Canavalia virosa Lectin and its Tissue Binding Pattern in Squamous Cell Carcinoma of Oral Cavity

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A lectin belonging to the general group of mannose/glucose binding was isolated from the seeds of *Canavalia virosa* using sephadex G-50 as its specific adsorbant. In order to establish a useful marker of malignancy of oral tissue, the binding sites of this *Canavalia virosa* lectin (CVL), peanut agglutinin (PNA), Soybean agglutinin (SBA) and *Bandeiraea simplicifolia* agglutinin-1 (BSA-1) were comparatively examined in surgical specimens from the benign and malignant lesions of oral cavity by lectin histochemistry method. The carcinomatous cells showed much more variable lectin binding towards CVL than normal controls which generally had uniform binding. But loss of receptor for PNA, ABA and BSA-1 was observed in poorly differentiated squamous cell carcinoma: the combination of these lectins may be regarded as a useful marker for the objective evaluation of dysplastic grading and to the early detection of histologic evidence of oral epithelial malignancy.

Key Words: Lectin binding, *Canavalia virosa* lectin, Oral cancer

The carbohydrate chains of glycoconjugates are major constituents of cellular membranes and probably confer physiological information relevant to cell interaction, growth regulation and cell differentiation (5, 15, 13). Analysis of endogenous receptors for cellular glycoconjugates may prove clinically useful for tumor diagnosis (7). Lectins, the divalent or multivalent carbohydrate-binding proteins of defined specificity, have proven valuable for defining changes in the structure and composition of cellular glyconjugates upon neoplastic transformation (3, 1). Fluorescein or peroxidase labelled lectins with different sugar specificity applied as histochemical reagents have provided valuable data on both normal and pathologically altered tissue.

Recently we have purified a new lectin from the seeds of *Canavalia virosa*. It has similar characters to those of Con A from *Canavalia ensiformis*, but the primary structure revealed differences in the sequence (6). The present investigation was carried out to study the *Canavalia virosa* lectin binding behaviour in normal, benign and squamous cell carcinoma of oral cavity in comparison with other known lectins such as peanut agglutinin (PNA), soybean agglutinin (SBA) and *Bandeiraea simplicifolia* agglutinin-1 (BSA-1). The degree of malignancy are objectively identified by staining combination of these lectins.

Materials and Methods

The surgical specimens of normal, precancerous lesions and oral squamous cell carcinomas were collected from Medical College Hospital, Trichur, during the years 1991 and 1992. There were 7 normal tissue, 8 cases of oral leukoplakia and 26 cases of oral squamous cell carcinoma which in turn was classified into poor (8 cases), moderate (8 cases) and well differentiated (10 cases). Samples were fixed in 10% formalin and routinely processed and embedded in paraffin. Serial 5 μm sections were cut, one

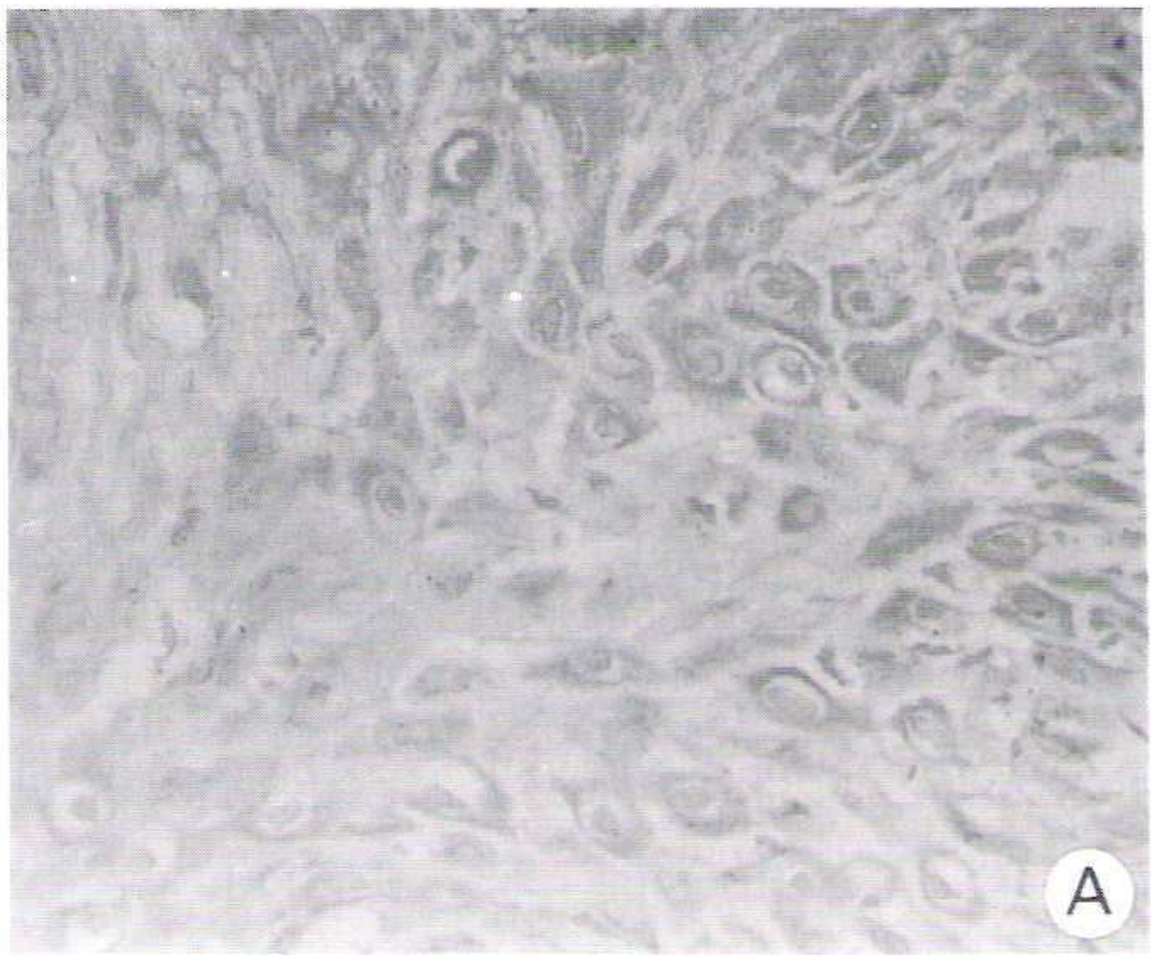
Table I - Lectin panel employed

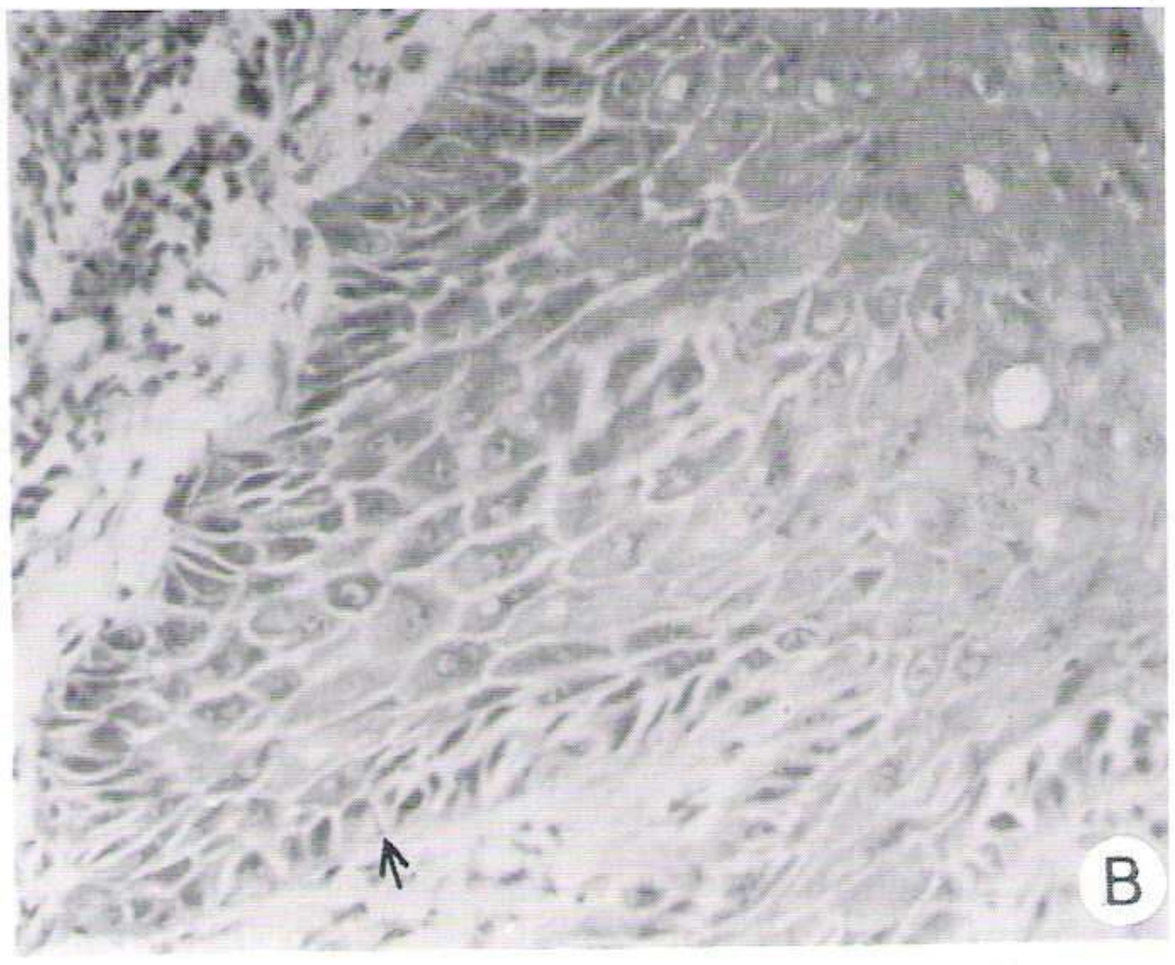
Lectin Abbreviation	Lectin Common name	Origin	Carbohydrate specificity
CVL	*Canavalia virosa* lectin	*Canavalia virosa*	α-D-mannopyranose, α-D-glucopyranose
PNA	Peanut agglutinin	*Arachis hypogaea*	D-galactose, β-(1-3)N-acetyl-D-galactosamine
SBA	Soybean agglutinin	*Glycine max*	N-acetyl-D-galactosamine, D-galactose
BSA-1	*Bandeiraea simplicifolia* agglutinin-1	*Bandeiraea simplicifolia*	α-D-galactose

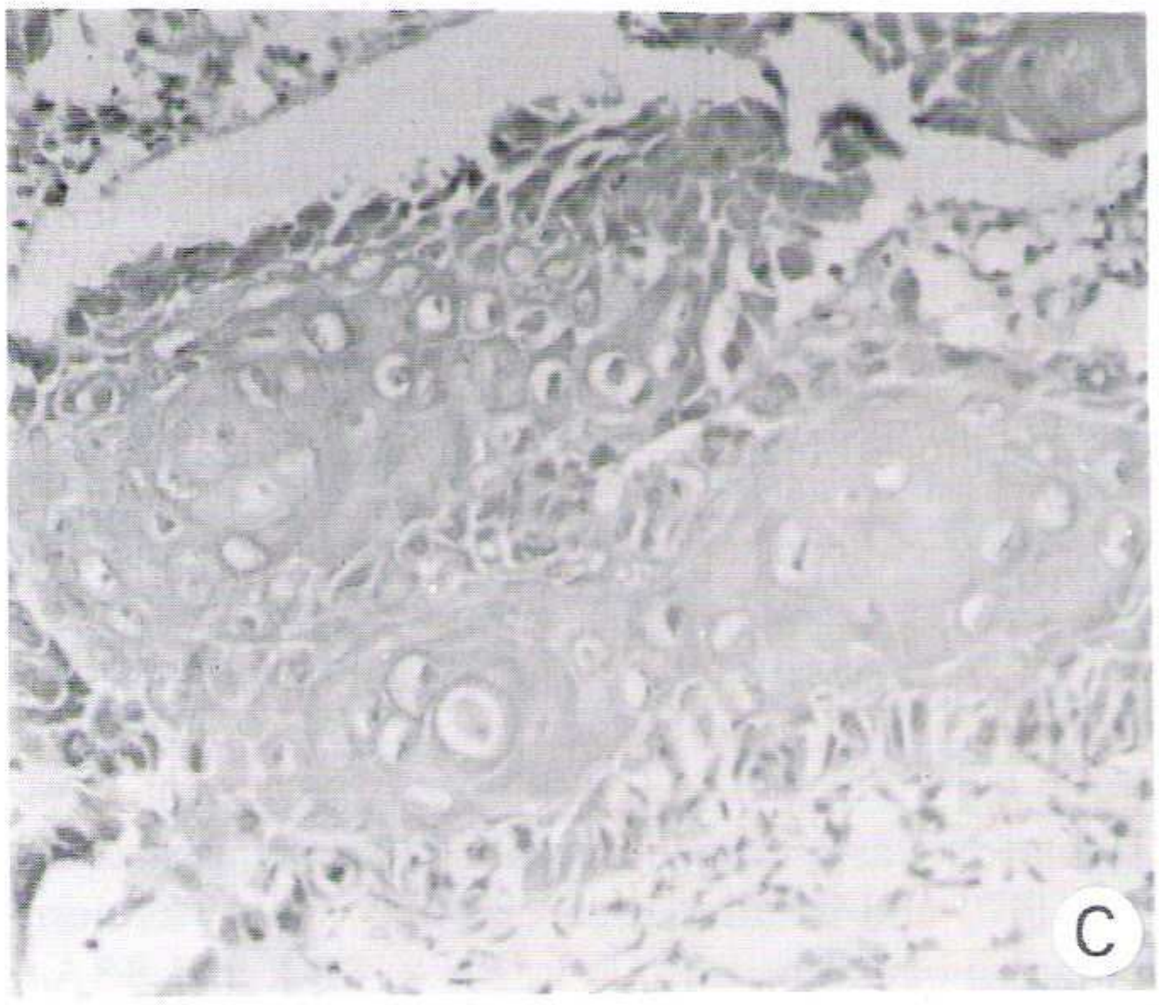
stained with hematoxylin eosin, the others used for lectin stainings. The origins, common names, abbreviations and carbohydrate specificities for the lectin panel employed in this study are detailed in Table I. The horseradish peroxidase (HRP) labeled lectins including PNA, ABA and BSA-1 and the sugars used to block lectin binding were purchased from Sigma Chemical Company (St. Louis, Missouri). The CVL was conjugated to HRP by the method of Nakane and Kawadi (10). The HRP-conjugates were incubated with rehydrated tissue sections for 60 minutes at room temperature in a moist chamber, washed in PBS and the peroxidase was localized by the addition of 3,3-diaminobenzidine dihydrochloride (DAB) and hydrogen peroxide mixture in PBS (18). The sections were counterstained lightly with hematoxylin, dehydrated with alcohol and mounted. For control inhibition studies, lectin solutions were treated with 0.2 M monosaccharides specific to them (60 minutes at 37°C) prior to incubation. The binding of the lectins was estimated using a scale of (+) to (+ + +) for degree of intensity or (−) for absence of staining.

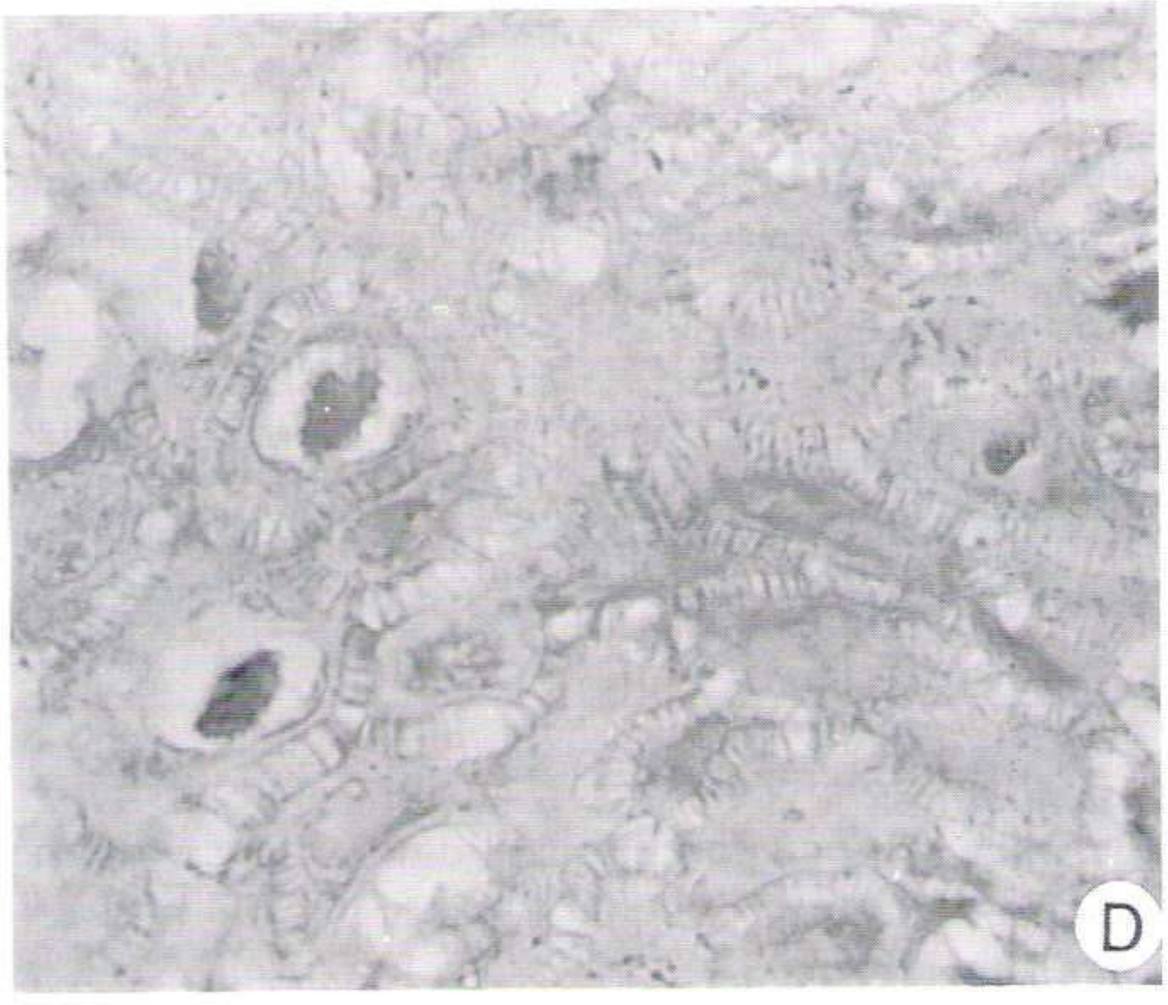
Results and Discussion

All the labelled lectins bound specifically to cells from histologically different tumors in the sections. This was ascertained by the absence of positive reaction in competitive inhibition experiments. The results of che CVL binding in oral tissues are given in Table II and Figure 1. Most prominent reactivity of CVL was seen in keratinized and intercellular areas. The intensities of staining in normal, benign and malignant conditions were different. CVL bound to intercellular regions of normal tissue with an even distribution of staining. Such even distribution of CVL in normal tissue contrasted with a more heterogeneous staining pattern found when this lectin was reacted with malignant tissue. In benign tissue, CVL demonstrated a different cytoplasmic pattern. The differences in binding may be due to the alterations of the normal cells during the malignant transformation (12). Peroxidase labelled lectins have proved to be a useful tool to visualize the presence of lectin receptors in routine pathological specimens (16, 17). The lectin binding method is based on the fact that the cells contain higher amount of oligosaccharide residues on their surface, and the number of cells with such binding sites differs significantly between healthy individuals and cancer patients (4, 11). The affinity of CVL to basal cells was prominent in keratinized epithelium. The basal cells in dysplastic and regenerating epithelia seem to be in active phase of proliferation and in disturbed condition of cellular maturation. The reactivity of CVL in keratinized area seemed to represent the major difference between the normal and neoplastic tissue. The dyskeratotic cells which were prominent during neoplastic changes get deeply stained with CVL. The overall staining pattern of CVL in well differentiat-









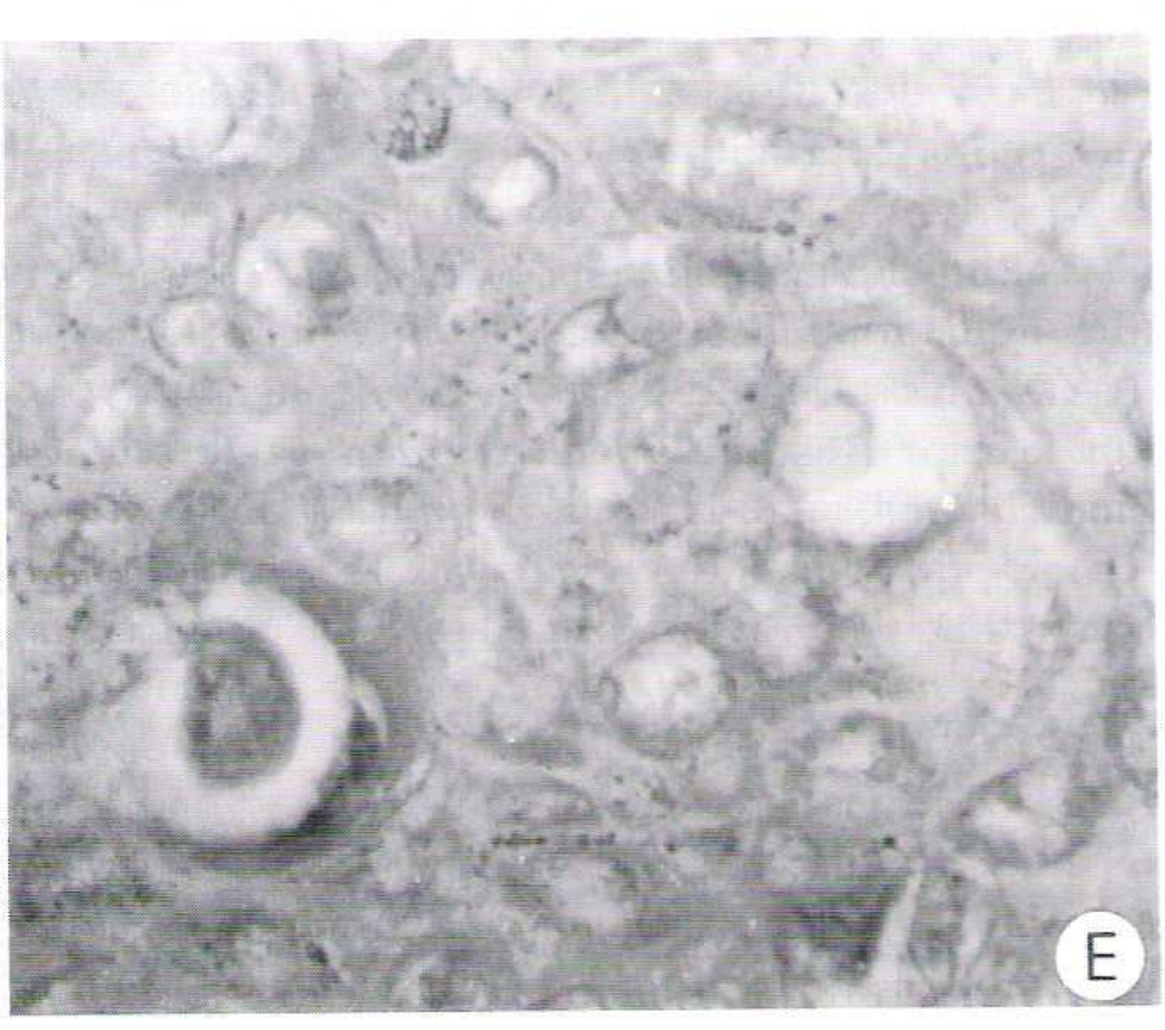


Fig. - (A) Oral leukoplakia tissue stained with CVL - Irregular with variations in intensity of binding (CLV×400).
(B) Moderately differentiated squamous cell carcinoma irregular cytoplasmic binding, weak binding in basal cells (arrow) and intense binding in superficial and acanthotica layers (CVL×400).
(C) Well differentiated squamous cell carcinoma - Intense finding in keratin pearls (XVL×250).
(D) Normal tissue stained with SBA - Uniform binding in intercellular regions (SBA×1000).
(E) Squamous cell carcinoma stained with PNA - Intense binding in intercellular areas and cytoplasm of keratinized cells (PNA×1000).

Table II - CVL binding pattern in oral tissue

Tissue	Cytoplasm	Intercellular area	Keratinized area	Cells at basement membrane
Normal (n=7)	Uniform +(7/7(	Uniform +(7/7)	Nil	Uniform +(6/7) −(1/7)
Oral leukoplakia (n=8)	Irregular + +(6/8) Uniform + +(2/8)	Uniform +(5/8) −(3/8)	Uniform + +(6/8) Irregular + +(2/8)	Uniform +(5/8) −(3/8)
Poorly differentiated squamous cell carcinoma (n=8)	Irregular + +(7/8) −(1/8)	Irregular +(6/8) Uniform +(2/8)	Irregular + +(8/8)	Uniform +(4/8) −(4/8)
Moderately differentiated squamous cell carcinoma (n=8)	Irregular + + +(3/8) + +(4/8) −(1/8)	Irregular + +(2/8) +(4/8) −(2/8)	Irregular + + +(6/8) + +(2/8)	Uniform + +(4/8) +(2/8) −(2/8)
Well differentiated squamous cell carcinoma (n=10)	Irregular + + +(5/10) + +(3/10) +(2/10)	Irregular + +(4/10) +(6/10)	Irregular + + +(8/10) + +(2/10)	Uniform + + +/ +(7/10) −(3/10)

ed squamous cell carcinoma did not differ from the pattern described in moderately, or poorly differentiated tumors.

The reactivity of PNA, SBA and BSA-1 was not resembled with CVL but similar heterogeneous reactivity was observed with carcinoma cells (Fig. 1E). Moreover, it is to be noted that despite having a common sugar for PNA, SBA and BSA-1, more intense reactivity of BSA-1 was observed in the intercellular areas of normal tissue and a loss of cytoplasmic reaction with SBA in normal tissue (Fig. 1D), which suggests that there may be differences in the binding sites of these lectins. Semiquantitative differences in the intensity of histochemical reactions in cytoplasm could also be detected with these probes between different subtypes of malignancies. A loss of binding property with PNA, SBA and BSA-1 was observed in poorly differentiated carcinoma. Carcinoma cells with poor differentiation may be associated with further oligosaccharide changes. Similar observation was obtained in oral chemical carcinogenesis (10) and irradiated carcinomas (14). The combination of the lectins CVL, PNA, SBA and BSA-1 employed in this study may be used as an aid to the early detection of histological evidence and to the dysplastic grading of oral epithelial malignancy.

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