Evaluation of Lectin Level as New Tumor Marker for Urinary Tract and Prostate Cancers

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Abstract
Lectins, simply; are proteins or glycoproteins that are probably present in all eukaryotic cells, and many bacterial species, as well as in some viruses. Lectins was first discovered as a highly toxic protein that was isolated from castor tree seeds (Ricinus communis) and named ricin, this protein showed the ability to agglutinin erythrocytes. Several lectins are investigated for their use in cancer research and therapy. The present study was designed to detect lectin levels in serum and tissue of patients with urinary tract system and prostate diseases. Using the hemagglutination process, the lectin activity was measured in cases of patients with malignant and benign kidney, bladder, and prostate tumors, in addition to those with non tumoral kidney diseases. Results of the present study showed a significant increase ($p < 0.001$) of lectin levels in patients with malignant tumors when compared with those of benign tumors, non tumoral diseases, and healthy individuals. The highest serum and tissue lectin levels were found in patients with advanced malignant stages, regardless their genders.
Key Words: lectin, kidney, bladder, prostate, hemagglutination, serum, tissue.

الخلاصة
اللكتينات، بشكل مبسط هي بروتينات أو بروتينات سكرية، توجد في جميع الخلايا الحقيقة النواة وفي العديد من الأصناف البكتيرية، بالاظافة إلى عدد من الفيروسات.بدايةً اكتشفت اللكتينات كمركبات بروتينية شديدة السمية تم عزلها من بذور Castor tree وسمي Ricin .أوردت عدد من البحوث السرطانية والعلاجات الدور الحيوي لبعض اللكتينات في حدوث هذا النوع من الأمراض وفي علاجها. صممت الدراسة الحالية للكشف عن مستويات اللكتين في أمصال وأنسجة مرضى اعتلالات القناة البولية والبروستات. تم قياس الفعالية اللكتينة باستخدام طريقة التلازن الدموي في كل من حالات الأورام الخبيثة والحميدة لكل من الكلية و المثانة والبروستات إضافة إلى المصابين بالاعتلالات الكلوية غير الورمية.أظهرت نتائج الدراسة الحالية ارتفاعا معنويا ($p < 0.001$) في مستوى اللكتين لدى المصابين بالأورام الخبيثة مقارنة بمرضى الأورام الحميدة منها وبالاعتلالات الكلوية اللاورمية إضافة إلى الأفراد الأصحاء. وجدت أعلى مستويات اللكتين عند مرضى المراحل المتقدمة من الإصابات السرطانية، بغض النظر عن جنس المريض.

Introduction
Lectins, simply; are ubiquitous proteins or glycoproteins that are probably present in all eukaryotic cells [1-5], and many bacterial species [6], as well as in some viruses [7, 8]. They are capable to bind mono - and oligosaccharides with high affinity [9, 10], and usually agglutinate cells or precipitate polysaccharides and glycoconjugates specifically and reversibly [11]. The binding involves hydrophobic interactions as well as hydrogen bonds [12]. Lectins was first discovered as a

highly toxic protein that was isolated from castor tree seeds (*Ricinus communis*), and named ricin [13], this protein showed the ability to agglutinin erythrocytes [14]. In 1888; Peter Hermann Stillmark, had called this protein as hemagglutinin, or phytoagglutinin, because it was originally found in the extracts of some plants [15-17]. Because cells are "sugar coated," it is not surprising that lectins are important partner in biological recognition and the development of multitude biological functions [18,19]. The fields of lectins applications are variety and included: Cell identification and separation, mitogenic stimulation of lymphocytes [20], investigation of carbohydrates on cells and subcellular organelles, crystal structures of legume lectins have led to a detailed insight of the atomic interactions between carbohydrates and proteins [21], purification of carbohydrates or carbohydrates derivatives using suitable lectins [22, 23], detection, isolation, and structural studies of glycoproteins, blood typing [24], neuroscience [25], purging of bone marrow for transplantation, and in the drugs industrialization [26].

Cancer is heterogeneous diseases in most respects, including its cellularity, different genetic alterations and diverse clinical behaviors [27, 28]. Cancer cells are invasive [18], this invasion is happening either by direct growth into adjacent tissue through invasion or by implantation into distant sites by metastasis [29]. Several lectins are investigated for their use in cancer research and therapy. Preliminary findings suggest that some lectins can detect alterations of malignant cells as well as reduce the cancer cell tumorigenicity and thus may be helpful for prognosis of the immune status of the patients [30]. At early observation in the study of galectins (a family of lectins) and cancer was that various types of tumor cells express galectins on their surface. Experimental evidences also suggested that these galectins could be cross-linked by exogenous glycoprotein ligands resulting in the homotypic aggregation of tumor cells [31]. The elevation of several galectins expression significantly enhance tumor cell adhesion to common extracellular matrix proteins [32], increases the incidence of lung metastases, and protects cancer cells from apoptosis[30]. Furthermore, pretreatment of tumor cells with an anti-galectin-3 antibody reduces the incidence of metastatic lung colonies by up to 90% [32]. These data suggest that galectin-3 expression and interactions with its cognate carbohydrate ligands could be important in tumor metastasis. The present study was designed to detect lectins in serum and tissue of patients with urinary tract system and prostate diseases.

Patient and Control Individuals

During the period from the beginning of February 2007 to the end of July 2008, 223 patients and 46 healthy individuals, with the age range 10-80 years; were enrolled in the present study. The patients were classified into two fundamental groups, i.e., 155 patients with urinary tract diseases, 96 of them with different kidney diseases and 59 cases with the different bladder tumors. Sixty eight patients with the different prostate tumors represented the second study group. The host informations of the study patients groups were summarized in the present table.

Patient Groups (n.) Age Range (year)		Gender	
		Male (n.) Age Range (year)	Female (n.) Age Range (year)
Kidney	Malignant (55) (32 – 80)	36 (32 – 80)	19 (37 – 65)
	Benign (23) (10 – 62)	14 (10 – 66)	9 (25 – 62)
	Non Tumoral diseases (18) (12 – 68)	11 (27 – 62)	7 (12 – 68)
Bladder	Malignant (40) (27 – 90)	28 (32 – 90)	12 (27 – 76)
	Benign (19) (19 – 82)	13 (19 – 70)	6 (36 – 82)
Prostate	Malignant (44) (37 – 88)		
	Benign (24) (35 – 77)		

Materials and Methods

Isolation of Crude Lectins from Serum and Tissue Specimens:

Five milliliters of venous blood samples were collected from patients and the control groups. Samples were allowed to clot at room temperature, centrifuged at 3000 xg for 5 minutes, then sera were collected and stored at –15˚C. Different tissue specimens were removed from the patients by surgery carried out by specialist during surgical intervention, washed many times with 0.9% (w / v) NaCl, and stored immediately at –15˚C.

The frozen tissue (1g); after cutting into slices was homogenated by manual homogenizer in 3 ml of Tris-HCl buffer solution (20 mM, pH 8) on ice bath. The suspension was centrifuged and the supernatant was used for lectin isolation.

For isolation of serum and tissue crude lectins, 1 volume of serum was mixed with 2.5 volumes of petroleum ether, while; 2 volume of the homogenate supernatant were mixed with 3 ml of petroleum ether for defatting. The mixtures were shacked strongly, then, centrifuged at 3000 xg for 5 minutes. The organic phase was neglected and defatted serum and homogenate supernatant were stored at –15˚C to be used for determination of the hemagglutination activity.

Preparation of Standard Ttrypsinized Erythrocyte Suspension for Hemagglutination Test

Human blood group O+ erythrocytes were collected from the local blood bank in Al-Sadder Teaching Hospital in Najaf. Blood was centrifuged at 3000 xg for 5 minutes, the sera were discarded and erythrocytes were washed for 4 times with saline solution (5 ml saline: 1 ml packed erythrocytes). The washed erythrocytes were suspended in phosphate buffer saline solution (pH 7.4), and diluted with the

same buffer to give an absorbance of 2 at 620 nm. One part of trypsin solution (1%) was added to 10 parts of the final erythrocytes suspension. The mixture was incubated at 37˚C for 1 hour, then centrifuged at 5000 xg for 5 minutes. The trypsinized erythrocytes mixture was washed 3 – 5 times with saline solution to remove trypsin traces. Saline solution was added, until the absorbance of the erythrocyte suspension was 1.4 at 620 nm.

Protein Determination

Total proteins in the serum and tissue specimens and purified MBL were estimated using Bradford method [33]. Where bovine serum albumin was used as a standard protein.

Determination of Hemagglutination Activity of Crude Serum and Tissue Lectins of Patient and Control Groups

To determine the hemagglutination activity in serum and tissue Lis and Sharon [14] method was used, with essential modifications. The procedure involved three tubes, test (T), blank (B), and control (C). A set of control tubes (2 – 4) were used in each experiment and the assay was carried out as in the following:

Components	Test	Blank	Control
1) Diluted serum (1:20) with Tris-HCl buffer (20 Mm, pH 8) or Crude tissue lectins preparation	1 ml	1 ml	-
2) Trypsinized erythrocyte suspension	2 ml	-	2 ml
3) Saline solution	-	2 ml	1ml
4) Calcium chloride solution (60 mM)	1 ml	1 ml	1 ml
T, B, and C tubes were placed in exactly vertical position at 37˚C for 75 min.			
Cells were pelted after centrifugation at 3000 xg for 3 minutes, then re-suspended by gentle shaking and allowed to stand for another 75 minutes at 37˚C.			
The absorbance of 2 ml of the upper mixtures was measured at 620 nm.			

The reduction of optical density (ROD) in the test tube (in crude sera and tissues determination) was measured from the following equation:

$$ROD\% = \frac{A_C - A_{T-B}}{A_C} \times 100$$

Where: A_C: Optical density of cell suspension in the control tube; and A_{T-B}: Optical density of cell suspension in the test tube – Optical density of cell suspension in the blank tube.

Results and discussion:

Levels of the Specific Hemagglutination Activity in Patients and Control Groups

In Serum

The optimized conditions of the hemagglutination protocol were used for estimation of individual serum lectin activity in the studied groups. It was expressed as specific hemagglutination activity unit (SHU). Figure 1, demonstrates that patients of malignant kidney tumors (except 3 cases only) have a

hemagglutination activity higher than 6 SHU, those of non tumoral kidney diseases and healthy individuals (except one individual in each group) have less than 6 SHU, while those of benign kidney tumors also have less than 6 SHU. These results suggest that 6 SHU could be used as a cutoff value for the specific hemagglutination activity when it is used as a biomarker for discriminating of patients with malignant kidney tumors.

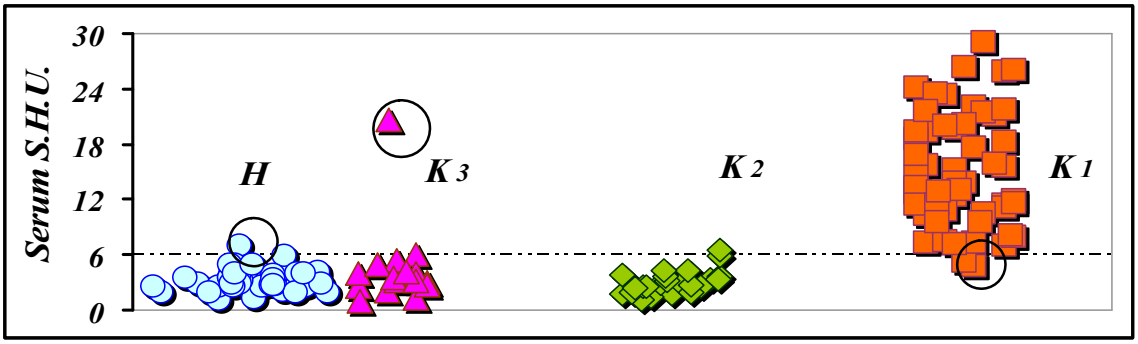

Fig.1: Distribution of the Serum Hemagglutination Activity in Patients of Malignant Kidney Tumors (K1), Benign Kidney Tumors (K2), Non Tumoral Kidney Diseases (K3), and Healthy Individuals (H). The symbol – - – - refer to the cut off malignant kidney tumors value

The evaluation of the specific hemagglutination activity in the various groups revealed a significant increase ($p < 0.001$) in patients of malignant kidney tumors when compared with those of benign tumors, non tumoral kidney diseases, and healthy individuals. However, non significant variations were obtained when other groups were compared together (table1). The sensitivity and specificity of serum lectin activity in detection of malignant kidney tumors were 94.54 % and 95.65 % respectively.

Table 1: Serum Specific Hemagglutination Activity Levels in Patients of Malignant Kidney (K1) and Benign Kidney (K2) Tumors, Non Tumoral Kidney Diseases (K3), and Healthy Individuals (HK1 and HK2).

Groups	Age (year) Mean ± S.D. Range	SHU Mean ± S.D.	Range	p- value
K1 (55)	54.93 ± 12.50 32 – 80	14.99 ± 6.21	4.79 – 29.08	0.000** for K1 vs K2
K2 (23)	45.04 ± 15.33 10 – 66	3.04 ± 1.31	1.17 – 6.49	0.000** for K1 vs K3
K3 (18)	42.39 ± 16.60 12 – 68	4.44 ± 4.27	0.99 – 20.70	0.309 forK2 vs K3
HK1 (32)	47.38 ± 10.92 32 – 80	4.27 ± 1.87	1.09 – 9.09	0.000**]for K1 vs HK1
HK2 (43)	39.77 ± 13.77 10 – 66	3.94 ± 1.71	1.09 – 9.09	0.491 for K2 vs HK2
				0.724 forK3 vs HK2

The mean difference is significant at the 0.001 level. **Refers to significant difference between variables.

In the present study, 21 patients with malignant kidney tumors followed up for hemagglutination activity levels, 72 hours after surgical operation, of the removal of the tumor. These patients exhibited decreased serum hemagglutination activity after the removal of the tumors (data not shown). Bladder and prostate patients groups illustrated the same statistical results as kidney patients groups when they were subjected to ANOVA test. The results revealed a significant elevation ($p < 0.001$) of the specific hemagglutination activity levels in patients with malignant bladder and prostate tumors when they were compared with those of benign tumor, and healthy controls groups (table 2). The sensitivity and specificity of serum lectins activity measurement for detection of malignant bladder tumors were 92.5 % and 78.94 % respectively, while these for detection of malignant prostate tumors were 81.81 % and 83.33 % respectively. On the other hand, non significant variations were observed when benign tumor groups were compared with healthy individuals.

Table 2: Serum Specific Hemagglutination Activity Levels in Patients with Bladder and Prostate (Malignant and Benign) tumors, and Healthy Individuals.

Groups		Age (year) Mean ± S.D. Range	SHU Mean ± S.D.	Range	p – value
Bladder	B1 (40)	61.43 ± 16.08 27 – 90	16.53 ± 9.25	3.54 –36.46	0.000** for B1 vs B2 0.000** for B1 vs HB1 0.816 for B2 vs HB2
	B2 (19)	55.05 ± 14.99 19 – 82	4.03 ± 1.84	0.54 – 7.07	
	HB1 (36)	46.75 ± 13.67 26 – 87	4.26 ± 1.86	1.09 – 9.09	
	HB2 (38)	46.90 ± 15.19 18 – 87	4.36 ± 2.01	1.09 - 9.50	
Prostate	P1 (44)	59.66 ± 13.50 37 – 88	15.29 ± 8.43	4.48 – 34.00	0.000** for P1 vs P2 0.000** for P1 vs HP 0.462 for P2 vs HP
	P2 (24)	57.21 ± 11.97 35 – 77	4.05 ± 2.24	0.82 – 9.09	
	HP (15)	49.20 ± 12.85 35 – 81	5.28 ± 2.42	1.09 - 9.50	

B1and P1: Malignant Bladder and Prostate Tumor Patients respectively, B2 and P2: Benign Bladder and Prostate Tumor Patients respectively, HB1: healthy controls for comparison with Malignant Bladder Tumor Patients, and HB2: healthy controls for comparison with Benign Bladder Tumor. HP: male healthy individuals. The mean difference is significant at the 0.001 level. **Refers to significance between variables

The cut off value of malignant kidney tumors was higher than those of malignant bladder and prostate tumors (figure 2).

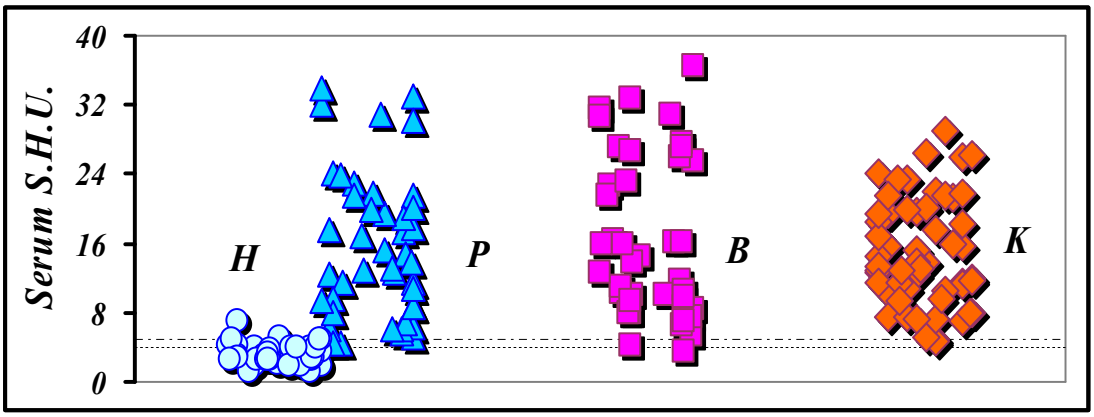

Fig. 2: Distribution of Serum Hemagglutination Activity Levels in Patients of Malignant Kidney (K), Bladder (B), Prostate, and Healthy Individuals (H)The symbol — - — - —refers to the cutoff Malignant Kidney Tumor Value, and the - - - - - refers to the cut off Malignant Bladder and Prostate Tumors Values

In Tissue

Figure 3 shows that patients of malignant kidney tumors (except one case only) have a tissue hemagglutination activity level higher than 0.13 SHU, while those of benign kidney tumors and non tumoral kidney diseases have less than 0.13 SHU. These results suggest that 0.13 SHU could be used as a cutoff value for the SHU when it is used as a biomarker for discriminating patients of malignant kidney tumors.

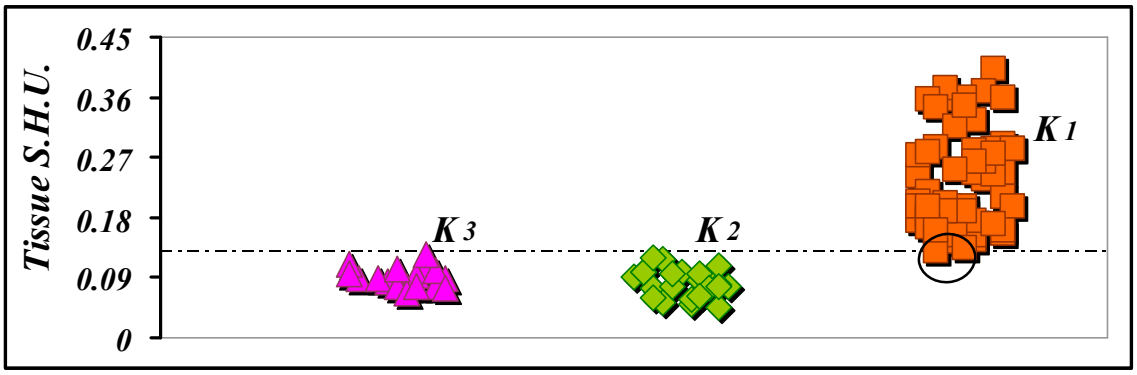

Fig. 3: Distribution of Tissue Hemagglutination Activity Levels in Patients of Malignant Kidney Tumors (K1), Benign Kidney Tumors (K2), and Non Tumoral Kidney Diseases (K3). The symbol – - – - refer to the cut off malignant kidney tumors value.

The evaluation of the SHU in the various kidney groups revealed a significant increase ($p < 0.001$) in patients of malignant kidney tumors when compared with those of benign tumors, and non tumoral kidney diseases, while, non significant difference was found when the benign kidney tumors and non tumoral kidney diseases groups were compared together (table 3).

Table 3: Tissue Specific Hemagglutination Activity Levels of Tumoral (Malignant and Benign) and Non Tumoral Kidney Patients.

Patients	Age (year) Mean ± S.D. Range	SHU Mean ± S.D.	Range	p- value
K1 (55)	54.93 ± 12.50 32 – 80	0.24 ± 0.08	0.12 – 0.40	0.000** for K1 vs K2
K2 (23)	45.04 ± 15.33 10 – 66	0.08 ± 0.02	0.05 – 0.12	0.000** for K1 vs K3
K3 (18)	42.39 ± 16.60 12 – 68	0.09 ± 0.02	0.07 – 0.12	0.627 for K2 vs K3

K1:Malignant Kidney Tumor Patient group, K2: Benign Kidney Tumor Patients and K3: Non Tumoral Kidney Patients. The mean difference is significant at the 0.001 level. **Refers to significance between the variables

Malignant bladder and prostate tumors groups also express high specific hemagglutination activity levels. Significant differences ($p < 0.001$) were observed

when malignant bladder and prostate tumors were compared with their corresponding benign groups (table 4).

Table 4: Tissue Specific Hemagglutination Activity Levels in Malignant and Benign Bladder and Prostate Tumor Patients

Patients		Age (year) Mean ± S.D. Range	SHU Mean ± S.D.	Range	p-value
Bladder	B1 (40)	61.43 ± 16.08 27 – 90	0.23 ± 0.09	0.10 – 0.49	0.000**
	B2 (19)	55.05 ± 14.99 19 – 82	0.11 ± 0.06	0.06 – 0.32	
Prostate	P1 (44)	59.66 ± 13.50 37 – 88	0.24 ± 0.08	0.11 – 0.38	0.000**
	P2 (24)	57.21 ± 11.97 35 – 77	0.09 ± 0.02	0.06 – 0.13	

The mean difference is significant at the 0.001 level. **Refers to significance between the variables

The cutoff values for malignant kidney, bladder, and prostate were clarified in figure 4. Malignant kidney tumors illustrated a high cutoff value in comparison with malignant bladder and prostate tumors patients.

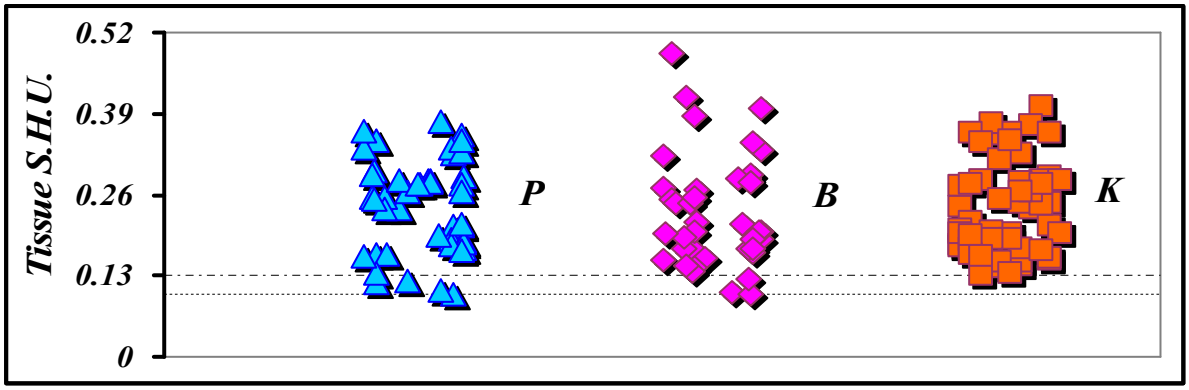

Fig. 4: Distribution of Tissue Hemagglutination Activity Levels in Patients of Malignant Kidney (K), Bladder (B), and Prostate (P) The symbols – - – - refer to the cut off malignant kidney tumor value and - - - - to the cut off malignant bladder and prostate tumors value

Correlation of Serum and Tissue Hemagglutination Activities of Urinary Tract and Prostate Patient Groups:

The correlation of lectin contents (specific hemagglutination activity) in serum and tissue from malignant urinary tract and prostate tumor patients in addition to pathological tissues (benign tumors and non tumoral kidney tissues) was evaluated using the linear regression analysis. Figure 5 A, B, and C, illustrates the significant positive correlation ($r = 0.89$ at $p < 0.001$) of the specific hemagglutination activity

of serum and tissue samples of patients suffered from malignant kidney tumors. However, those of benign tumors and non tumoral diseases failed to do so.

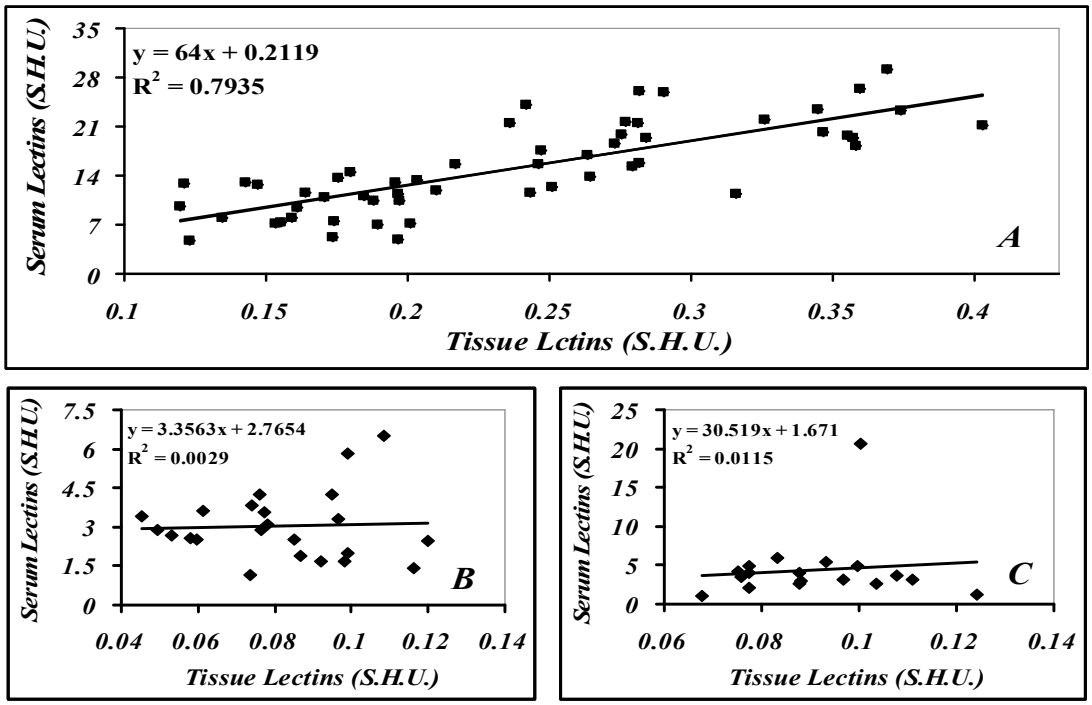

Fig. 5: Correlation of Serum and Tissue Hemagglutination Levels in Patients of A: Malignant tumors, B: Benign tumors, and C: Non Tumoral Kidney Diseases

Significant positive correlations were also observed for patients of malignant bladder tumors ($r = 0.639$ at $p < 0.0005$), and those of benign bladder tumors ($r = 0.503$ at $p < 0.0005$), (figure 6 A, and B).

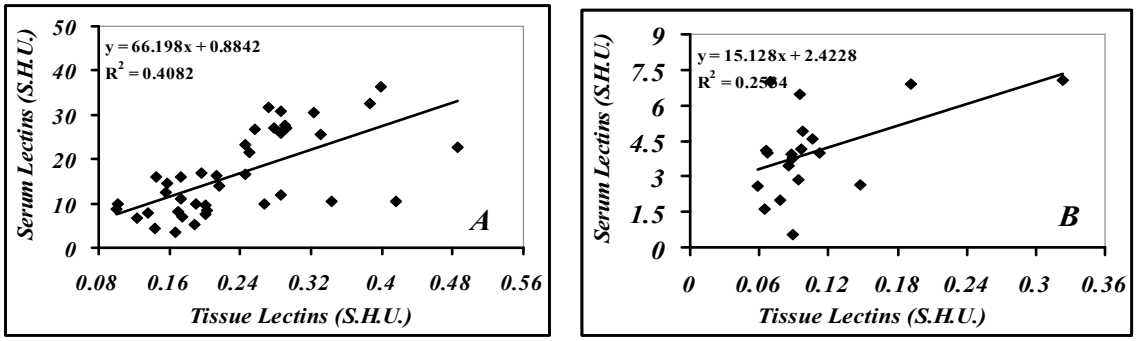

Fig. 6: Correlation of Serum and Tissue Hemagglutination Levels in Patients of A: Malignant Tumors and B: Benign Tumors of Bladder

Prostate tumor patients demonstrated significant positive correlation in those of malignant tumors ($r = 0.597$ at $p < 0.0005$), but not in those of benign tumors (figure 7 A, and B).

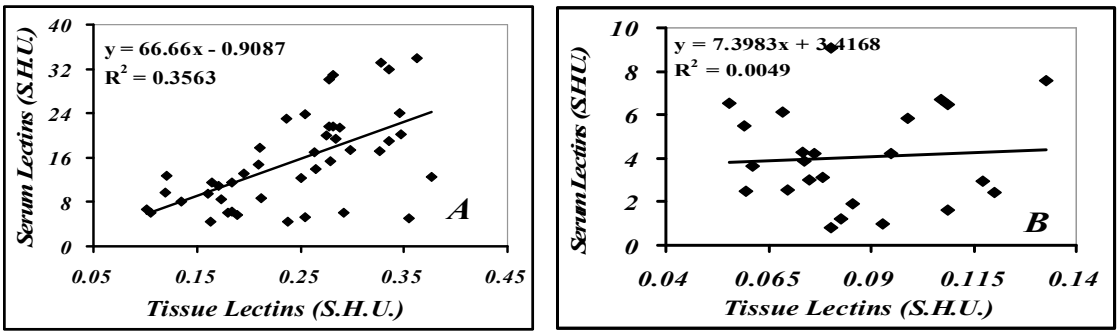

Fig. 7: Correlation of Serum and Tissue Hemagglutination Levels in Patients of A: Malignant Tumors and B: Benign Tumors of Prostate

Stage Differences in the Hemagglutination Activity of Malignant Groups

Implication of Stages of Malignancy in Serum Specific Hemagglutination Activity

In order to verify the changes of the hemagglutination activity with the advancing of malignancy, patients were subdivided on the base of the stage of the diseases into stage I, II, III, and IV. From the statistical analysis of the malignant kidney tumors of different stages, a positive correlation between the serum specific hemagglutination activity with the malignant tumor progression ($r = 0.767$ at $p < 0.0005$) was observed (figure 8).

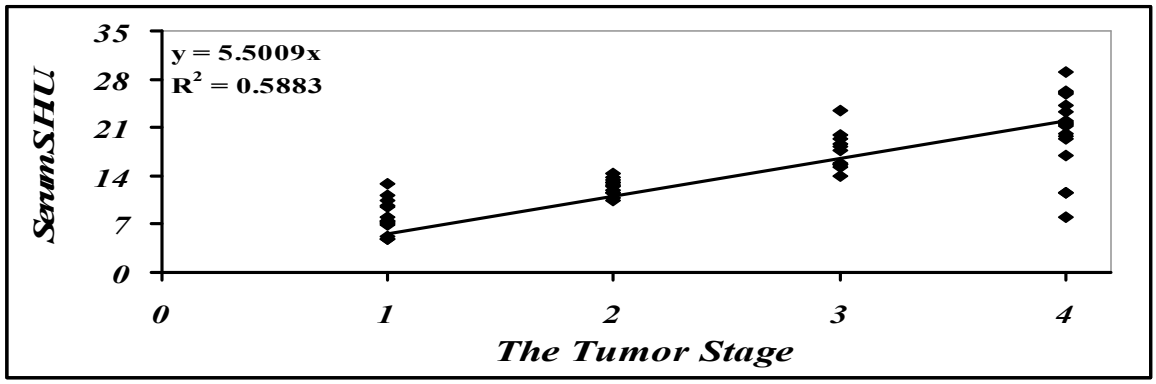

Fig. 8: Correlation of Serum Hemagglutination Activity with Stages of Malignant Kidney Tumors

The mean levels of specific hemagglutination activity in patients of the 4 stages of malignant kidney tumors are illustrated in table 5. Significant elevations ($p < 0.001$) of the specific hemagglutination activity were observed when the data of each two stages (except III and IV) were compared.

Table 5: Stage Differences in Serum Specific Hemagglutination Activity of Malignant Kidney Tumor Patients

Subjects	Age (year) Mean± S.D. Range	SHU Mean± S.D.	Range	p- value
Stage I (14)	49.07 ± 11.94 32 – 74	8.03 ± 2.40	4.79 – 12.80	0.000** for (1, 2, 3, 4, and 5) 0.011 for (6)
Stage II (12)	55.67 ± 13.85 34 – 79	12.40 ± 1.21	10.37 – 14.42	
Stage III (11)	53.73 ± 9.71 43 – 75	17.58 ± 2.73	13.87 – 23.47	
Stage IV (18)	59.72 ± 12.40 41 – 80	20.55 ± 5.57	7.97 – 29.08	

The mean difference is significant at the 0.001 level. **Refers to significance between the variables.

Stage I vs Stage II 4) Stage II vs Stage III
Stage I vs Stage III 5) Stage II vs Stage IV
Stage I vs Stage IV 6) Stage III vs Stage IV

The comparison of serum hemagglutination activity levels of malignant bladder tumor patients of different stages revealed significant ($p < 0.011 – 0.001$) elevations in the advanced stages when compared with those of early stages (table 6). Similar results were obtained when the data of serum hemagglutination activity levels of malignant prostate tumor patients of different stages were compared together (table 6).

Table 6: Stages Differences in Serum Specific Hemagglutination Activity of Malignant Bladder and Prostate Tumor Patients

Subjects		Age (year) Mean± S.D. Range	SHU Mean± S.D.	Range	p- value
Bladder	Stage I (10)	**56.20 ± 20.93 27 – 87**	**7.50 ± 2.21**	**3.54 – 10.00**	**0.002 for (1) 0.000** for (2, 3, and 5) 0.004 for (4) 0.009 for (6)**
	Stage Π (8)	**61.25 ± 17.64 40 – 90**	**13.55 ± 2.91**	**8.05 – 16.45**	
	Stage III (10)	**58.30 ± 7.62 44 – 68**	**19.22 ± 8.51**	**5.27 – 27.57**	
	Stage IV (12)	**68.50 ± 14.98 40 – 83**	**23.81 ± 9.46**	**9.95 – 36.46**	
Prostate	Stage I (9)	**53.00 ± 12.54 38 – 70**	**7.97 ± 2.50**	**4.48 – 12.80**	**0.710 for (1) 0.000** for (2, 3, 4, and 5) 0.138 for (6)**
	Stage Π (11)	**59.09 ± 14.88 37 – 82**	**8.70 ± 3.23**	**4.53 – 13.09**	
	Stage III (8)	**59.88 ± 11.67 43 – 75**	**19.16 ± 6.12**	**12.61 – 30.12**	
	Stage IV (16)	**63.69± 13.52 41 – 88**	**21.99 ± 7.51**	**5.09 – 33.10**	

The mean difference is significant at the 0.001 level. **Refers to significance between variables.

Stage I vs Stage II 4) Stage II vs Stage III
Stage I vs Stage III 5) Stage II vs Stage IV
Stage I vs Stage IV 6) Stage III vs Stage IV

Correlation of Tissue Hemagglutination Activity with Stages of Malignancies

Figure 9 demonstrates a significant ($r = 0.781$ at $p < 0.0005$) positive correlation between tissue hemagglutination activity of kidney tumors with the progression of the malignancy.

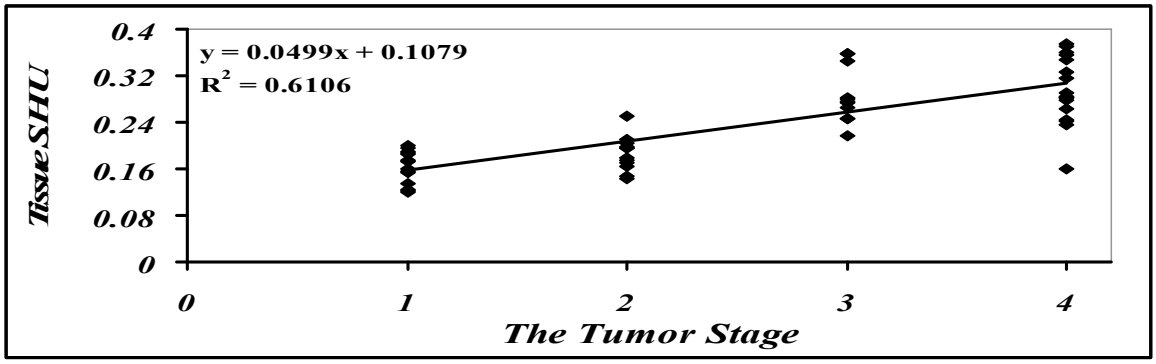

Fig. 9: Correlation of Tissue Hemagglutination Activity with Stages of Malignant Kidney Tumors

The evaluation of tissue specific hemagglutination activity levels in patients of malignant kidney tumors of different stages demonstrated a trend of gradual rise as malignancies were advanced (table 7). Similar results were obtained when tissue specific hemagglutination activity levels of patients with malignant bladder and prostate tumors of various stages were compared (table 8).

Table 7: Levels of Tissue Specific Hemagglutination Activity of Different Stages of Malignant Kidney Tumors

Subjects	Age (year) Mean± S.D. Range	SHU Mean± S.D.	Range	p- value
Stage I (14)	49.07 ± 11.94 32 – 74	0.16 ± 0.03	0.12 – 0.20	0.000** for (2, 3, 4, and5) 0.103 for (1) 0.299 for (6)
Stage II (12)	55.67 ± 13.85 34 – 79	0.19 ± 0.03	0.14 – 0.25	
Stage III (11)	53.73 ± 9.71 43 – 75	0.29± 0.50	0.22 – 0.36	
Stage IV (18)	59.72 ± 12.40 41 – 80	0.30 ± 0.06	0.16 – 0.40	

The mean difference is significant at 0.001 level. **Refers to significance between variables.

Stage I vs Stage II
Stage I vs Stage III
Stage I vs Stage IV
4) Stage II vs Stage III
5) Stage II vs Stage IV
6) Stage III vs Stage IV

Table 8: Levels of Tissue Specific Hemagglutination Activity in Different Stages of Malignant Bladder and Prostate Tumors

Subjects		Age (year) Mean± S.D. Range	SHU Mean± S.D.	Range	p- value
Bladder	Stage I (10)	**56.20 ± 20.93 27 – 87**	**0.15 ± 0.04**	**0.10– 0.20**	**0.352 for (1) 0.001 for (2 and 6) 0.000** for (3 and 5) 0.019 for (4)**
	Stage Π (8)	**61.25 ± 17.64 40 – 90**	**0.18 ± 0.03**	**0.15 – 0.25**	
	Stage III (10)	**58.30 ± 7.62 44 – 68**	**0.25 ± 0.05**	**0.19 – 0.34**	
	Stage IV (12)	**68.50 ± 14.98 40 – 83**	**0.33 ± 0.08**	**0.21 – 0.49**	
Prostate	Stage I (9)	**53.00 ± 12.54 38 – 70**	**0.16 ± 0.06**	**0.10 – 0.29**	**0.019 for (1) 0.000** for (2, 3, 4 and 5) 0.037 for (6)**
	Stage Π (11)	**59.09 ± 14.88 37 – 82**	**0.20 ± 0.03**	**0.16 – 0.26**	
	Stage III (8)	**59.88 ± 11.67 43 – 75**	**0.27 ± 0.05**	**0.21– 0.38**	
	Stage IV (16)	**63.69± 13.52 41 – 88**	**0.41 ± 0.04**	**0.21 – 0.36**	

The mean difference is significant at 0.001 level. **Refers to significant between variables

Stage I vs Stage II 4) Stage II vs Stage III
Stage I vs Stage III 5) Stage II vs Stage IV
Stage I vs Stage IV 6) Stage III vs Stage IV

Gender Involvement in Kidney Lectins Hemagglutination Activity Changes: In Serum

The effect of gender on the kidney hemagglutination activity (SHU) levels in patients of cancerous tumors, benign tumors, and non tumoral kidney subgroups was evaluated. Student's t-test failed to exhibit significant changes among male and female subgroups (table 9)

Table 9: Gender Differences of Serum Specific Hemagglutination Activity in Tumoral and non Tumoral Kidney Disease Patients and Healthy Individuals.

Type	Gender	Age (year) Mean ± S.D. Range	SHU Mean ± S.D.	Range	p- value
K1 (55)	M (36)	57.31 ± 13.69 32 – 80	15.48 ± 6.94	4.79 – 29.08	0.259
	F (19)	50.79 ± 9.19 37 – 65	14.08 ± 4.55	7.97 – 21.69	
K2 (23)	M (14)	43.93± 16.73 10 – 66	2.40 ± 0.77	1.17 – 3.59	0.377
	F (9)	47.44 ± 12.28 25 – 62	4.04± 1.38	2.45 – 6.49	
K3 (18)	M 11)(	47.36 ± 11.33 27 – 62	3.95 ± 1.23	2.05 – 6.00	0.550
	F (7)	34.57 ± 21.22 12 – 68	5.21 ± 6.92	0.99– 20.70	
H (46)	M (21)	44.24 ± 9.57 10 -81	4.69 ± 2.08	1.09– 9.09	0.432
	F (25)	44.88 ± 17.10 11– 87	3.53 ± 1.14	1.09 – 6.13	

K1:Malignant Kidney Tumor Patient group, K2: Benign Kidney Tumor Patient group, K3: Non Tumoral Kidney Patients, and H: total healthy individuals. M: Male, F: Female. The mean difference is significant at 0.001 level

In Tissue

Gender was found to have no effect on tissue hemagglutination activity in patients and control groups (table 10).

Table 3.20: Gender Differences of Tissue Specific Hemagglutination Activity in Tumoral and non Tumoral Kidney disease Patients

Type	Gender	Age (year) Mean ± S.D. Range	SHU Mean ± S.D.	Range	p-value
K1 (55)	M (36)	57.31 ± 13.69 32 – 80	0.24 ± 0.08	0.12 – 0.40	0.338
	F (19)	50.79 ± 9.19 37 – 65	0.23 ± 0.07	0.12 – 0.35	
K2 (23)	M (14)	43.93± 16.73 10 – 66	0.09 ± 0.05	0.05 – 0.25	0.798
	F (9)	47.44 ± 12.28 25 – 62	0.09 ± 0.02	0.06 – 0.12	
K3 (18)	M 11)(	47.36 ± 11.33 27 – 62	0.09 ± 0.01	0.08 – 0.11	0.948
	F (7)	34.57 ± 21.22 12 – 68	0.09 ±0.02	0.07 – 0.12	

K1 refer to Malignant Kidney Tumor Patients, K2 refers to Benign Kidney Tumor Patient, and K3 refer to Non Tumoral Kidney Patients. M: Male, F: Female. The mean difference is significant at 0.001 level

Results of the current study demonstrated an elevation of hemagglutination activity levels in sera of malignant tumor patients when compared with levels of healthy individuals, regardless of the studied organs. On the other hand, the levels of serum hemagglutination activity in patients with benign tumors remained within the hemagglutination activity of normal individuals, while serum of patients with non tumoral kidney diseases did not show significant changes when compared with healthy individuals. In addition, patients of benign tumors and non tumoral diseases exhibited approximately comparable results with those of the healthy individuals. The significant positive correlation of serum and tissue lectins of patients with malignant tumors suggest a direct relationship of lectin from the two sources, perhaps malignant tumors are the sources of lectin in sera of patients.

Increased levels of tissue lectins in malignant tumor specimens may be explained through several hypotheses: During malignancy, an increased expression of oncogene proteins due to chromosomal translocation, amplification, or mutation is considered one of the main alteration in the cancer cells. Lectin may be one of these proteins. In malignant tumor cells, the loss of tumor suppressor gene protein products due to deletion or mutation, may lead to increase the oncogene proteins, lectin may be among these proteins. Alterations in enzyme patterns may suggest that malignant cells have increased levels of enzymes involved in nucleic acid synthesis. The key enzymes in the de novo and salvage pathways of purine and pyrimidine biosynthesis are increased, the opposing catabolic enzymes are decreased during malignant transformation and tumor progression, lead to increase

malignant cells number, and the production of several proteins will increase too. Genetic imprinting errors and genetic instability leading to progressive loss of regulated cell proliferation, increased invasiveness, and increased metastatic potential. Expression of lectins is completely controlled by the machinery system of protein synthesis. It is prone for alteration during malignant transformation [4]. The elevation in several carbohydrates concentrations in malignant cells and the aberrant glycosylation of glycoproteins can be considered one of the causes for lectin production [34]. The chemical basis for some of the changes in tumor cell glycoproteins may be attributed to the fact that the N-linked oligosaccharides of tumor cells contain more multiantennary structures than the oligosaccharides derived from normal cells [4].

The source of increased serum lectins in cancer patients remains unclear [35]. In the present study, removal of the tumors, decreased serum hemagglutination activity, thus tumor tissues are most likely to produce and secrete lectins in sera. The agglutination test of cancerous tissues showed that lectin was found not only on malignant cells but also in macrophages and stromal cells (mainly fibroblasts) near cancer focus, and the stromal cells immediately adjacent to cancer nests have higher levels of the hemagglutination activity in comparison to cells far from the nests. These results suggest that circulating lectins are generated not only by tumor cells but also from peritumoral inflammatory cells and stromal cells.

Some human lectin genes are expressed constitutively, whereas others are induced by gene activation under specific biological circumstances [36]. Membrane-bound and many soluble lectins are synthesized on ER-bound ribosomes and delivered to their eventual destinations via the ER-Golgi pathway. However, a significant subset of soluble lectins (galectins, heparin-binding growth factors, and some cytokines) are synthesized on free ribosomes and delivered directly to the exterior of the cell by a poorly understood mechanism involving extrusion through the plasma membrane. Some of these lectins can recognize biosynthetic intermediates that occur in the Golgi-ER pathway (e.g., galactosides and high-mannose oligosaccharides) [37].

Different modalities have been proposed to explain how lectins might be involved in the metastatic process: Lectins act as a bridge molecule enhancing the adhesive interactions between tumor cells and the extracellular matrix. Several lectins are able to mediate homotypic cell-cell adhesion through interaction with complementary glycoproteins depending on the hypothesis that lectins are involved in the formation of tumor emboli and dissemination of tumor cells in the circulation. Lectins are able to protect the malignant cell against apoptosis induced by the loss of cell anchorage. The expression of lectins in tumor cells may provide a critical determinant for cell survival of disseminating cancer cells in the circulation during metastasis [35].

In patients with benign tumors serum hemagglutination activity was found to remain within values of healthy individuals, this is due to the differences of benign from malignant tumors. In contrast to the malignant cells, benign tumor cells are

under control. On the other hand, during benign tumor formation, several lectins, which extend normally on the cell surface, are degraded and others are built, these processes are contributed in keeping lectin concentration balance [38]. Lectins provide way for one molecule to stick to another one without any immunity involved. They play a wide role in health, but their ability to influence the inflammatory process indicates that they are involved in inflammatory diseases, e.g.: bowel disease, systematic lupus erythematosus, rheumatoid arthritis, and even weight gain [10, 39, 40].

Majority of lectin researches have focused on the using of lectins from different sources (other than human) in human medical fields. Somewhat, working with human lectins was surrounded by difficulties, as a result of that, human endogenous lectins studies were, rather, few [41-43]. Various lectins from different species are studied for evaluation of their roles in cancer treatment, and therapy. Preliminary findings suggest that some lectins, but not all; can detect alterations of malignant cells as well as reduce the cancer cell tumorigenicity, thus may have benefits for the immune status of the patients. A lectin from Viscum album (mistletoe) for instance is known to increase the reactivity of the lymphocytes of tumor-bearing mice to the mitogens in vitro, thus indicating its immune stimulating effects for cancer-immunosuppressed lymphocytes. It also inhibits the protein synthesis in various malignant cell lines. Similarly, because of the cytostatic/apoptotic and immunomodulatory effects of the mistletoe lectin, the extracts are often applied in the treatment of tumor bearing patient [37].

In vivo study using mice, galectin-3 has been implicated in tumorigenicity and metastasis of breast cancer. John et al., have found that cancerous mice treated with galectin-3C (which produced by NH2-terminally truncated form of galectin-3) showed reduced tumor size and weight in comparison with those without such treatment, as well as with reduction of lymph nodes involvement. For this reason, NH2-terminally truncated form of galectin-3 may be efficacious for reduction of tumor growth and prevention of metastases [44]. Iurisci et al., have estimated galectin-3 levels in sera of normal individuals and patients of metastatic breast, gastrointestinal, lung, ovarian, melanoma cancers, and non-Hodgkin's lymphoma. They have observed elevated levels of this lectin in patients relative to the control individuals [35].

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