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EVALUATION OF DYSPLASTIC EXPRESSION IN ORAL LEUKOPLAKIA BY CLINICAL, HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY BY CYTOKERATIN 19 MARKER (CK 19) – A COMPARATIVE STUDY

Oral Medicine

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ABSTRACT

Introduction: Oral cancers contribute to over 30 % of all malignancies in India. Leukoplakia being one of the most common precancerous lesions can be detected clinically by in vivo staining procedures there by helping in early identification of this pre cancerous lesions and aid in early treatment initiation. Therefore the present study was conducted to compare the dysplastic expression in oral leukoplakia by toluidine blue staining, histopathology and immunohistochemistry (IHC) using CK 19 marker expression. **Objectives:** To compare dysplasia in oral premalignant lesion leukoplakia by clinical, histopathological and IHC using CK 19 marker. **Materials and methods:** Prospective comparative study was conducted on 15 patients who were found positive clinically with leukoplakia and there after comparing the dysplasia using toluidine blue staining, histopathology and Immunohistochemistry using CK 19 marker. Statistical analysis was done using SPSS version 16 **Results:** Of the 15 patients who were clinically suspected to have oral leukoplakia, 12 (80%), 10 (66.7%) and 8 (53.33%) cases were found to be positive on toluidine blue staining, histopathology and immunohistochemistry respectively. **Conclusion:** In the present study Toluidine blue was found to be sensitive in identifying precancerous lesions and can be used an adjunct to clinical diagnosis there by helping in early detection of oral cancer. This diagnosis can be confirmed using histopathology and IHC using CK 19 marker in detecting mild to moderate dysplasia at the early stages.

KEYWORDS

Chronic liver disease, Liver cirrhosis, Non-alcoholic fatty liver disease.

INTRODUCTION:

In the Western world, Oral cancers contribute to approximately 3% of all the cancers whereas in India, it accounts to more than 30%. The most common malignant precursor oral mucosal lesion is Leukoplakia which is defined clinically as white patch that cannot be characterized clinically or pathologically as any other disease. Furthermore, in most of the cases it becomes difficult to differentiate premalignant lesion like leukoplakia from reactive and inflammatory diseases. It is important to detect oral premalignant lesions early to improve the survival rate and quality of life. , ,

In many cases, clinicians have difficulty in recognizing patients at high risk of developing oral cancer more so when there is a need to identify the site where biopsy has to be taken from the suspected lesions which depends on clinical ability to differentiate pre-malignant from other reactive and inflammatory lesions.316% of the cases that appear as leukoplakia would have already progressed to be malignant while dysplastic leukoplastic lesions have 46% risk of development into malignancy in a mean time of 8.6 years., ,

Clinical diagnosis being primarily by visual inspection and a manual palpation, various techniques have been developed to support clinical examinations, such as toluidine blue staining which when used in vivo selectively stains acidic tissue components such as DNA and RNA.

In the present study, the use of toluidine blue staining was taken into consideration to identify clinically doubtful oral leukoplakia and to compare with histopathologial examination and cytokeratin 19 expression through immunohistochemistry.

Objectives:

To compare dysplasia in oral premalignant lesion leukoplakia by clinical, histopathological and IHC

Materials and methods:

A prospective study was conducted in Department of Oral Medicine and Radiology (OMR) of Narayana Dental College in Nellore district of Andhra Pradesh, India during April to October year 2012. All patients attending out patient department of OMR department during the study period were examined clinically for oral leukoplakia lesions and further included in the study if found positive for oral leukoplakia.
Inclusion criteria: Patients aged above 18 years, clinically found positive for oral leukoplakia and agreeing to participate in the study and willing for incisional biopsy and with no other systemic diseases were included in the study after obtaining written informed consent.

Exclusion criteria: Patients with HIV and Hepatitis positive, diabetes, hypertension and patients who are contraindicated for obtaining biopsy were excluded from the study.

Ethical permission was obtained from the institution's ethical committee.

A pretested structured questionnaire was used to collect demographic information such as age, sex, occupation and relevant clinical details.
A total of 15 patients clinically diagnosed with leukoplakia were subjected to 2 % toluidine blue staining and found positive were subjected for incisional biopsy for histopathological examination and Immunohistochemistry by cytokeratin(CK19) marker . Data was entered into Microsoft excel and analyzed using SPSS version 16 .

Clinically diagnosed leukoplakia patients were subjected to staining with local application of 2% toluidine blue with cotton applicator and left for 10 seconds and destained with 1% Acetic acid the degree of dysplasia was accessed by the intensity of strain retention.

Incisional biopsy was done on the area which is deep blue in colour under local anesthesia and followed by placing removable sutures appropriately tissue was immediately transferred into 2 bottles containing 10% formalin and were sent for histopathological examination and IHC by CK 19 marker. Suture removal was done a week later after the procedure.

The tissue specimens were classified as dysplastic on histopathology based on cellular abnormality i.e variation in cell size, morphological characteristics, cell orientation, alteration in cellular maturation thickness of epithelium involved . The immunohistochemistry (IHC)were stated as positive depending on the focal positivity of the CK19 marker in the tissues specimens.

Results:

Out of 15 subjects included in the study, majority of them (11, 73.3%) were in the age group of 40 to 49 years (Table 1). 13, (86.6%) of them were male and 2 (13.33%) of them were female subjects. (Table 2) Of the total 15 patients suspected clinically for oral leukoplakia 12 (80%) of them were found positive for toluidine blue staining and 3(20%) were negative.

Incisional biopsy of all 15 patients was obtained and subjected for histopathological examination. Of the 12 cases that were found to be positive for toluidine blue staining, 10(83.3%)cases showed some degree dysplasia on histopathological examination and 2 (16.6%) were

found to be negative. Cases that stained negative for toluidine blue were also negative on histopathological examination. This was found to be statistically significant (p value of .04) (Table 3) Out of 12 specimens that stained positive subjected to Immunohistochemistry 8 were found to be positive for IHC CK 19 marker and 4 were negative. Three cases that were negative with toluidine blue were also negative on IHC. This was not found to be statistically significant (p value of .06)

Table 1: Distribution of study subjects by age group and leukoplakia.

Age group	Leukoplakia	Percentage
30-39 years	1	6.67
40-49 years	11	73.33
>50 years	3	20
Total	15	100
mean age: 45.2		

Table 2: Distribution of study subjects by sex and leukoplakia

Sex	Leukoplakia	Percentage
Male	13	86.67
Female	2	13.33
Total	15	100

Table 3: Comparison of toluidine blue and histopathology in oral leukoplakia

Toluidine blue	Histopathology				
	Positive	Percentage	Negative	Percentage	Total
Positive	10	83.3	2	16.6	12
Negative	0	0	3	100	3
Total	10		5		15
P value: 0.04					

Table 4: Comparison of toluidine blue and Immunohistochemistry in oral leukoplakia

Toluidine blue		Immunohistochemistry			
	Positive	Percentage	Negative	Percentage	Total
Positive	8	67	4	34	12
Negative	0	0	3	100	3
Total	8		7		15
P value: 0.1					

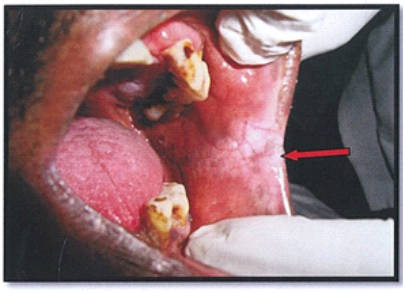

Fig 1: Speckled leukoplakia of oral cavity

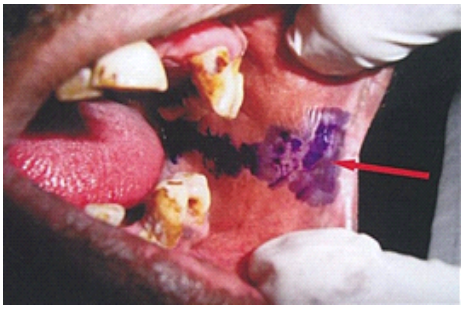

Fig 2: Toluidine Blue application on the lesion

Figure 3: Retention of Toluidine blue after Destaining with 1% Acetic acid

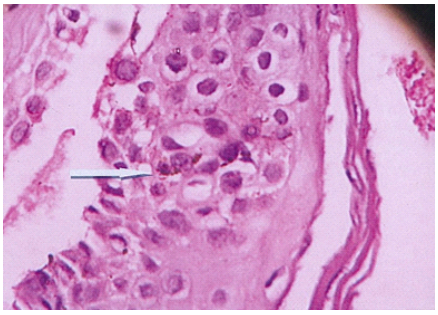

Figure 4: Histopathologically showing mild dysplasia in leukoplakia

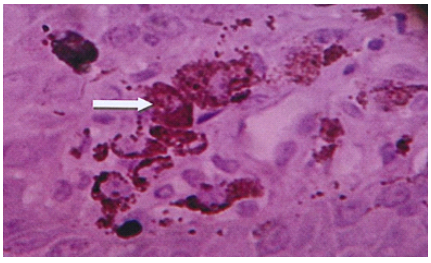

Figure 5: focal positivity of cytokeratine 19 marker in leukoplakia

Discussion

The mean age of the study population was 45.2 years for leukoplakia, this was in accordance with the studies conducted earlier by Banoczy et al. 8 In the present study found to be higher in male patients since smoking habit is more prevalent among male patients which is found to be similar to study conducted by Jindal et al.

Of the cases suspected as precancerous by toluidine blue staining, confirmation of dysplasia was found in 83.3% of cases by histopathological examination this is nearly sensitive.

The present study intends to prove that even mild to moderate dysplastic expression in a premalignant lesion can be done with toluidine blue with high sensitivity henceforth toluidine blue is an adjunct to clinical examination but histopathology being gold standard.

Dysplastic changes were found in 67% of the cases by Immunohistochemistry by CK 19 marker among those that were found positive by toluidine blue staining. This was in found to be similar to another study conducted by Ramprasad et al which concluded that increased expression of CK 19 marker in all grades of dysplasia. The overall CK19 positive cells decreased in number from mild to severe dysplasia which was due to changes taking place during terminal differentiation. While other studies conducted by Vigneswaran et al, Morgan et al, Angus et al detected complete absence of CK19 expression in carcinomas.

Conclusion:

Although one can clinically identify the nature of the lesion weather benign or malignant still lot of focus is given for histopathological revelations for dysplastic expression in oral premalignant lesions like leukoplakia. In vivo strains like toluidine blue can be prompt resources in diagnosing the molecular changes or some specific chemical changes taking place within cells or tissues during the process of

carcinogenesis in premalignant lesions and conditions. IHC CK 19 can be of significance along with histopathology in detecting mild to moderate dysplastic changes of premalignant lesions like leukoplakia and thus it's a help in early detection of precancerous lesions.

REFERENCES

1. Dangi J, Kinnunen TH, Zavras AI. Challenges in global improvement of oral cancer outcomes: Findings from rural Northern India. Tob Induc Dis. 2012;10:5
2. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007;36:575–80.
3. World Health Organization Collaborating Center for Oral Precancerous Lesions. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol 1978;46:518-39.
4. Allegra E, Lombardo N, Puzzo L, Garozzo A. The usefulness of toluidine staining as a diagnostic tool for precancerous and cancerous oropharyngeal and oral cavity lesions. Acta Otorhinolaryngol Ital. 2009;29(4):187–190.
5. Jemal A, Thimas A, Murray T, Thun M. Cancer statistics, 2002. CA Cancer J Clin 2002;52:181-2.
6. Woolgar JA, Scott J, Vaughan ED, Brown JS, West CR, Rogers S. Survival, metastasis and recurrence of oral cancer in relation to pathological features. Ann R Coll Surg Engl 1995;77:325-31
7. Banoczy J, Sugar L. Longitudinal studies on oral leukoplakia. J Oral Pathol 1972;1:265-9.
8. Chiesa F, Tradati N, Sala L, Costa L, Podrecca S, Baracchi P, et al. Follow-up of oral leukoplakia after carbon dioxide laser surgery. Arch Otolaryngol Head Neck Surg 1990;116:177-80.
9. Silverman S Jr, Gorsky M, Lozada F. Oral leukoplakia and malignant tranformation. Cancer 1984;53:563-8.
10. Carrard VC, van der Waal I. A clinical diagnosis of oral leukoplakia; A guide for dentists. Med Oral Patol Oral Cir Bucal. 2018;23(1):e59–e64. Published 2018 Jan 1. doi:10.4317/medoral.22292
11. S. K. Jindal, A. N. Aggarwal, K. Chaudhry, S. K. Chhabra, G. A. D'Souza, D. Gupta, S. K. Katiyar, R. Kumar, B. Shah, V. K. Vijayan, et al. Tobacco smoking in India: prevalence, quit-rates and respiratory morbidity.
12. Indian J Chest Dis Allied Sci. 2006 Jan-Mar; 48(1): 37–42
13. V. V. S. Ram Prassad, N. Rao Nirmala, M. S. Kotian. Immunohistochemical evaluation of expression of cytokeratin 19 in different histological grades of leukoplakia and oral squamous cell carcinoma. Indian J Dent Res. 2005 Jan-Mar; 16(1): 6–11.