DIFFERENTIAL ERYTHROCYTE AGGLUTINATION PATTERN IN NORMAL AND CANCER PATIENTS WITH SYNADENIUM GRANTII ROOT (HOOK F) LECTIN

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ABSTRACT

In the present study the property of lectin agglutination in blood on normal and different cancer patients has been observed. The purified *Synadenium grantii* root lectin was non blood group specific and its utility as a diagnostic tool in malignancy was studied. Hemagglutination (units/ml) of red blood cells of different types of cancer was compared with the normal control's red blood cells. Out of 113 total cancer patients, only a group of 29 breast cancer patients showed significant increase in titre value ($P < 0.05$) compared to normal control.

KEY WORDS : Lectin, Red cell membrane, Cancer Hemagglutination.

INTRODUCTION

Lectins are proteins or glycoproteins that bind specifically to saccharide moieties in glycoproteins or glycolipids in the same cell surface without modifying it chemically. Several studies have documented that particular carbohydrate residues on primary cancers are associated with metastasis. Identification of such residues is possible using lectins that includes helix pomatia agglutination (HPA) and Phaseolus vulgaris leucoagglutinin (PHA-L) (1). Both lectins have been reported to be valuable prognostic markers in breast & colon cancers and their the binding patterns of human breast cancer have been studied (2). All the above mentioned approaches for cancer diagnosis and management involve invasive and painful procedures. Therefore a simple and noninvasive blood based marker is required for early diagnosis as well as for monitoring progress of treatment. Hence simple hemagglutination titre test is employed in biological and biomedical research. Since hemagglutination is reversible and RBCs have more than one specific carbohydrate binding site various lectins can be used (2,3). Hence to test the utility of *S. grantii* root lectin, we have designed our preliminary study.

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Nagda and Morde (4) have shown increase in haemagglutination titre in patients with GI tract cancer as compared to control using *Pedilanthus titthy morloides* lectin. Erythrocyte membrane alterations in cancerous condition have been studied using different lectins (7).

To the best of the authors' knowledge no other study had been carried out of *S. grantii* root lectin except one on *S. grantii* latex lectin for mitogenic activity of lymphocytes (7).

MATERIALS AND METHODS

Preparation of Lectin

S. grantii root lectin purified on affinity chromatography columns (7) was used for present study. The root lectin has no absolute blood group specificity but preferentially binds with 'O' erythrocytes.

Preparation of red blood cell suspension

The blood samples of 60 controls and 113 study group were collected in potassium oxalate bulbs. Red blood cell (RBCs) were centrifuged and washed thrice with excess of phosphate buffered saline (PBS). The packed red cells were resuspended in PBS to prepare 5% suspension.

Hemagglutination Test

Frozen lectin was thawed at room temperature just before the experiment. Hundred microlitres erythrocyte suspension was added to equal volume of various concentrations of (different titre) lectin in a porcelain tile cavity. The reaction was observed after 30 minutes with naked eyes and microscope was used to confirm the doubtful cases. Hemagglutination pattern was scored from +1 to +4 according to Marsh (8), and +1 agglutination titre was noted to calculate units/ml.

Statistical analysis

The hemagglutination activity of normal subjects and various types of cancer cases is compared by students 't' test, the univariate analysis. The difference in control and cases was considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

The blood samples from 113 study subjects with different types of malignancies and 60 normal healthy controls were categorized into following groups —

cancer of (i) breast (29), (ii) maxillia (22), (iii) lung (110), (iv) cervix (10), (v) cheek (8), (vi) rectum (8), (vii) stomach (6), (viii) thyroid (6), (ix) tongue (5), (x) Larynx (4), (xi) male genital organ (4). The diagnosis of the above mentioned malignant cases was made by the oncologist and with necessary clinical laboratory investigations. The age and sex distribution of control group and the study group was matched.

Table 1 Depicts the hemagglutination activity units (HAU) shown by the control group and cancer patients. It is clear that the hemagglutination activity units was found to be significantly increased in cancer breast cases ($P < 0.001$). However in the other types of cancer patients this activity was not significantly increased. Differences in lectin agglutination between normal control red blood cells and cancerous condition were reported earlier (9). The differences or alterations are on the molecular basis. Although mechanism for these alterations is not yet understood, it was suggested that altered glycosyltransferases or glycoprotein acceptors might be responsible for the changes observed on the erythrocyte surface. In case of cancer breast cases there may be increase in galactose epitopes as

Table 1. Distribution of cases as per type of cancer with hemagglutination activity

Cases	No.	Mean $\pm$ S.D.	Range of hemagglutination activity (minimum and maximum) units/ml	Statistical comparison ϵ control v/s cancer
Control (Normal)	60	188.00 $\pm$ 199.96	30 — 480	
Cancer cases				
Breast	29	300.7 $\pm$ 293.7	80-480	2.13*
Maxilla	22	199.6 $\pm$ 188.9	80-320	0.24
Lung	11	228.5 $\pm$ 195.6	80-480	0.62
Cervix	10	240.0 $\pm$ 160.0	80-480	0.78
Chick	08	264.0 $\pm$ 200.2	1-640	1.01
Rectum	08	150.0 $\pm$ 66.7	80-240	0.53
Stomach	06	213.0 $\pm$ 211.2	80-640	0.29
Thyroid	06	246.6 $\pm$ 89.1	120-320	0.71
Tongue	05	216.0 $\pm$ 180.2	40-480	0.3
Larynx	04	250.0 $\pm$ 94.5	120-320	0.61
Male genital	04	320.0 $\pm$ 113.6	160-480	1.3

ϵ Student's 't' test value

* $P < 0.05$ i.e. significant

compared to other study group individuals.

Though the difference between HAU due to lectin in normal and cancer breast cases was significant, the observations are overlapping. So taking this into account, 95% confidence limit for normal subjects was calculated by taking $\text{mean} \pm 1.96$ standard error, which was 137.4 to 238.6 units /ml and more than 50% HA values showed their trend clustering towards the lower bound of normality range (95%) confidence limit. However, HA values of majority of the cases were around and above the upper bound of normality range (Table 2). The sensitivity and specificity of hemagglutination titre with *S. grantii* root lectin with cut off value of 238.6 upper bound of 95% confidence limits was 58.6% & 63.3% respectively . It is indicative of increased sugar receptors on the surface. Glycosylation variations are observed in different stages of cancer. It was found that the proportion of such subjects was almost doubled (48.3%) in study

Table 2. Hemagglutination activity (units/ml) and 95% confidence limit wise distribution of control group and breast cancer cases

	Normal n (%)	Cases n (%)
Below Upper Bound	38 (63.33)	12 (41.38)
Above Upper Bound	22 (36.67)	17 (58.62)

group compared to control (25.0%).

Studies associated with cell membrane changes and lectin agglutination showed that binding sites differ in different stages (10). In our study although cases at different stages were present, stage wise analysis was not carried out. Whether this microheterogeneity at cell surface is the basis of effective means of defence or simply another manifestation of disease still remains to be determined. Hence *S. grantii* root lectin along with other lectins with different specificities may help in stage wise diagnosis of breast cancer.

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