written. Half of the numbers were allotted acetic acid test and half were allotted toludine blue test randomly. Patients within the inclusion criteria were then asked to chose a number from 1-50 and the test written against that particular number was performed on the patient.

For Toluidine Blue staining the area of mouth, where application was to be done, was dried. Photograph of the lesion was taken. Cotton pellet soaked in 1% solution of toluidine blue was kept over the lesion and retained there for a minute. After removal of toluidine blue pellet, lesion was photographed. Following another cotton pellet soaked in 1% acetic acid (decolorizing agent) was kept over the area for a minute previously stained

by toluidine blue. After a minute this pellet was removed and lesion was photographed again. Retained blue color over the areas of lesion after the application of 1% acetic acid was considered to be positive test result for presence of dysplasia or malignancy.

For acetic acid test patient was asked to rinse the mouth thoroughly using water. The area of mouth, where application was to be done, was dried. Photographed of the lesion was taken. Cotton pellet soaked in 5% acetic acid was kept on the lesion for one minute. After removal of cotton pellet lesion was photographed again. Change in the appearance of lesion to opaque white was considered as positive test result for dysplasia or malignancy.

Incisional biopsy of the suggestively dysplastic areas of lesion was obtained for all the study subjects. Two sections of sample were made. One was used for histopathology and other one was sent for immunohistochemical study for expression of p53.

Results

In Toluidine Blue group 20 (80%) patients were male while 5 (20%) patients were female. Similarly in Acetic Acid group there were 20 (80%) male and 5 (20%) were females.

All lesions included in study were of 5 types Homogenous leukoplakia, Speckled Leukoplakia, Reticular Lichen Planus, Erosive Lichen Planus, Oral Squamous cell carcinoma. There were 21 (42%) cases of Homogenous leukoplakia, 2 (4%) cases of Speckled Leukoplakia, 11 (22%) cases of Reticular Lichen Planus, 4 (8%) cases of Erosive Lichen Planus and 12 (24%) cases of OSCC.

In our study, patients' age range was 27-71 years with mean age of 45.86 years. Leukoplakia was most commonly present in the age group of 46 – 55 years (33.33%). Cases of lichen planus were most prevelant in age group of 36 – 45 years (46.66%). Oral squamous cell carcinoma cases were most commonly present in age group of 56- 65 years (33.33%).

Out of all 50 patients 32 patients has lesions involving single site while 18 patients has lesion involving more than 1 site. In whole study the most commonly involved intra oral site was buccal mucosa (60.29%).

Homogenous Leukoplakia were most commonly present on buccal mucosa (73.91%) while both the cases of speckled leukoplakia were also present in buccal mucosa. Following buccal mucosa, labial mucosa was the most common site of involvement for homogenous leukoplakia.

Most lesions of lichen planus were present on more than 1 intra-oral site. The most common site of involvement for reticular lichen planus was also buccal mucosa.

The most common site for the presense of oral squamous cell carcinoma was gingivobuccal sulcus(75%) while 2 (16.67%) & 1 (8.33%) cases were limited to only buccal mucosa & tongue respectively.

All patients with leukoplakia had history of use of tobacco in any form. Out of 23 patients 15 had history of use of both smoking and chewing tobacco. 5 patients had history of use of smoking tobacco only while 3 patients had history of use of chewing tobacco only.

8 patients out of all cases of leukoplakia also had history of use of alcohol.

All patients of OSCC except 1 had history of use of chewing tobacco. Out of these chewing tobacco users 4 were smokers also.

In our study, sensitivity of toluidine blue in detection of Oral Squamous Cell Carcinoma was found to be 100% and the specificity was 45%.

In our study, sensitivity of acetic acid in detection of oral squamous cell carcinoma was found to be 85.7% and specificity of acetic acid was found to be 55.55%. Sensitivity of Toluidine Blue (100%) was found to be higher than that of Acetic Acid (83.3%) in detection of OSCC but specificity of Acetic acid Test (55.5%) was higher than that of Toluidine blue (45%).

No significant association was observed between Toluidine Blue test and Acetic acid test. ($p > 0.05$ i.e. 0.564) (χ^2**=.333**). A significant association was observed between Toluidine blue test and p53 immunohistochemistry. ($p< 0.05$ i.e. 0.010) (χ^2**=6.618**). No significant association was observed between Acetic acid and p53 immunohistochemistry. ($p > 0.05$ i.e. 0.10) (χ^2 **= 2.707**)

Discusssion

In our study, sensitivity of toluidine blue in detection of Oral Squamous Cell Carcinoma was found to be 100%.(Table-12) These results were in accordance with the findings of study conducted by Epstein JB et al[7] who reported sensitivity of toluidine blue in detection of OSCC is 100%. Other studies conducted by Warnakulasuriya KAAS, Johnson NW[8(29)] and Onfer MA, Spasto MR, Navarro CM[9] also reported sensitivity of 100% . Some other studies conducted by Mashberg A[10]; Silverman S, Migliorati C &Barbora J[11] found to be

sensitivity of toluidine blue to be 97.5% and 98% respectively.

In our study, the specificity of toluidine blue in detection of oral squamous cell carcinoma is 45% (Table 12) which was quite low when compared to the results of other studies conducted in past. This was because of reason that toluidine blue has a tendency to stain dysplastic lesions (leukoplakia, erosive lichen planus) also positive. Our results were near to specificity found in study conducted by Warnakulasurya KAAS, Johnson NW[8] & Onofre MA, Sposto MR, Navarro CM[9] which were 62% & 67% respectively.

In our study, sensitivity of acetic acid in detection of oral squamous cell carcinoma was found to be 85.7% (Table 13) which was closely related with the result of study conducted by Kanokporn Bhalang, Anocha Suesuwal, Kittipong Dhanuthai, Phakdee Sannikorn, Lakana Luangjarmekorn & Somporn Swasdison (2008) [1] in which sensitivity of acetic acid was 83.3%.

In our study the specificity of acetic acid in detection of oral squamous cell carcinoma was found to be 55.55% (Table 13) which was not in accordance with the study conducted by Kanokporn Bhalang, Anocha Suesuwal, Kittipong Dhanuthai, Phakdee Sannikorn, Lakana Luangjarmekorn & Somporn Swasdison (2008) [1].

In our study, sensitivity of Toluidine Blue (100%) was found to be higher than that of Acetic Acid (83.3%) in detection of OSCC. But, specificity of Acetic acid Test (55.5%) was higher than that of Toluidine blue (45%).

Conclusion

Toluidine Blue vital staining is highly efficient in detection of OSCC but not able to clinically differentiate between malignant and dysplastic lesion efficiently.5% Acetic acid is also efficient in detection of OSCC but not as Toluidine Blue. Acetic acid is more able to clinically differentiate between malignant lesion and dysplastic lesion but still not as highly as to be a standard test. There is no significant association between results of Toluidine Blue and Acetic acid results. There is a significant association of between Toluidine Blue test and immunohistochemistry for tumor marker p53. There is no significant association between results of Acetic acid test and immunohistochemistry for tumor marker p53

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Tables

Table 1 Distribution of lesions for different tests

LESIONS	Toluidine Blue	Acetic Acid	Total
Homogenous Leukoplakia	12 (48%)	9 (36%)	21 (42%)
Speckled Leukoplakia	2 (8%)	0(0%)	2 (4%)
Reticular Lichen Planus	5(20%)	6(24%)	11(22%)
Erosive Lichen Planus	1(4%)	3(12%)	4(8%)
Oral squamous cell carcinoma	5(20%)	7(28%)	12(24%)
Total	**25**	**25**	**50**

TABLE 2 : EFFICACY OF TOLUIDINE BLUE FOR DETECTION OF CARCINOMA

	HISTOLOGICAL DIAGNOSIS		TOTAL
TOLUIDINE BLUE DYE RETENTION	MALIGNANCY +	MALIGNANCY -	
+	5	11	16
-	0	9	9
TOTAL	**5**	**20**	**25**

Table 3 : EFFICACY OF ACETIC ACID FOR DETECTION OF CARCINOMA

ACETIC ACID TEST	HISTOLOGICAL DIAGNOSIS		TOTAL
	MALIGNANCY +	MALIGNANCY -	
+	6	8	14
-	1	10	11
TOTAL	**7**	**18**	**25**

TABLE 4: COMPARISON OF RESULTS FROM TB AND AA TESTS

Test	Result		Total	χ^2	P-Value
	Positive	Negative			
TB	16 (64%)	9 (36%)	**25**	0.333	0.564
AA	14 (56%)	11 (44%)	**25**		
Total	**30**	**20**	**50**		

TABLE 5: ASSOCIATION BETWEEN p53 IMMUNOHISTOCHEMISTRY AND TB TESTS

TB Test result	p53 Result		Total	χ^2	P-Value
	Positive	Negative			
Positive	8 (50%)	8 (50%)	**16**	6.618	0.010*
Negative	0 (0%)	9 (100%)	**9**		
Total	**8**	**17**	**25**		

*denotes significant association

TABLE 6: ASSOCIATION BETWEEN p53 IMMUNOHISTOCHEMISTRY AND AA TESTS

AA Test result	p53 Result		Total	χ^2	P-Value
	Positive	**Negative**			
Positive	7 (50%)	7 (50%)	**14**	2.707	0.100
Negative	2 (18%)	9 (82%)	**11**		
Total	**9**	**16**	**25**