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JACK FRUIT LECTIN AS A DIAGNOSTIC MARKER IN CYTOLOGY AND HISTOLOGY

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Abstract: Jack fruit lectin (JFL) was isolated from the seeds of Jack fruit (Artocarpus integrifolia) and purified using an immoblized column of N-acetyl-D-galactosamine. The purified JFL was conjugated to Horseradish-peroxidase (HRP) and the conjugate was used for the cytochemical and histochemical studies of benign, premalignant and malignant lesions of oral cavity, breast, thyroid and uterine cervix using diaminobenzidine (DAB) as substrate. Three hundred and sixty formaldehyde fixed, paraffin embedded tissues and 115 cytological smears from normal, benign, premalignant and malignant lesions of oral cavity, breast, thyroid and uterine cervix were used for the study. Normal cells showed uniform weak binding in the membrane as well as cytoplasm whereas carcinomatous cells showed strong binding towads JFL in contrast to normal cells. Dysplastic cells showed difference in binding in mild, moderate and severe dysplasia. The intensity fo binding was increased with the severity of the dysplasia. The nature and intensity of binding of Jack fruit lectin with cancer tissues suggest that this lectin may be used as a diagnostic marker in neoplasia.

1. Introduction

Lectins, a group of proteins found in a wide variety of plants, animals and microorganisms has recently moved to the forefront of biologic research. The basis of their use as reagents in pathology and cell biology is their ability to recognise complex carbohydrate structures in glycoprotein and glycolipids, in particular those of cell membranes.

Lectins have been used frequently to detect abnormal glycoconjugates as markers of malignancy with a diagnostic relevance for tumour biochemistry and cancer therapy (Calafat and Jansen, 1983). Binding of lectins to normal, benign and cancerous tissues of oral cavity, breast, thyroid and cervix have been studied by various workers (Vigneswaran et al 1988, Sen. 1989, Fukutomi et al. 1989, Sobreno-Simoes & Damjanov 1986).

Jack fruit lectin has been reported to be of use in the study of cell membrane in tissues of murine and human origin (Remani et al, 1990). In this study we used Horse radish peroxidase (HRP) conjugated Jack Fruit Lectin (JFL) to study the distribution of lectin receptors in normal, benign and malignant tissues of oral cavity, breast, thyroid and uterine cervix.

2 Materials and Methods

Three hundred and sixty formaldehyde fixed, paraffin embedded tissues and 115 cytological smears from normal benign,

Table 1. JFL Binding pattern in oral tissues

Tissue	Cytoplasm	Intracellular area	Keratinized area	Cells at base-ment membrane
Normal (n=10) Oral Leukoplakia (n=20)	Uniform + (10/10) Uniform + (20/20)	Uniform ++ (10/10) Uniform ++ (14/20) + (6/20)	Uniform ++ (10/10) Uniform ++ (12/20) Irregular ++ (8/20)	Diffuse (10/10) Uniform ++ (12/20) Irregular ++ (8/20)
Oral submucous fibrosis (n=20)	Uniform ++ (20/20)	Uniform +++ (15/20) ++ (5/20)	Uniform ++ (16/20) Irregular +++ (4/20)	Uniform ++ (20/20)
Squamous cell carcinoma (n=25) Verrucous carcinoma (n=15)	Irregular +++ (19/25) ++ (6/25) Focal ++ (15/15)	Irregular +++ (20/25) ++ (5/25) Irregular ++ (15/15)	Irregular +++ (17/25) ++ (8/25) Irregular ++ (15/15)	Focal +++ (20/25) ++ 5/25) Uniform ++ (15/15)

Table 2. JFL Binding patterns in breast tissues

Type of tissues	Carcinomatous cells		Epithelial cells		Stromal cells	Apocrine cells
	Membrane	Cytoplasm	Membrane	Cytoplasm		
Normal (n=10)	-	-	++ (6/10) + (4/10)	++ (7/10) + (3/10)	+++ (8/10) ++ (2/10)	+++ (8/10) ++ (2/10)
Fibroadenoma (n=15)	-	-	++ (10/15) + (5/15)	++ (8/15) + (7/15)	++ (7/15) + (8/15)	++ (7/15) + (8/15)
Lactating breast (n=15)	-	-	+++ (12/15) ++ (3/15) +/- (3/20	+++ (11/15) ++ (4/15) +/- (3/20)	+++ (9/15) ++ (6/15)	+++ (8/15) ++ (7/15)
Infiltrating duct Carcinoma (n=25)	+++ (18/25) ++ (7/25)	+++ (14/25) ++ (9/25)	++ (11/25) + (14/25) - (2/25)	++(2/25) + (5/25) - (18/25)	+++ (8/25 ++ (4/25) + (13/25)	+++ (9/25) ++ (5/25) + (11/25)
Lobular carcinoma (n=20)	+++ (12/20) ++ (8/20)	+++ (16/20) ++ (4/20)	++ (8/20) + (12/20)	++ (2/20) ++ (4/20) - (14/20)	+++ (7/20) ++ (5/20) + (8/20)	+++ (8/20) ++ (6/20) + (6/20)

Table 3. JFL Binding pattern in cervical tissues

Histology

Tissue	Cytoplasm	Intracellular area	Keratinized area	Cells at basement membrane
Normal (n=15)	Uniform + (15/15)	Uniform ++ (10/15) + (5/15)	Uniform + (15/15)	Diffuse + (15/15)
Carcinoma in situ (n=10)	Irregular +++ (5/10) ++ (5/10)	Irregular +++ (6/10) ++ (4/10)	Irregular +++ (5/10) ++ (5/10)	Focal ++ (6/10) + (4/10)
Squamous cell carcinoma (n=30)	Irregular +++ (24/30) ++ (6/30)	Irregular +++ (18/30) ++ (8/30) + (4/30)	Irregular +++ (20/30) ++ (10/30)	Focal ++ (15/30) + (8/30) - (7/30)
Verrucous carcinoma (n=20)	Focal ++ (12/20) + (8/20)	Irregular ++ (14/20) + (6/20)	Irregular ++ (15/20) + (5/20)	Uniform ++ (8/20) + (7/20) - (5/20)

premalignant and malignant lesions of oral cavity, breast, thyroid and uterine cervix were used for the study.

JFL was isolated, purified and conjugated with horse radish peroxidase (HRP) type VI and the purified conjugate was used for the cytohistochemical studies as described by Vijayakumar et al (1987) using diaminobenzidine dihydrochloride (DAB) as substrate. After staining, the slides were mounted and observed under light microscope. Depending upon the intensity of staining the slides were graded + to +++.

3. Results and Discussion

The results of the JFL binding in histology specimens of normal, benign, premalignant and malignant tissues from oral cavity, breast, thyroid and uterine cervix are given in Table I to IV. The results of the cytological smears are given in Table V.

Neoplastic cells showed increased affinity to the JFL. In normal oral tissues the binding was weak and uniform. In oral leukoplakia and oral submucous fibrosis (OSMF) the binding was uniform in the cytoplasm as well as intercellular area. In squamous

Table 4. Lectin binding in different thyroid tissues

Tissues	Jack fruit lectin		
	N	P	I
Normal Thyroid (n=10)	++++	D/F	+
Multinodular goitre (n=10)	+++	D/F	+ to ++
Hashimotos Thyroiditis (n=5)	++++	D	++
Atrophic follicles (n=5)	+to++	D/F	+to++
Follicular adenoma (n=20)	++++	D/F	+to++
Follicular Carcinoma (n=20)	++++	D/F	+++
Papillary carcinoma (n=10)	++++	D/L	+++
Medullary carcinoma (n=10)	++++	D	+++

D - Diffuse cytoplasmic binding
F - Focal cytoplasmic binding
I - Intensity of binding
L - Luminal binding
P - Pattern of binding
N - Number of cells stained

cell carcinoma of the oral cavity all the cells were intensely stained and the binding pattern was irregular. Another important observation was that four of the oral submucous fibrosis cases showed an irregular binding pattern similar to that obsrved in squamous cell carcinoma. Two of them turned into malignancy after two years.

In breast tissues JFL showed significant difference in binding patterns and staining intensities in benign and malignant tissues. In benign lesion the lectin binding was weak and was detected predominantly on the luminal surface of the mammary epithelium but in malignant tumours strong binding was observed in both membrane and cytoplasm.

The binding of JFL in thyroid tissues also showed difference in normal, benign and malignant tissues. Follicular carcinoma cells showed more intense staining than follicular adenoma cells.

In cervical tissues the binding patterns was similar to that observed in oral squamous cells. The binding was intense and irregualr in carcinomatous cells. The normal cervical cells showed uniform weak binding.

The cytology specimens showed significant difference in staining in normal, dysplastic and malignant tissues. Carcinomatous cells showed intense membrane as well as cytoplasmic binding whereas normal cells showed weak binding. Dysplastic cells showed difference in binding in mild, moderate and severe dysplasia. The intensity of binding was increased with the severity of the dysplasia. Carcinoma in situ cells also showed same binding pattern as in the case of carcinoma.

The ability of plant lectines to bind carbohydrate has been well documented (Barry and Goldstein, 1979). The peroxidase labelled lectins have proved to be a useful tool to visualize the presence of lectin receptors in routine pathology specimens (Davina et al, 1985).

Paymaster (1956) observed transformation of OSMF into squamous cell carcinoma of the oral cavity in one third of his patients. Rajendran et al (1986) have reported that OSMF can be an intermediary stage in the transformation process of normal cell into malignancy. In our study, four out of twenty OSMF tissues showed a binding pattern similar to that of squamous cell carcinoma towards JFL. The similarity in the binding pattern shown by these four cases of OSMF is strongly suggestive of their malignant potentiality as is emphasized by the fact that two of these four later developed into frank carcinoma.

In breast lesions the results showed that the binding is different in benign and malignant lesions. Walker (1985)

Table 5. JFL binding pattern in cytological smears

Tissue	Cytoplasm	Membrane
Uterine Cervix		
Normal	- (8/15)	- (4/15)
Squamous cells (n=15)	+ (7/15)	+ (11/15)
Dysplastic Squamous cells	+ (14/16)	+ (12/16)
(i) Mild (n=16)	++ (2/16)	++ (4/16)
(ii) Moderate (n=12)	+ (5/12)	+ (4/12)
	++ (7/12)	++ (8/12)
(iii) Severe (n=7)	++ (5/7)	++ (3/7)
	+++ (2/7)	+++ (4/7)
Epidermoid Carcinoma In situ (n=10)	++ (4/10)	++ (3/10)
	+++ (6/10)	+++ (7/10)
Invasive squamous Cell carcinoma	++ (5/20)	++ (4/20)
	+++ (15/20)	+++ (16/20)
Oral Cavity		
Normal squamous cells (n=5)	+ (5/5)	+ (5/5)
Carcinoma cells	+++ (5/5)	+++ (5/5)
Breast		
Normal/Benign (n=10)	-/+ (10/10)	-/+ (10/10)
Carcinoma (n=5)	+++ (5/5)	+++ (5/5)
Thyroid		
Normal/Benign (n=5)	+ (5/5)	+ (5/5)
Carcinoma cells (n=5)	+++ (5/5)	+++ (5/5)

demonstrated the selective staining of breast carcinoma cells by plant lectins. A few studies have indicated that lectin binding can provide prognostic information on breat cancer (Fenlon et al 1987), although in another study their use as prognostic markers appears to be limited. In this study benign cells showed luminal binding while carcinoma cells showed intense irregular cytoplasmic and membrane binding. From this study it seems that the JFL may be of use in distinguishing carcinomatous tissues from benign tumour tissues.

The JFL showed difference in binding pattern in normal benign and malignant thyroid tissues. This study is in agreement with that of Sasano et al (1989) who observed similar binding characteristics of thyroid tissues using lectins from peanut, Helix pomatia and Ulex europaeus.

The results of the JFL staining in cytology specimens showed that the binding pattern of JFL in normal tissue is different from that of malignant tissues. The difference in the binding may be due to the alternation during malignant transformation (Davina et al, 1985). JFL showed difference in the binding pattern in severe dysplasia and carcinoma in situ. These results indicate that a more optimal discrimination is possible and reflect the occurrence of an essential alteration during the transformation from severe dysplasia to carcinoma in situ (Copplesson and Brown 1981).

Thus, Jack fruit lectin exploits a more fundamental difference between benign and malignant lesions and will be better able to predict the malignant potential of suspected lesions. The ready availability, the ease of preparation in purified form and the fact that the lectin can be conjugated to diagnostic markers makes the JFL a potential reagent in cytology and histology.

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