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Research Article

TO DETERMINETHE DIAGNOSTIC EFFICIENCY OF TOLUIDINE BLUE WITH LUGOL'SIODINE IN ORAL PREMALIGNANCIES AND ORAL SQUAMOUS CELL CARCINOMA, BY IN VIVO STAINING - ORIGINAL RESEARCH

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A B S T R A C T

In vivo stains are the prompt resources, which have emerged, in the recent years so as to aid as clinical diagnostic tools in detecting early premalignant and malignant lesions. Toluidine blue by its property of retaining in the increased DNA and RNA cellular activity are as, aids in delineating the suspicious are as where as Lugol's Iodine reaction is pertained to the amount of glycogen content in the squamous epithelial cells and thus both the stains when used consecutively aids not only in delineating the margins of the suspicious lesions but also addscontrast to the early dysplastic lesions.

The aim of the study was to determine the diagnostic efficiency of Toluidine blue with Lugol's Iodine in Oral Premalignancies and OralSquamous Cell Carcinoma.

Methods: Study group comprised of 30 subjects, out of which 15 were with clinically suspicious leukoplakia and 15 with clinically suspicious oral squamous cell carcinoma lesions. All the lesions were stained consecutively with Toluidine blue and Lugol's Iodine and the dye retention was recorded with photographs and depending on the retention of the dyes biopsy site was determined. The biopsy specimens were sent for histopathological evaluation and the results were statistically analyzed.

Results: Lugol's Iodine when used with Toluidine blue helped in delineating the inflammatory lesions and was a mean source in determining clinically the degrees of differentiation of malignant lesions as the moderately differentiated malignant lesions in which glycogen content failed to show Lugol's Iodine retention.

Conclusion: Toluidine blue with Lugol's Iodine can be used a sapre-therapeutic assessment of the biologic aggressiveness of the disease. Further studies with larger sample size are required to draw substantial conclusion.

INTRODUCTION

Cancer is one among the most dreadful disease human race is suffering. Cancers involving different organs and structures of human body have become a prime concern in the field of medicine due to their significant marks inmorbidity and mortality rates and oral cancers are no exception to it[1]. It often arises from premalignant lesions and conditions such as Leukoplakia, Erythroplakia and Oral Lichen Planus (OLP), OralSubmucous Fibrosis. Detecting oral malignant and potentially malignant lesions in early stages dramatically affects survival rates. By far clinical examination and histopathological studies have been used for detection of precancerous and cancerous lesions. As with other fields of medicine, in oral cavity diagnostic approaches are going toward noninvasive, simple, inexpensive, painless and accessible methods such as cytology, brush biopsy, toluidine rinses, chemiluminiscent devices, and auto fluorescence, spectroscopy.[2,3]

Toluidine blue by its property of retaining in the increased DNA and RNA cellular activity areas, aids in delineating the suspicious areas whereas Lugol's iodine which is retained in the normal and mild dysplastic squamous epithelial cells not only delineates the margins of the suspicious lesions but also adds contrast to the dysplastic lesions[4,5]. The biological potential of evaluating precancerous lesions still relies on light microscopic histological examination, so an appropriate tissue is a prerequisite[6].

An early detection of premalignant lesions with this method, underelegant specialists, can be conducted in mass screening programmes and when aided with necessary treatment the progression of the lesion into malignancies can potentially be halted. This foregoing madeit imperative to study the

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diagnostic efficiency of Toluidineblue and Lugol's iodine in Oral Premalignancy and Oral Squamous Cell Carcinoma

MATERIALS & METHOD

The present study was conducted in the Department of Oral Medicine and Radiology, Pacific Dental College And Hospital, Udaipur, Rajasthan and Department of ENT, RNT Medical College And Hospital Udaipur, Rajasthan.

Study Design

The study group included30 subjects of both the sexes reported to the out patient department of Oral Medicine and Radiology, Pacific Dental College and Hospital, Udaipur , Rajasthan and Department of ENT, from RNT Medical College And Hospital Udaipur, Rajasthan.

15subjects who were clinically suspected as Premalignant Lesions (Homogenous leukoplakia, Speckled leukoplakia) and 15 subjects as malignant lesions (Oral Squamous Cell carcinoma) were selected who fulfilled the following criteria for the study:-

1. Leukoplakia: Non scrappable elevated white patchorplaque with a history of to bacco chewing or smoking and smooth or wrinkled surface and sometimes traversed by small cracks or fissures[7].
2. Speckled Leukoplakia: Mixedred and white lesion with keratotic white nodule (or) specks (or) patches distributed over an atrophic erythematous background[8,9].
3. Oral Malignancy[10]: White patch like lesion with ulcerated area within(or)

Adjacen tto it. Anulceratedarea with rolled bordersand hard indurated edges with velvety red irregular base (infiltrative variety). A proliferative growth with single (or) multiple ulcers around it within duration (exophytic–verrucousvariety),white patch like lesion with interspersed reddish areas, which ulcerates.

Interpretation of the Toluidine blue stain

Lightblueretentionwasconsideredaspositiveforpremalignantlesi onsunlessprovedo ther wise by biopsy and dark blue stain was considered as positive for lesions suspicious of malignancy, while lesions without any retention of stain were considered as negative[11].

Interpretation of the Lugol's iodine stain

Brown stain was considered as positive for lesions while without any retention of stain were considered as negative[12]. Biopsy site was selected on basis of clinical appearance and dyes retention and in the sites where no retention of the stain occurred, clinical judgment directed the biopsy

RESULTS

In the present study 30 subjects with clinical diagnosis of 15 each premalignant lesions and oral squamous cell carcinoma, were chosen to assess the efficiency of In Vivo staining using Toluidine Blue with Lugol's Iodine. Of 15 Premalignant lesions13 (86.6%) subjects were clinically diagnosed with homogenous leukoplakia,2(13.3%)subjects with speckled leukoplakia.

The dataobtained from the study , which comprised of clinical details, In Vivo staining pattern and histolopathogicalgrading was recorded and the staining pattern of Toludine Blue and Lugol's Iodine stains in relation to histopathological diagnosis was statistically analyzed.

In the oral leukoplakia study group, subjects were selected with clinical diagnosis of homogenous leukoplakia and speckled leukoplakia. The agedistribution ranged between 25 to 65 years with mean age as 40 years. While the gender distribution followed, 13 Male patients with clinical diagnosis of Homogenous Leukoplakia and 2 female patients, each with clinical diagnosis of Homogenous Leukoplakia and Speckled Leukoplakia.

In the Oral Squamous Cell Carcinoma study group, 15 cases were selected, which included patients with age range between 25-75 years and mean age as 50 years. The gender distribution revealed predominance of Male subjects showing 14 cases out of 15 as males while only 1 female patient in study group.

Among 15clinically diagnosed oral leukoplakia cases,13 cases (86%)presented lesions on the sites likebuccal mucosa,retro-molar and commissural areas, while 1 case (7%) presented on the hard palate and 1 case (7%) on other sites like vestibular area/alveolar ridge

Among 15 clinically diagnosed cases of oral squamous cell carcinoma10 cases (66.6%) were presented on the buccal mucosa, vestibule and alveolar ridge, while 2 cases (14%)were located on the ventro-lateral aspects of the tongue, and 3 cases (20%) were on the gingiva.

Among 15 cases of oral leukoplakia lesions,12 cases showed retention of Toluidine blue staining while 3 cases failed to retain the stain. Out of 12 positive stained cases, 11 were clinically diagnosed as homogenous leukoplakia with staining pattern observed was, uniform light blue with diffuse boundaries. The 1 positive stained case was clinically diagnosed as speckled leukoplakia, revealed minute intense spots of dark blue pattern with uniform light blue background and diffuse boundaries. The rest 3 cases showed negative staining pattern. When Lugols Iodine stain was consecutively applied along with Toluidine Blue Stain, 11 cases out of 15 oral leukoplakia, revealed positive staining for both the stains. Among these 11 cases, 8 revealed dye changes histolopathologically while 3 revealed inflammatory changes with no dysplasia. On the other hand 4 cases which failed to retain Lugol's Iodine stain along with Toluidine Blue Stain, were diagnosed histologically as inflammatory lesions(LP) with no dysplastic changes. The sensitivity of Toluidine Blue with Lugol's Iodine stainin determining the dysplastic changes was found to be 100% and the specificity as 33.5%.The positive predictive value and the negative predictive value were 58.3% and 100%respectively. The diagnostic accuracy of toluidine blue with lugol's iodine stainin distinguishing early premalignant lesions was 60%.

Ofthe 15 cases of oral squamous cell carcinoma, all 15 (100%) cases retained the toluidine blue stain. Staining pattern observed in all the oral squamous cell carcinoma cases was, dark royal blue with clear demarcation of the lesion.When Lugol's Iodine stain was consecutively applied along with Toluidine Blue Stain, 13 cases out of 15 revealed positive staining for both stains, while two cases failed to retainLugol's

Iodine stain. Stain retained lesions were histologically confirmed as demonstrating carcinomatous changes with 6 diagnosed as well differentiated oral squamous cell carcinoma, and 7 as moderately differentiated oral squamous cell carcinoma .The 2 Lugol's Iodine negative stained cases were diagnosed histopathologically as moderately differentiated oral squamous cell carcinoma. The sensitivity and positive predictive value of both stains was determined as 100%.The diagnostic accuracy of toluidine blue staining in distinguishing malignant lesions was100%**.**

The above data was statistically analyzed using chi square test which revealed p-value of < 0.001, hence revealing significance of the diagnostic use of both stains in oral leukoplakia and Oral Squamous Cell Carcinoma Cases.

DISCUSSION

The selective character of staining the intact mucosa with Lugol'siodine is dependenton the glycogen content present in the normal epithelium and this selective character of staining helps in delineating the inflammatory or carcinomatous epithelium from normal epithelium where glycogen content is low[4].

The safety of toluidine blue and Lugol's iodine as a vital stain has been assessed and confirmed over numerous studies. In the present study too, we observed no harmful effects or persistent staining in any of the lesions or in the adjacent mucosa.
Study performed by MiuraA,OhbaY(1967) was the first one which reported the acceptance of Toluidine blue staining technique after the application on patients with suspected oral carcinoma for seven years.Later Rosen IB, Cornish M, EdelsonJ (1971)screened 45 cases of oral cancer with Toluidine blue and found no after effects of stains which can be considered as harmful to use it as in vivo stain. A study conducted byVahidyNA, ZaidiSHM, JafareyNA (1972)on 1190 patients and of the 535 cases of SCC,481cases stained with Toluidine blue, here also Toludine blue was considered as reliable and safe stain for in vivo staining. In the same year SigurdsonA,WillenR(1972) stained 54 suspicious lesions of oral cavity with toluidine blue, and confirmed the safety of toluidine blue. If we consider the studies in which both stains were used, the first one to use them as together was by MandardAM,TournexJ,GignouxM in (1981), they used Lugol'ssolution and Toluidine blue solution in the diagnostic assessment of 151 consecutive patients at risk of esophageal disease. The safety protocol they mentioned in their article clearly suggests that these stains are safe on oral tissue as well.
In our study a male predominance was observed in both oral leukoplakia and oral squamous cell carcinoma study groups. Among 15 cases of oral leukoplalakia (homogenous leukoplakia, speckled leukoplakia), 13:2 male female ratio was observed. While on the other hand, in oral squamous cell carcinoma group14:1 male female ratio was seen. Similar ratio for males and females were also observed in studies done by E Allegra et al in 2011, FareediMukram Ali, et al in 2012 and Sapnamadhani et al in 2013.

Clinically when oral leukoplakia/ oral squamous cell carcinoma tissues were stained using cotton applicator with 1% of toluidine blue, we observed retention of the dye in the form of royal blue color to light blue color. In the present study these color retentions were taken as positive and no retention were considered as negative.

Mahesh Chandra Hegde et al (2006), used1% toluidine blue (TB) on 90 cases of oral lesions or mucosal alterations suspicious of malignancy in the oral cavity and or pharynx. They interpreted dark blue (royal or navy) stain as positive,(either the entire lesion being stained or a portion of it is stained or stippled). A light blue staining was considered doubtful but still considered as positive until proved negative histopathologically. If there was no colour absorbed by the lesion, the stain was interpretatedas negative.

E. Allegra et al in 2009 also used Toluidine blue 1% on 45 oral mucosa lesions from 32 patients. But they proposed that lesions that showed dark blue staining were considered to be positive for premalignant or malignant tissue, while those with light staining, or totally not coloured, were considered negative. This was different to what the present study has followed[12].

Kamarthi Nagaraju et al in 2010 in their study also observed58 cases out of 60 (premalignant lesions and malignant) toluidine blue positivity in the form of dark blue to light blue color, clinically when applied with 1%Toluidineblue dye[13].

In oral squamous cell carcinoma, 13 cases retained both stains clinically while 2 cases failed to retain lugols iodine stain. Histopathologically out of 13 cases 6 were diagnosed as well differentiated squamous cell carcinoma while 7 were diagnosed as moderately differentiated squamous cell carcinoma. The two clinically unstained cases were diagnosed histopathologically as moderately differentiated squamous cell carcinoma. This result of clinically unstained lesion can be attributed to the previously explained phenomenon of low content of glycogen and emphasizes the importance of vital staining using toluidine blue and lugols iodine stains in oral squamous cell carcinoma.[13]

Therefore, Lugols iodine when used consecutively with Toluidine blue the sensitivity of the stain in detecting oral squamous cell carcinoma was 86.6%while the diagnostic accuracy was found to be also 86.6%. As all 15 cases showed histopathologically, diseased state there was no scope to calculate the positive and negative predictive values in this category

Further studies are suggested with larger samples, performed longitudinally to evaluate the progress of the lesions based on the intensities and patterns of retention of the stans and to draw substantial conclusion.

CONCLUSION

In the present study, diagnostic efficiency of Toluidine Blue and Lugol's Iodine indetecting the premalignant and malignant lesions was assessed

- A significant difference was observed in the staining pattern of toluidine blue in homogenous leukoplakias and speckled leukoplakias owing to the differences in the epithelial alterations in both the stages of the disease.
- Consecutive use of toluidine blue and Lugol's iodine identified the inflammatory lesions echoing premalignant lesions.

- A significant difference was observed in the dye retention of Lugol's iodine in premalignant lesions and malignant lesions owing to the differences in the glycogen content of cells, which gets minimized as the mitotic activity increases.

Further studies analyzing a combination of sound clinical judgment and diagnostic adjuncts such as Toluidine Blue and Lugol'sIodine could be more representative of cellular proliferation. Further studies with more number of cases are suggested to establish these stains as sound diagnostic adjuncts.

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