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Clinical utility of the interaction between lectin and serum prostate specific antigen in prostate cancer

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The present investigation was a lectin-based diagnosis of malignant prostate cancer (PC) by the interaction of phytohemagglutinin (PHA lectin) from *Phaseolus vulgaris* with the glycan part of serum prostate specific antigen (PSA) of patients with prostatic disorder. This was confirmed by the interaction between PHA and purified PSA obtained from serum by electrophoretic separation and finally by HPLC chromatography. The precipitate of carbohydrate content after binding of PHA with purified PSA of PC was significantly higher than that of benign prostate hyperplasia (BPH) and/or normal serum PSA. The results suggest that there may be a striking difference in glycosylation pattern of PSA between BPH and PC. The cut off value ≥ 10 μg/ml of the carbohydrate content of PHA-PSA precipitate indicates strong suspicion for PC irrespective of total serum PSA cut off level ≥ 4.0ng/ml by conventional immunoassay method and this may be taken as a guideline in differentiating PC and BPH.

Key words: prostate cancer, BPH, PSA, lectin.

The interest of the sugar specific proteins, lectins, have greatly intensified with the realization that they react with a variety of glycoproteins [1, 2]. The interaction takes place between the glycan moiety of glycoproteins and the carbohydrate binding receptors of lectins. The reactions of biomedical glycoprotein markers with lectins were studied as valuable tools for clinical diagnosis [3]. It was observed that PHA lectin (*Phaseolus vulgaris*), reacts with different serum proteins and glycoproteins [4]. Estimation of serum prostate specific antigen (PSA) for detection of prostate cancer (PC) is determined by the sensitive immunoassay method. A low serum PSA cut-off level of 4.0 ng/ml is used during screening procedure to detect PC at an early stage but an appreciable risk of false positive results was observed with this low cut off value resulting in unnecessary biopsies for those with BPH [5]. Some serum PSA samples of patients with clinically proven benign prostate hyperplasia (BPH) showed higher value than 4ng/ml [6] and a few histologically proven PC patients indicated normal serum PSA level [7] in immunoassay method. The techniques currently used in immunodetection of serum PSA concentration are of limited clinical value in the early detection of PC and its distinction from BPH had already been reported by us [8]. PSA is known to be a glycoprotein and preliminary observations indicate that PSA binds with PHA.Further,a few studies have discussed the changes in sugar-chain structure of PSA associated with malignant transformation [9].Therefore, the present investigation was designed to quick identification of suspected PC by interaction of PHA lectin with the glycan part of serum PSA glycoprotein and differentiation of PC from BPH.

Patients and methods

Chemicals. PHA lectin (Phaseolus vulgaris) was purchased from Sigma Chemical Co. All other chemicals were of analytical grade.

Samples. Serum samples of 22 male histologically proven prostate cancer (PC) patients (age between 42-75 yr) and 24 benign prostatic hyperplasia (BPH)(age between 40-70yr) were collected from Urology Clinic of B.R.Sing Railway

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Hospital, Govt. of India, Kolkata and Nightingale Hospital, Kolkata-71.Patients underwent digital rectal examination, transrectal ultrasound, and transrectal ultrasound-guided biopsy of the prostate. 18 healthy elderly male subjects (age between 45-75 yr) without any complication were included in this investigation as normal control. Serum from 10 normal female subjects (age 40-65 yr) was also collected from the hospital as control. Venous blood samples for PSA measurements were drawn before rectal examination or instrumentation and stored at -20° C until assays were performed. Concentration of serum PSA in normal, BPH and PC were assayed by Enzyme Immunoassay (EIA) method in the hospital.

Purification of PSA. The clear serum samples of healthy male, PC and BPH patients were taken separately for 60% $(NH)_4SO_4$ cut. The precipitate obtained in each case after overnight store at 4 C was centrifuged at 15000 rpm for 10 minutes and the precipitate was dissolved in minimum volume of phosphate buffered saline, pH 7.2 (PBS). The sample thus obtained was applied to 7.5% polyacrylamide gel electrophoresis (PAGE) [10] several times to collect a good amount of PSA in a particular type of band. Different bands obtained in the gel were collected by cutting the gel and dissolved in PBS. All the extracts were centrifuged at 10,000 rpm for 10 minutes to get clear supernatants and concentrated by freeze drying. The PSA in a particular band extract was identified by EIA test as described earlier [8]. The extract that contained PSA was applied to HPLC for further purification in 20mMsodium phosphate buffer at pH 7.5.

Hemagglutination test. The assay was performed by serial dilution technique by using 2% washed rabbit RBC suspension in PBS as described in our earlier publication [11]. The agglutination was assessed after 60- minute incubation at 37 C. The activity was expressed as the titre, the reciprocal of the greatest dilution at which agglutination could be detected. The inhibition by PSA in serial dilution was done by the preincubation with PHA lectin (50µg protein) for 15 minutes and then incubation was done with RBC (2%) for 60 minute at 37°C.

Precipitation test. Precipitaion reactions of 0.30ml PHA (2mg/ml) with purified PSA (10ng/ml) of different conditions and 0.50ml serum samples were performed as described by So and Goldstein [12] in presence of PBS. To correct the value for precipitation between PHA and other glycoproteins present in serum, normal female serum devoid of PSA, omit this printed line was kept as blank only. The reaction mixture was incubated at 0° C for 60 minute. The reaction mixtures after incubation were centrifuged at 5000rpm to sediment the precipitate and it was washed by PBS. Tubes, after removing the supernatant, were kept upside down on the filter paper to absorb the last drop. The precipitate was estimated spectrophotometrically at 480nm in terms of carbohydrate by the method of Dubois et al [13] using mannose as standard.

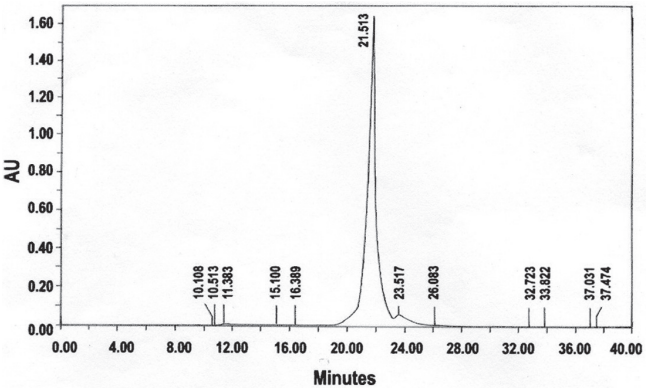

Figure 1. HPLC chromatography of electrophoretically separated serum PSA. Column was protein PAK I-125. Flow rate 0,5 ml/min. The column effluent was monitored at 280 nm.

Results and Discussion

The purified PSA of three different types of serum samples (normal, BPH and PC) were obtained by $(NH_4)_2SO_4$ fractionation, electrophoretic separation and finally by HPLC column chromatography. All three purified PSA samples thus obtained were identified by Enzyme Immunoassay (EIA) method. The peak pattern (Figure 1) was same in three cases.

Table 1presents the result of hemagglutination test conducted on 2% rabbit RBC by PHA lectin. The agglutination was inhibited by the minimum concentration of purified PSA protein. This confirms that glycan part of PSA binds with PHA lectin receptor. Further it was observed that minimum inhibitory concentration of PSA in PC patient was less than those of normal and BPH patients. This may be due to altered structure of PSA in which binding sugar of glycan part was more exposed than other two types of PSA samples. The mean carbohydrate content in the precipitate for interaction of PHA with total serum glycoproteins and serum PSA was shown in Table 2. PHA can form precipitate with other serum glycoproteins. Total serum PSA in normal female serum determined by EIA was negligible (0.07±0.02ng/ml) but other glycoproteins were same with male. So normal female serum which is almost devoid of PSA was taken

Table 1. Inhibition of PHA lectin activity by the purified serum PSA protein in different systems

Systems	Minimum conc. of means PSA-protein inhibition of agglutination (µg)
Control	nil
Control + N-PSA*	3.2
Control + BPH-PSA	3.5
Control + PC-PSA	1.2

* N-PSA – Normal PSA. Control system contains only PHA and 2% washed rabbit RBC. Method is given in the text.

Table 2. Mean carbohydrate content in the precipitate for interaction of PHA with total serum glycoprotein and serum PSA.

System	Carbohydrate content for PHA-serum glycoprotein binding (μg)/ml	*Carbohydrate content for PHA-serum PSA binding (μg)/ml
Normal female (10)	6.6 ± 1.0 (A)	–
Normal male (18)	10.9 ± 1.5 (B)	4.3 ± 1.4
BPH (24)	11.6 ± 1.2 (C)	5.0 ± 1.8
PC (22)	21.6 ± 1.6 (D)	15.0 ± 1.1

*Carbohydrate contents in PHA and serum-PSA binding for normal male, BPH and PC were obtained by subtracting (B – A), (C – A) & (D – A) respectively. Figure in the parenthesis indicate number of subjects taken.

to correct the actual binding of serum PSA with PHA in normal male, BPH and PC. Thus the value of the carbohydrate content of the precipitate obtained after reaction of female serum glycoprotein with PHA was subtracted from total precipitate formed between PHA and normal male serum, PHA and BPH serum, and PHA and PC serum glycoproteins (table2). It was observed that the mean carbohydrate content (15.0 ± 1.1 μg/ml) in precipitate after binding of serum PSA of PC patient with PHA was significantly higher ($p<0.001$) than those of BPH (5.0±1.8 μg/ml) and normal elderly male (4.3±1.4 μg/ml) but the mean precipitate value of BPH did not show any significant change with normal male(table 2). This was also confirmed when same amount HPLC purified PSA of three different types (BPH,PC and normal) reacted separately with a definite amount of PHA protein. The precipitate obtained was higher with the purified PSA in PC than those of other two types. The amount of carbohydrate contents of normal and BPH remained almost same. The distinct separate binding pattern of PHA in PC and BPH patients may be due to altered post transitional glucosylation of PSA during transformation in malignant form of human prostate [9, 14]. The value of PHA-PSA binding in PC was significantly higher than that of BPH in the present study and it was the reverse of ConA-PSA binding in our earlier report [8]. This may be due to configuration of PSA and lectin. Binding of lectin to the sugar of the glycan chain depends on various factors like linkage position of sugar to the oligosaccharide chain, the number and distribution of receptor site or presence of sugar in the side chain of glycan part [15, 16]. ConA binding specific sugars are α-Man> α-Glc>GlcNAc but sugar specificity of PHA is Galβ1, 4GlcNAcβ1, 2Man [17]. Galactose binding lectin cannot bind all terminals 'Gal' of the oligosaccharide chain.

Binding depends on linkage position of galactose, anomeric configuration and axial or equatorial portion of OH of galactose with which lectin receptor will bind [16]. Suhei et al [9] observed that the sugar moiety structure of PSA increased in PC is a multiantennary complex type and a fraction of this asparagines linked sugar chain structure of seminal fluid binds PHA more than ConA.

The present findings suggest that there is a striking difference in sugar moieties between BPH and PC of PSA. The values shown in table 2 were the mean carbohydrate contents in the precipitate for interaction of PHA with PSA. Among the serum samples of 22 PC patients, the lowest and the highest values were 10 μg/ml and 18 μg/ml carbohydrate contents in the precipitate respectively. So we consider the cut off level of PC patient in terms of carbohydrate content as ≥ 10μg/ml carbohydrate content in the precipitate for binding of PHA with serum PSA may lead to the development of a lectin-based diagnostic tool for the prostate cancer and thus the rate of unnecessary biopsies will be reduced.

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