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Jack Fruit Lectin Binding Pattern in Carcinoma of the Uterine Cervix

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Abstract

A lectin was isolated and purified from the seeds of Jack Fruit (Artocarpus integrifolia) using a column of immobilized N-acetyl D-Galactosamine. The Jack Fruit lectin (JFL) was conjugated to horse radish peroxidase (HRP). The purified conjugate was used to study the binding properties of tissues from carcinomas of the uterine cervix. The binding to cancer tissues was compared with that of normal controls. The carcinomatous cells showed varying degrees of binding towards JFL as controls to normal controls which generally had uniform binding. The nature and intensity of binding of the lectin with the cancer tissues suggest that this lectin may be used as a diagnostic marker in carcinoma of uterine cervix.

Introduction

Lectins are carbohydrate-binding proteins or glycoproteins of non-immune origin that can precipitate glyco-conjugates and/or agglutinate cells (1,2). Due to these properties, lectins are widely used in various biochemical and cellular studies (3-5). Individual lectin is bound to visualants like fluorescent dyes,

enzymes, electron dense substances, peroxidase, radio-iodine, etc., have been used as histochemical markers to identify and locate specific carbohydrate residues (6-8).

Walker (9) demonstrated the selective staining of malignant cell by peroxidase labelled Peanut lectin. Gonzalez Campora et al (10) and Sobrinho-Simoes and Denjanov (11) employs Ulex europaeus agglutinin (UEA-1) for the diagnosis of follicular carcinoma of the thyroid. Fukutomi et al (12) used lectin from Helix pomatia (HPL) and UEA for histochemical staining of breast carcinomas.

Cervical cancer constitutes about 14% of the total cancer incidence in Kerala, South India (13). Many of the patients come to the hospital for treatment at an advanced stage. Any method for the earlier diagnosis of the disease would make the treatment more effective. It was reported that peroxidase labelled Con-A labelling could be considered as a supplementary technique for the early detection of cervical cancers (8,14-16). We have successfully employed JFL as a histochemical marker for the differential diagnosis of premalignant and malignant lesions of the oral cavity an breast (17,18). These studies prompted us to evaluate the use of JFL as a histochemical marker in carcinomas of the uterine cervix.

Materials and Methods

Twenty squamous cell carcinomas, 20 verrucous carcinomas of the uterine cervix, and the cervical tissue of 10 normal patients were examined. Wax-embedded tissues were cut into 3-5 μ slices, dewaxed using 3 changes of xylene, and rehydrated with graded alcohol. The JFL was prepared as reported earlier by Vijayakumar and Forrester (19). The lectin was conjugated to HRP type VI and used for staining as described by Vijayakumar et al (20) with diaminobenzidine dihydrochloride (DAB) as a substrate. After staining, the slides were mounted and examined under a light

Table - I

Jack Fruit Lectin Binding Patterns in Carcinoma of Uterine Cervix

Tissue	Cytoplasm	Intracellular area	Keratinized area	Cells at basement membrane
Normal (n=10)	Uniform +(10/10)	Uniform ++(10/10)	Nil	Diffuse
Verrucous carcinoma (n=20)	Irregular ++(10/20) +(10/20)	Irregular ++(15/20) +(5/20)	Irregular +++(16/20) ++(4/20)	Uniform +/++(15/20) -(5/20)
Squamous cell carcinoma (n=20)	Irregular +++(10/20) ++(10/20)	Irregular +/++(10/20) -(10/20)	Irregular +++(15/20) ++(5/20)	Focal +/++(15/20) -(5/20)

\- = No staining
\+ = Weak staining
++ = Strong staining
+++ = Strong intense staining

microscope. Depending on the intensity of staining, slides were graded + to +++. The HRP was purchased from Sigma Chemical Company, USA and DAB from BDH, England.

Results

The results of the JFL binding in cervical tissues are presented in Table-I and Figures 1-5. The neoplastic cells showed increased affinity towards the JFL. The normal cells on the other hand had uniform binding in the cytoplasm. The cells at the basement membrane shoed diffused binding. In the case of neoplastic cells, the binding was irregular; keratinized area showed intense irregular binding. Twenty-four percent of the cells of the basement membrane did not show any staining in carcinomas. In 50 percent of the squamous cell carcinomas in intracellular area showed irregular binding. The binding in the keratinized area seemed to be the major difference between the normal and neoplastic tissues.

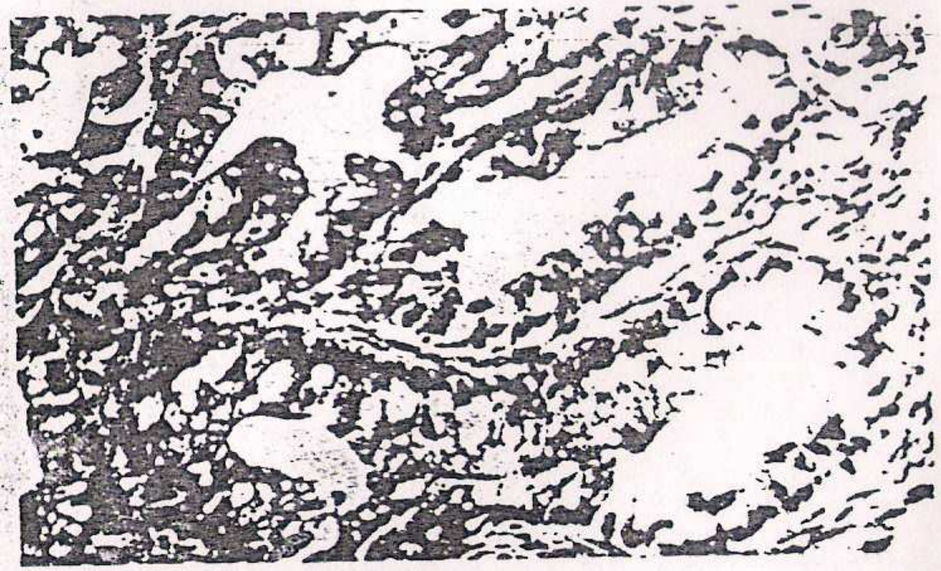

Fig. 1 Normal endocervical glands

Discussion

The JFL is an N-acetyl D-galactosamine specific, non-glycosylated protein, isolated from the seeds of Jack fruit. Peroxidase-conjugated JFL has been successfully employed as a histochemical marker (17,18,20). Using a crude preparation of JFL, Vijayan et al (21) had shown that this lectin may be of use as a diagnostic marker in exfoliative cytology.

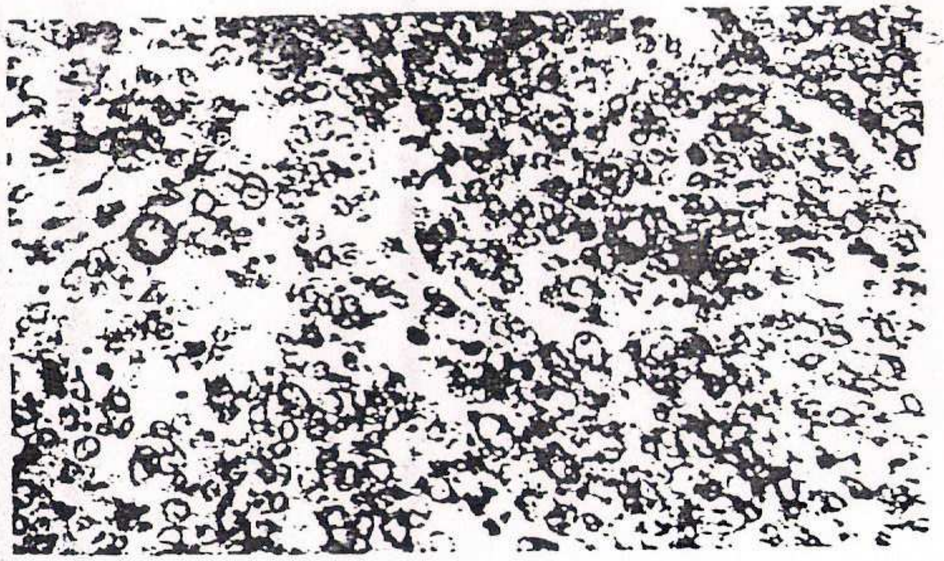

Fig. 2 Poorly differentiated squamous cell carcinoma showing irregular intense binding.

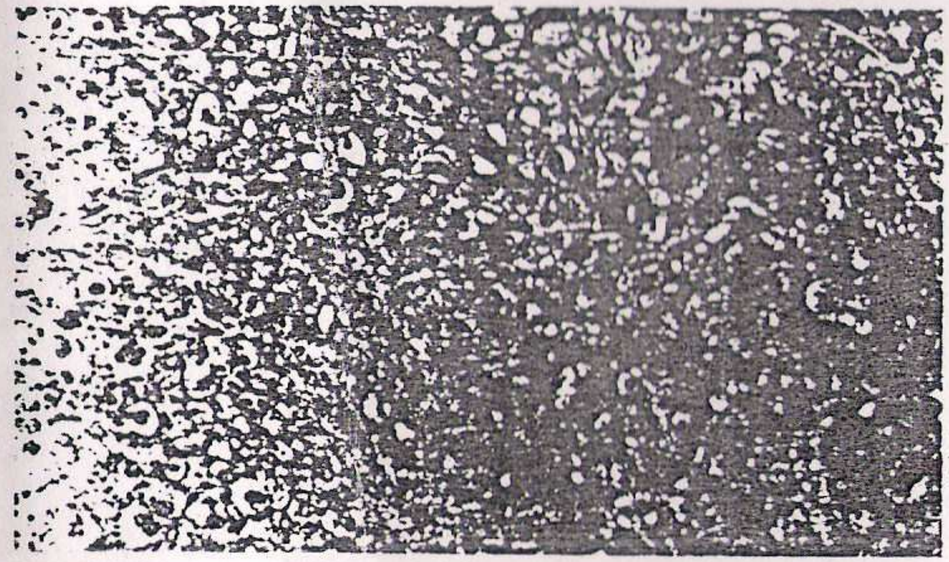

Fig. 3 Moderately differentiated squamous cell carcinoma. Large polymorphic cells showing intense cytoplasmic binding.

In the present study the affinity purified JFL, conjugated to HRP, was employs to study the binding pattern in histopathologically proved tissues form carcinomas of the uterine cervix. The result shows that the binding pattern of JFL in normal tissues is different from that of malignant cells of the uterine cervix. The difference in the binding may be due to the

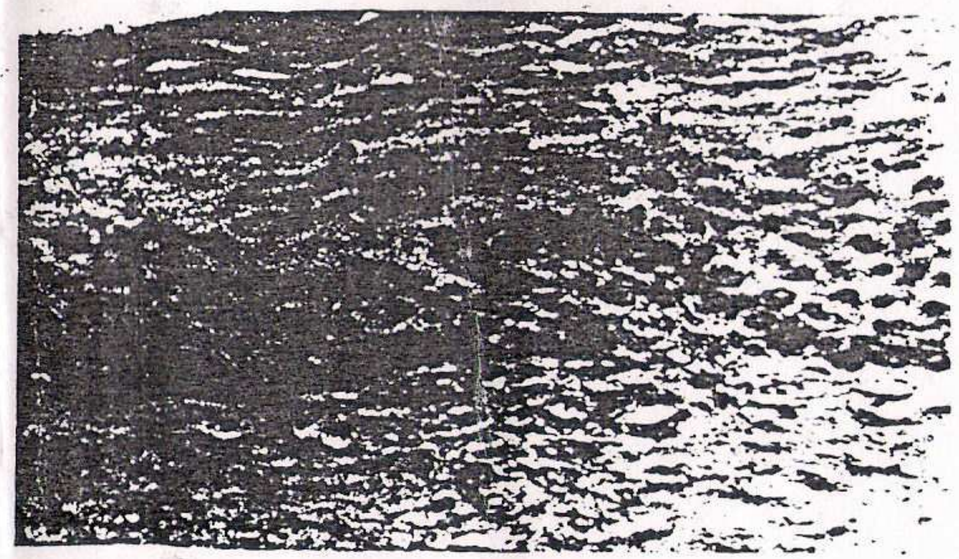

Fig. 4 Squamous epithelium showing parakeratosis. Intense lectin binding in keratinized areas.

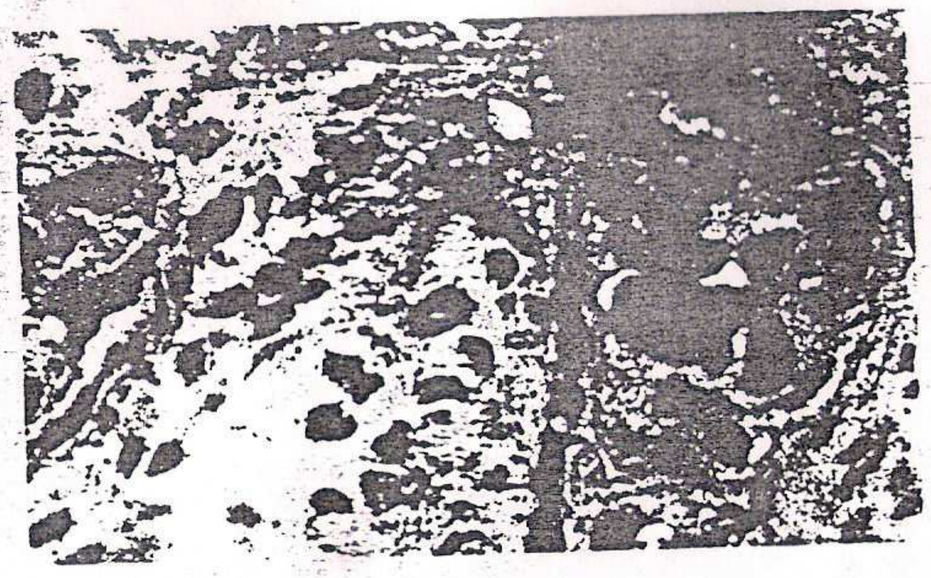

Fig. 5 Moderately differentiated squamous cell carcinoma. Intense cytoplasmic binding in the miotic cells.

alteration of the normal cells during the malignant transformation in the uterine cervix (14). The nonsialated forms of membrane glycoconjugates may appear on the surface of cells during malignant transformation (22), and the JFL may identify these glyconjugates, a possible reason for the difference in the binding.

The lectin binding method is based in 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 (8). In the present study 2 cases of carcinomata in situ showed binding similar to that shown by the normal tissues. Since only two cases were available we are unable to comment on it; but, it seems probable that JFL binding also depends on the amount of oligosaccharides on the cell surfaces, a hypothesis in agreement with the finding of Ross et al (22). The JFL is a stable lectin which can be easily separated and conjugated to any marker. This makes the lectin a useful histochemical reagent.

Acknowledgments

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