ORIGINAL RESEARCH

COMPARISON OF GLYCOGEN CONTENT, BASEMENT MEMBRANE INTEGRITY AND MITOTIC INDEX IN STAGES OF ORAL DYSPLASIA PROGRESSION TO CANCER AND IN ORAL LICHEN-LICHENOID REACTIONS: A HISTOCHEMICAL STUDY

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ABSTRACT:
Introduction: The glycogen content and basement membrane play significant roles as of energy reservoir, keeping cells adhered and also as a mechanical barrier against invading cells. The potential of a cell to regenerate or clone marks its existence and also its neoplasticity. The sequential progression of changes in a cell that render it dysplastic is owing to the changes in attributes of the basement membrane integrity, the energy metabolism and the cell turnover. Aims & Objectives: To study the glycogen content, basement membrane integrity & mitotic index with the use of Lillie allochrome stains to assess its role in predicting the gradual transformation. Materials & Methods: A retrospective study using the archives from the department. Study involves 15 samples each of mild, moderate, severe dysplasia and invasive carcinoma of the oral cavity and also of lichenoid disease and lichen planus. Result: The study showed a regressive trend in distribution of glycogen content across progression of cancer with a statistical significance of $p<0.005$. The loss of basement integrity of invasive cases was evident. The mitotic index parameter revealed significant results with progressive increase across the stages. Conclusion: A combination of simple and different parameters helps in early and accurate diagnosis of the pre-malignant lesions and conditions. Clinical Relevance: Evaluation involves a histochemical stain and thus provides for easy affordability, visual dexterity and quick results for both the patient and doctor.
Keywords: Glycogen content, basement membrane integrity, cancer, mitotic index, Dysplasia

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INTRODUCTION

Cancer is the second most common cause of morbidity and mortality in the world today after cardiovascular problems. Oral cancer is a significant component of the global burden of cancer. [1] Though diagnostic techniques have improved over years, yet survival rates continue to remain poor i.e. with a 5-year survival rate in approximately 50% cases of oral squamous cell carcinoma (OSCC) from the time of diagnosis. [2] Oral carcinogenesis is a multi-factorial process that involves numerous changes at the genetic and phenotypic levels that alter normal functions of oncogenes and tumor suppressor genes. Also, a fine balance exists between proliferation of cells and programmed cell death disruption of which relates to the development of cancer marked by uncontrolled proliferation.[3] The transition of normal epithelium to invasive cancer is progressive and accompanied by "multiple hits" or with the up and down regulation of proliferation, angiogenesis, local invasion and, eventually, distant metastasis. [4]

Hence, in the present research an attempt is made to study and analyse the glycogen content, basement

membrane integrity and mitotic index across the stages of oral dysplastic progression of cancer and in oral lichen-lichenoid reactions and to predict or formulate effective strategies and better treatment protocols against pre-cancer conditions. Research has, for many years now, sought effective treatment for this disease. The single point of agreement is that all forms of treatment work best when the cancer is diagnosed early. This statement is the basis of a ceaseless search for increasingly accurate diagnostic methods to improve or aid in treatment adequacy.

The study aimed at analysing and comparing the glycogen content, basement membrane integrity and mitotic index across several stages of oral dysplasia namely mild, moderate and severe and invasive carcinoma along with pre malignant conditions like lichen planus and lichenoid diseases i.e. reactions and dysplasias. It may provide for a valuable adjunct for early diagnosis or analysing its progression towards cancer.

MATERIALS & METHODS

The study group involved a total of 90 formalin fixed paraffin embedded (FFPE) tissue specimens; 15 each of mild (n=15), moderate (n=15) and severe (n=15) dysplasia and invasive carcinoma (n=15) along with oral lichenoid diseases (n=15) and oral lichen planus (n=15) that were retrieved from the archives of the department of oral pathology and microbiology, Manipal College of Dental Sciences, Manipal University. The diagnosis of the study group was confirmed histologically by routine hematoxylin-eosin (H&E) staining. The institutional ethical board has given clearance to conduct the study. Cases with primary diagnosis of oral squamous cell carcinoma, or under treatment and recurred cases of OSCC were excluded. The histochemical stain used staining and analysing of the sections was modified Lillie's allochrome. The procedure involved using periodic acid Schiff, hematoxylin, picric acid and methyl blue combination which together worked as a differential stain imparting varying colours to the epithelium and stroma namely glycogen is stained magenta, nucleus brown-black-gray, cytoplasm and muscle cells are green to greenish yellow, the basement membrane is characteristic red with collagen and reticulin in blue. Other features of keratinisation can be appreciated as yellow or yellow green, extravasated RBCs are golden yellow and inflammatory cells in purple with muscle laden glycogen showing different shades of magenta and green. [5] The analysis involved the estimation of the relative distribution of glycogen in the epithelium under following categories [Table I] The basement integrity was highlighted preferentially owing to its differential staining and by close observation and tracing of epithelial membrane against the stroma and categorised as follows [Table II].

Mitotic index is defined as the ratio between the number of cells in a population undergoing mitosis to the number of cells not undergoing mitosis. So its calculations involved assessing 5 different fields in a slide for mitotic activity and then obtaining the average for a relative mitotic capacity. The association of variables were evaluated using Fischer exact test for discrete variables and with parametric tests like ANOVA for continuous data variables. Test of significance involving $p<0.05$ were considered statistically significant. The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 16.0.

RESULTS

The normal non-keratinized alveolar mucosa contains about 0.4-0.5% wet weight of intraepithelial glycogen. The glycogen is distributed in the full thickness of the epithelium except for the basal and parabasal cells layers. [Fig 1] It was observed that the glycogen distribution decreased by varying amounts across the dysplastic lesions. The distribution across mild, moderate, severe dysplasia and invasive carcinoma showed a regressive trend of glycogen content [Fig 2 & Fig 3], [Table III].The Fischer exact test done for association and post hoc comparison data showed significant results ($p<0.05$). The same assessed across lichenoid planus and diseases had a value of 0.07 there but not significant. The basement membrane integrity was intact with no breach noted in cases of mild, moderate and severe dysplasias and lichen planus but with apparent breach noted in invasive carcinomas and some lichenoid dysplasias [Fig 2 & 3] [Table IV]. The Fischer Exact test done for comparing these lesions showed significant results approaching nil ($p<0.001$). The mitotic index being a continuous variable was analysed using one way Anova comparing across dysplastic lesions revealing an increased mitotic activity and capacity across mild, moderate and severe dysplasia and invasive carcinoma was evident [Fig 4]. Also, notable change was witnessed in lichen planus and lichenoid dysplasias that were assessed with independent t tests. The results were significant with $p<0.001$.

Table I: Distribution of glycogen

Sr No.	Score	Distribution
1	0	0 – 15%
2	1	15 – 60%
3	2	More than 60 %

Table II: Basement Membrane Integrity Scoring

Sr No.	Score	Basement Membrane Integrity
1	0	Intact Basement Membrane
2	1	Apparent breach
3	2	Loss of basement membrane

Table III: Assessment of Distribution of Glycogen in Lesions

Sr No.	Glycogen content	Mild Dysplasia	Moderate Dysplasia	Severe Dysplasia	Invasive Carcinoma	Total	Value	Significance (p<0.05)
1	0	0	0	4	7	11	27.614	0.00
2	1	7	9	11	8	35		
3	2	8	6	0	0	14		
Total		15	15	15	15	60		

Table IV: Assessment of Basement Membrane Integrity

Sr No.	Basement Membrane Integrity	Mild Dysplasia	Moderate Dysplasia	Severe Dysplasia	Invasive Carcinoma	Total	Value	Significance (p<0.05)
1	0	14	10	1	0	25	44.33	0.000
2	1	1	5	10	8	24		
3	2	0	0	4	7	11		
Total		15	15	15	15	60		

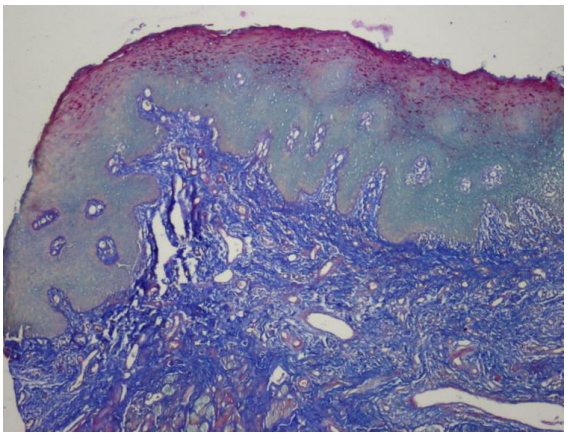

Figure 1: Glycogen content can be appreciated in the suprabasal layers in a case of moderate dysplasia (Lillie Allochrome Stain - 10x)

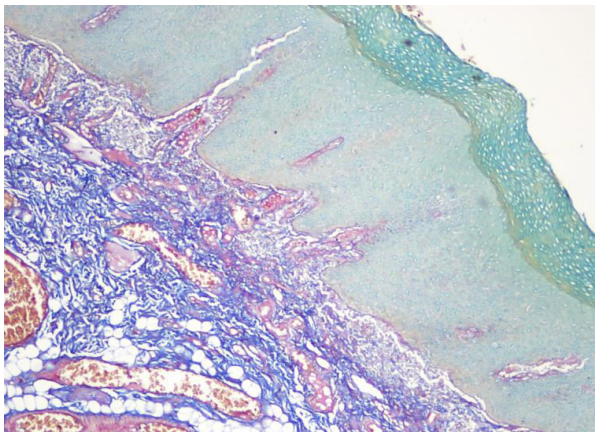

Figure 2: Distinct basement membrane integrity but little or none glycogen content noted in a case of severe dysplasia. (Lillie Allochrome Stain - 10x)

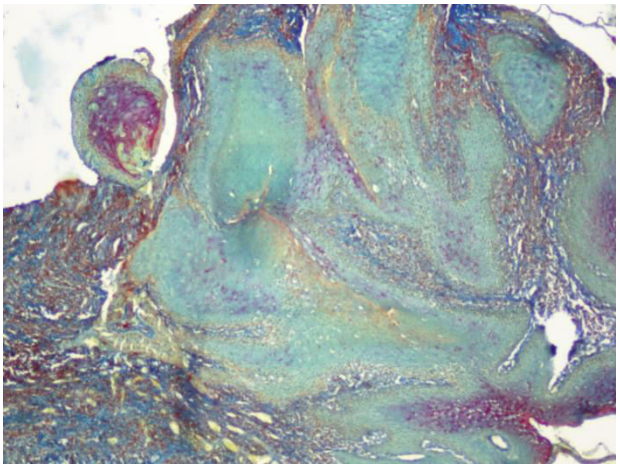

Figure 3: The basement membrane integrity is completely lost with little or none glycogen noted in a case of invasive carcinoma. (Lillie Allochrome Stain - 10x)

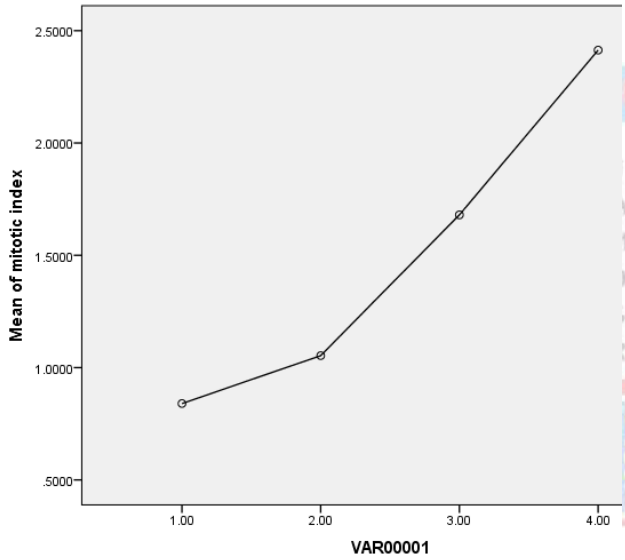

Fig 4 The chart indicates the relative increase in mitotic capacity across mild (1.00), moderate (2.00) severe dysplasia (3.00) and invasive carcinoma (4.00)

DISCUSSION

Oral cancer would be considered a sequential progression i.e. initially the normal mucosa undergoes slight alterations which may be characterized as pre-malignant lesions and conditions which progress though stages of mild, moderate and severe dysplasia, invasive carcinoma and subsequently oral squamous cell carcinoma or lichenoid dysplasia to lichen planus. Dysplasia is regarded as the earliest form of pre-cancerous lesion wherein there is an expansion of immature cells along with decrease in number and location of mature cells. Other characteristic features are anisocytosis, poikilocytosis, hyperchromatism and presence of mitotic figures. However, they remain localized, and has not invaded past the basement membrane into tissues below the surface. Thus, invasive carcinoma may be considered as one of the final steps in progression of cancer with an apparent breach in the basement membrane and denoting all the above features.[6][7]

Lichen planus is a lichenoid autoimmune mucositis with a clinically different but microscopically similar dermal counterpart. It is characterised by frequent oral ulcerations and blisters. Some authors, believe that the cancers do, in fact, arise from the longstanding lichenoid lesions, but others presume that the occurrence is simply the fortuitous simultaneous development of two independent entities. The controversy, unfortunately, is fueled more by opinion than substantial facts. Numerous case reports have not resolved this issue, but several follow up studies have found an increased frequency of cancer, and the cancers that develop are usually in areas of the lichenoid change.[7][8]

Glycogen and its products of metabolism are considered to be the most important and direct source of nutrient. However, its distribution has been shown to be variable across layers. According to previous studies by Bulow FV have revealed that the basal cell layer has relatively no glycogen but it tends to increase towards the surface area. Literature says that the distribution of epithelial glycogen is more in the suprabasal and parabasal cells. A similar trend has been noted in our study too.[9] Literature has previously reported that a decrease in glycogen content may be a feature of dysplastic epithelium although variations have been noted. According to a study by Doyle JL et al showed that 56% of their studied cases were parakeratotic and had decreased glycogen exhibiting epithelial atypia whereas in only eight of 17% in which parakeratosis was associated with an increased glycogen content and epithelial atypia. However, only 4% of nonkeratinized lesions with increased glycogen showed epithelial atypia.[10]

Also, Dick HM and Mcmuechy KA reported of a case of leukoplakia which revealed initial histologic evidence of dysplasia and later developed into epidermoid carcinoma. Also, noted was the associated glycogen content varied at different biopsies irrespective of the degree of dysplasia.[11] Cahn et al and Isaccson et al also reported an analysis of their cases that showed that the dysplastic and premalignant cases revealed parakeratosis without glycogen. [12] Our study involved similar cases that were progressing towards cancer i.e. the cascade of cancer progression through subsequential steps of mild,

moderate and severe dysplasia followed by invasive carcinoma and also among pre-malignant conditions. The glycogen content revealed a decreasing trend in distribution with increasing dysplasia and some cases with none as seen in carcinoma. However, many reasons have been proposed over years to justify this decreased content of glycogen in dysplastic epithelium as it may be either from increased metabolic activity that consumes the glycogen or its relatively decreased synthesis. Though, the exact reason is still unknown. [12]

The basement membrane is defined as a thin, fibrous, non-cellular region of tissue that separates the epithelium (skin, respiratory tract, gastrointestinal tract, etc), mesothelium (pleural cavity, peritoneal cavity, pericardial cavity, etc) and endothelium (blood vessels, lymph vessels, etc) from underlying connective tissue. The primary function of the basement membrane is to anchor the epithelium to lose connective tissue underneath. Also, it acts as a mechanical barrier, preventing malignant cells from invading the deeper tissues. The early and progressive stages of malignancy play a significant role in deciphering the stage of dysplasia or of malignancy. In our study the criterion is as per WHO specifications only and the results revealed are consistent with the guidelines.[13-15] The mitotic index is the fraction of cells in mitosis at any given time. Currently, Mitotic activity is also a part of tumor grading system in some cancers.

Chatterjee S study involved an analysis of the nucleus-associated parameters of mitotic and proliferation indices and nucleoli counts to predict the biological potential of potentially malignant tobacco-associated lesions like hyperkeratosis, mild, moderate and severe dysplasias and squamous cell carcinoma which highlighted the predominant role of mitotic index as an important and characteristic indicator of progress to malignancy. Our studies revealed similar results. [16]

A shortcoming of the present study was the relatively small sample size. The lichen planus diagnosed lesions were available and quantifiable. However, analysis of more number of cases of lichenoid diseases i.e. dysplasia or reactions could have shed more light in the regard.

CONCLUSION

A combination of different parameters namely glycogen content, basement membrane integrity and mitotic index add and equip the pathologist and clinician to give an accurate, reliable and early diagnosis of the lesions. Also, since the evaluation involves a histochemical stain to assess the various components; it serves and provides for the easy affordability, visual dexterity and quick results for the patient and doctor. Also, it may provide a good adjunct for explaining to the patient the severity of the disease and the chances of its conversion to malignancy. Thus, proving to be a valuable and important adjunct in diagnosis and educating.

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