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Detection, Isolation, Purification, and Characterization of Mannose Binding Lectin (ManBL) from Patients with Different Kidney Diseases and Healthy Individuals

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ABSTRACT
The present study was designed to investigate the presence of mannose binding lectin (ManBL) in the sera and tissue samples of patients with tumoral (benign & malignant) kidney in addition to patients with non tumoral kidney affection, in order to establish the possibility of using serum and tissue ManBL test for diagnosis and epidemiological testing of kidney cancers. Participants of the present study were 96 patients at the age range 12-80 years; they were attending by different hospitals in Najaf (basically) and Karbala, and 46 healthy individuals within the same age range of the studying patients groups. Specific hemagglutination activity levels were revealed a significant increased ($p < 0.001$) in the sera and tissues of the patients with the malignant tumors specimens in comparison to that of pathological controls and healthy individuals. Serum and tissue hemagglutination activity levels were significantly higher ($p < 0.001$) in patients with metastatic disease compared with patients with localized tumors. While, the levels of the hemagglutination activity were approximate at patients with the end malignant kidney tumors stages. Upon electrophoresis of the study samples, the results reflected presence of changes in some proteins and glycoproteins bands presents in these samples. Using hydrophobic affinity chromatography, ManBLs were purified from sera of malignant, benign kidney tumors and non tumoral kidney diseases in addition to healthy individuals groups. The purified lectins were found to be glycoprotein with 77.45 KD as an approximate molecular weight and its sugar content was equal to13.5%. The maximum concentration of purified ManBL was found at patients with malignant kidney tumors.The results revealed that the highest hemagglutination activity of purified ManBL occurred with O^+ RBCs, at pH 7.4, and 37˚C. ManBL (regardless of its origin) lost the hemagglutination activity, completely, in the presence of EDTA. A result that indicates that the present purified the human ManBLs is a calcium dependent type.

1. INTRODUCTION
Lectins, simply; are ubiquitous proteins or glycoproteins that are probably present in all eukaryotic cells[1-6], and many bacterial species[7, 8], as well as in some viruses[9, 10]. Theyare capable to bind mono - and oligosaccharides with high affinity[11, 12], and usually agglutinate cells or precipitate polysaccharides and glycoconjugates specifically and reversibly[13, 11]. The binding involves hydrophobic interactions as well as hydrogen bonds[14]. Lectin specificity for individual sugars or groups of sugars makes them powerful tools for detecting changes in the carbohydrate structure of the glycoproteins[15, 16].
Early observations of cancer patients who had fully recovered from an acute bacterial infection suggested that innate immunity has anti tumor activity. Complement activation may lead to the liberation of pro-inflammatory factors and the activation of inflammatory cells, which may have pro-carcinogenic effects. Activation of complement by mannose binding lectin (ManBL) is crucial for innate immune .This relationship has been revealed by analysis of common single nucleotide polymorphisms (SNPs) in the ManBL2 gene, which encodes ManBL. The frequency of SNP-determined ManBL deficiency is significantly higher in patients presenting with various infections and autoimmune disorders than it is in the general population, indicating the importance of ManBL in host defence. Thus, additional evidence suggests that ManBL function contributes to human cancer risk[17, 18].
Renal cancer is the third most common malignancy of the genitourinary system[19], and accounts for 3% of adult malignancies globally[20]. Limited early warning signs result in late recognition with metastases present in approximately one third of patients at the time of diagnosis[21], with 210,000 new cases per year and more than 100,000 deaths occurring worldwide annually. The male to female ratio is 1.5:1, and the disease usually occurs in the sixth and seventh decades of life[22]. RCCs are highly vascularized tumors, which may explain the 30-40% prevalence of metastatic disease at initial diagnosis[23, 24], when systemic therapies are then necessary. In this group of patients, one-year survival rate are ~25%, illustrating the limited role of both chemotherapy and radiotherapy in the management of advanced stages of RCC[25].

2. EXPERIMENTAL

2.1 *Patient and Control Individuals*
The present study involved 96 patients (55 cases with malignant kidney tumors, 23 cases with benign kidney tumors, and 18 cases with non tumoral kidney diseases) with the age range 10-80 years, in addition to 46 healthy individuals, at the same age range.

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2.2 *Isolation of Crude Lectins from Serum and Tissue Specimens*

Ten millilitres 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, and 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.

2.3 *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 in Iraq. Blood was centrifuged at 3000 xg for 5 minutes, the sera were discarded. The erythrocytes were washed with saline solution (5 ml saline: 1 ml packed erythrocytes), then were suspended in phosphate buffer saline solution (pH 7.4), and diluted with the same buffer to give an absorbance of 2 ml 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, and 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.

2.4 *Protein Determination*

Total proteins in the studied samples were estimated using Bradford method[26], and bovine serum albumin was used as a standard protein.

2.5 *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[27] 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
Diluted serum (1:20) with Tris-HCl buffer (20 Mm, pH 8), or Crude tissue lectins preparation	1 ml	1 ml	-
Trypsinized erythrocyte suspension	2 ml	-	2 ml
Saline solution	-	2 ml	1ml
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 separated after centrifugation at 3000 xg for 3 minutes, then re-suspended in the above mentioned buffer 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.

A_{T-B} = Optical density of cell suspension in the test tube – Optical density of cell suspension in the blank tube.

2.6 *Purification of ManBL*

Affinity chromatography technique was applied for the purification of ManBL from patients' with tumoral and non tumoral kidney diseases, in addition to healthy individuals. Preparation of the affinity chromatography column was carried out according to the instructions of Hermanson[28], and Amersham handbook [Amersham Pharmacia Biotech].

2.7 *Determination of Carbohydrate Content in the Purified ManBL*

Dubois method[29] was followed for determination of carbohydrate amount in the purified ManBL. Where glucose was used as a standard sugar.

3. RESULTS AND DISCUSSION

3.1 *Levels of the Specific Hemagglutination Activity in Patients and Control Groups*

3.1.1 *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). Fig-1, demonstrates that 52 patients out of the 55 studied patients of malignant kidney tumors have a hemagglutination activity higher than 6 SHU, while those of non tumoral kidney diseases and healthy individuals (except one sample in each group) have less than 6 SHU. Also those of benign kidney tumors were found to have specific activity less than 6 SHU. These results suggest the possibility of using 6 SHU as a cut off value for the specific hemagglutination activity. A result showed the possibility of using this parameter as a biomarker for discriminating of patients with malignant kidney tumors among those with benign, non tumoral kidney diseases, and healthy individuals.

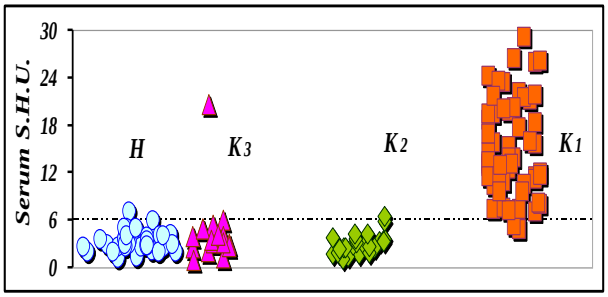

Fig-1: Distribution of the Serum Hemagglutination Activity in Patients of Malignant Kidney Tumors (K_1), Benign Kidney Tumors (K_2), Non Tumoral Kidney Diseases (K_3), and Healthy Individuals (H). The symbol – - – - refer to the cut off of malignant kidney tumors value

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 (Table-1). 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 (K_1) and Benign Kidney (K_2) Tumors, Non Tumoral Kidney Diseases (K_3), and Healthy Individuals (HK_1 and HK_2)

Group	Age (year) Mean ± S.D. Range	SHU Mean ± S.D. Range	P
K_1 (55)	54.93 ± 12.50 32 – 80	14.99 ± 6.21 4.79 – 29.08	0.000** for K_1vs K_2
K_2 (23)	45.04 ± 15.33 10 – 66	3.04 ± 1.31 1.17 – 6.49	0.000** for K_1vs K_3
K_3 (18)	42.39 ± 16.60 12 – 68	4.44 ± 4.27 0.99 – 20.70	0.309 for K_2vs K_3
H_{K1} (32)	47.38 ± 10.92 32 – 80	4.27 ± 1.87 1.09 – 9.09	0.000** for K_1 vsH_{K1}
H_{K2} (43)	39.77 ± 13.77 10 – 66	3.94 ± 1.71 1.09 – 9.09	0.491 for K_2vs H_{K2}
H_{K2} (43)	39.77 ± 13.77 10 – 66	3.94 ± 1.71 1.09 – 9.09	0.724 for K_3vs H_{K2}

In the present study, the hemagglutination activity levels in 21 patients with malignant kidney tumors were followed up for 72 hours after surgical operation, of the removal of the tumor. The result showed presence of a decrease in their serum hemagglutination activities after the removal of the tumors (data not shown).

3.1.2 *In Tissue*

Fig-2, 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 were less than 0.13 SHU. These results suggest that 0.13 SHU could be used as a cutoff value (biomarker) for discriminating, the tissue of patients of malignant kidney tumors from other kidney tumoral& none tumoral diseases.

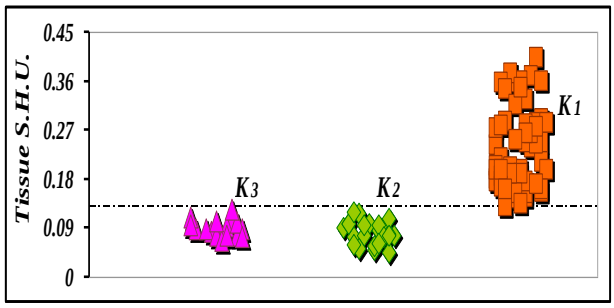

Fig-2: Distribution of Tissue Hemagglutination Activity Levels in Patients of Malignant Kidney Tumors (K_1), Benign Kidney Tumors (K_2), and Non Tumoral Kidney Diseases (K_3). The symbol – - – - refer to the cut off malignant kidney tumors value.

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-2).

Table-2: 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
K_1 (55)	54.93 ± 12.50 32 – 80	0.24 ± 0.08 0.12 – 0.40	0.000**for K_1vs K_2
K_2 (23)	45.04 ± 15.33 10 – 66	0.08 ± 0.02 0.05 – 0.12	0.000** for K_1vs K_3
K_3 (18)	42.39 ± 16.60 12 – 68	0.09 ± 0.02 0.07 – 0.12	0.627for K_2vs K_3

K_1: Malignant Kidney Tumor Patient group, **K_2:** Benign Kidney Tumor Patients and **K_3:** Non Tumoral Kidney Patients. The mean difference is significant at the 0.001 level. **Refers to significance between the variables

3.2 *Correlation of Serum and Tissue Hemagglutination Activities of kidney patients Groups*

The correlation of lectin contents (specific hemagglutination activity) in serum and tissue from malignant kidney tumor patients in addition to pathological tissues (benign tumors and non tumoral kidney tissues) was evaluated using the linear regression analysis. Fig-3 A, B, and C, illustrates presence of a significant positive correlation ($r = 0.89$ at $p < 0.001$) between the specific hemagglutination activity of serum and tissue samples in the cases of malignant kidney tumors. While such correlation was not observed in those of benign tumors and non tumoral diseases.

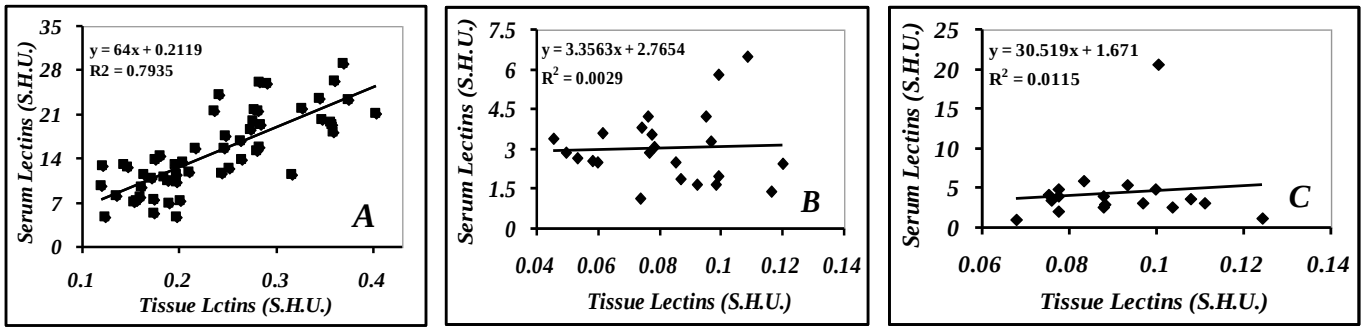

Fig-3: Correlation of Serum and Tissue Hemagglutination Levels in Patients of A: Malignant tumors, B: Benign tumors, and C: Non Tumoral Kidney Diseases

3.3 *Implication of Stages of Malignancy in Serum and Tissue Specific Hemagglutination Activity*

In order to verify the changes of the hemagglutination activity with the advancing of malignancy, the patients were subdivided according to 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 was observed between the serum specific hemagglutination activity & the malignant tumor progression ($r = 0.767$ at $p < 0.0005$) (Fig-4).

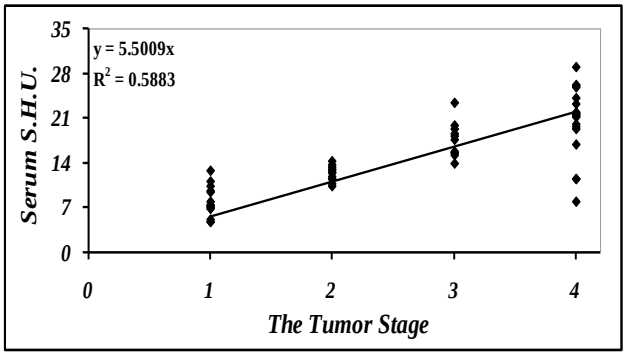

Fig-4: Correlation of Serum Hemagglutination Activity with Stages of Malignant Kidney Tumors

The mean levels of specific hemagglutination activity in patients of the four stages of malignant kidney tumors are illustrated in table 3. 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 3: The Differences in Serum Specific Hemagglutination Activity of Malignant Kidney Tumor Patients According to the Stage of the Disease

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

Fig-5 demonstrates presence of a significant ($r = 0.781$ at $p < 0.0005$) positive correlation between tissue hemagglutination activity of kidney tumors& the progression of the malignancy.

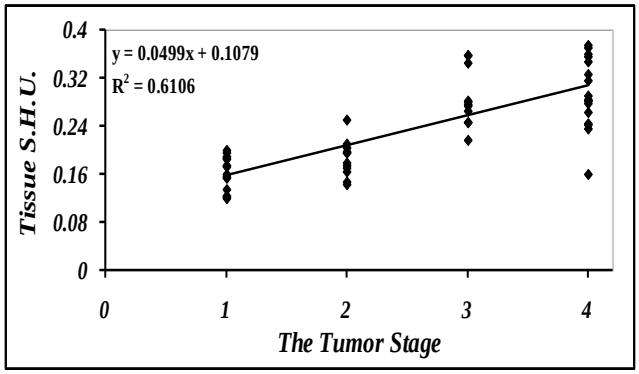

Fig-5: Correlation of Tissue Hemagglutination Activity with Stages of Malignant Kidney Tumors

The evaluation of tissue specific hemagglutination activity levels in patients of malignant kidney tumors at different stages of the disease demonstrated a trend of gradual rise as malignancies were advanced (Table-4).

Table 4: Levels of Tissue Specific Hemagglutination Activity at Different Stages of Malignant Kidney Tumors

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

The mean difference is significant at 0.00 The mean difference is significant at 0.001 levels. **Refers to significance between variables

1)Stage I vs. Stage II,**2)** Stage I vs. Stage III, **3)**Stage I vs. Stage IV, **4)**Stage II vs. Stage III, **5)** Stage II vs. Stage IV **6)**Stage III vs. Stage IV

3.4 *Gender Involvement in Kidney Lectins Hemagglutination Activity Changes*

The effect of gender on the kidney specific hemagglutination activity 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 patients (Table-5 and 6).

Table-5: 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
K_1 (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	
K_2 (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	
K_3 (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	

K_1: Malignant Kidney Tumor Patient group, **K_2:** Benign Kidney Tumor Patient group, **K_3:** Non Tumoral Kidney Patients, and **H:** total healthy individuals. **M:** Male, **F:** Female. The mean difference is significant at 0.001 level

Previously, the source of increased serum lectins in cancer patient was reported to be unclear[30].The significant positive correlations of serum and tissue lectins of patients with malignant tumors reported here suggest presence of a direct relationship between their lectins levels which means that the malignant tumors are the sources of lectin present in the sera of the malignant patients.

Increased levels of tissue lectins in malignant tumor specimens may be due to: (1) during malignancy, an increased expression of oncogene proteins due to chromosomal translocation, amplification, or mutation that is considered one of the main alterations in the cancer cells. Lectin may be one of these proteins. (2) In malignant tumor cells, the loss of tumor suppressor gene protein products due to deletion or mutation, may lead to increase the oncogene proteins, where lectin may be among them. (3) 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. Therefore, it is prone for alteration during malignant transformation[4]. (4) The elevation in several carbohydrates concentrations in malignant cells and the aberrant glycosylation of glycoproteins[16] can be considered one of the causes for lectin production.

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 was found to 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.

Table-6: 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
K_1 (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	
K_2 (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	
K_3 (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	

K_1: Malignant Kidney Tumor Patient group, **K_2:** Benign Kidney Tumor Patient group, **K_3:** Non Tumoral Kidney Patients, and **H:** total healthy individuals. **M:** Male, **F:** Female. The mean difference is significant at 0.001 level

3.5 *Purification of Serum Human ManBL*

Hydrophobic affinity chromatography was used for isolation and purification of ManBL from sera of patients with malignant kidney tumors, benign kidney tumors, non tumoral kidney diseases, and healthy individuals. The purification protocol was carried out by using sepharose 6B column activated with bis-oxirane (1, 4 – Butanedioldiglycidyl ether) (**$C_{10}H_{18}O_4$**). The chromatograms of the purified ManBLs were demonstrated in figure 3 (A, B, C, and D).

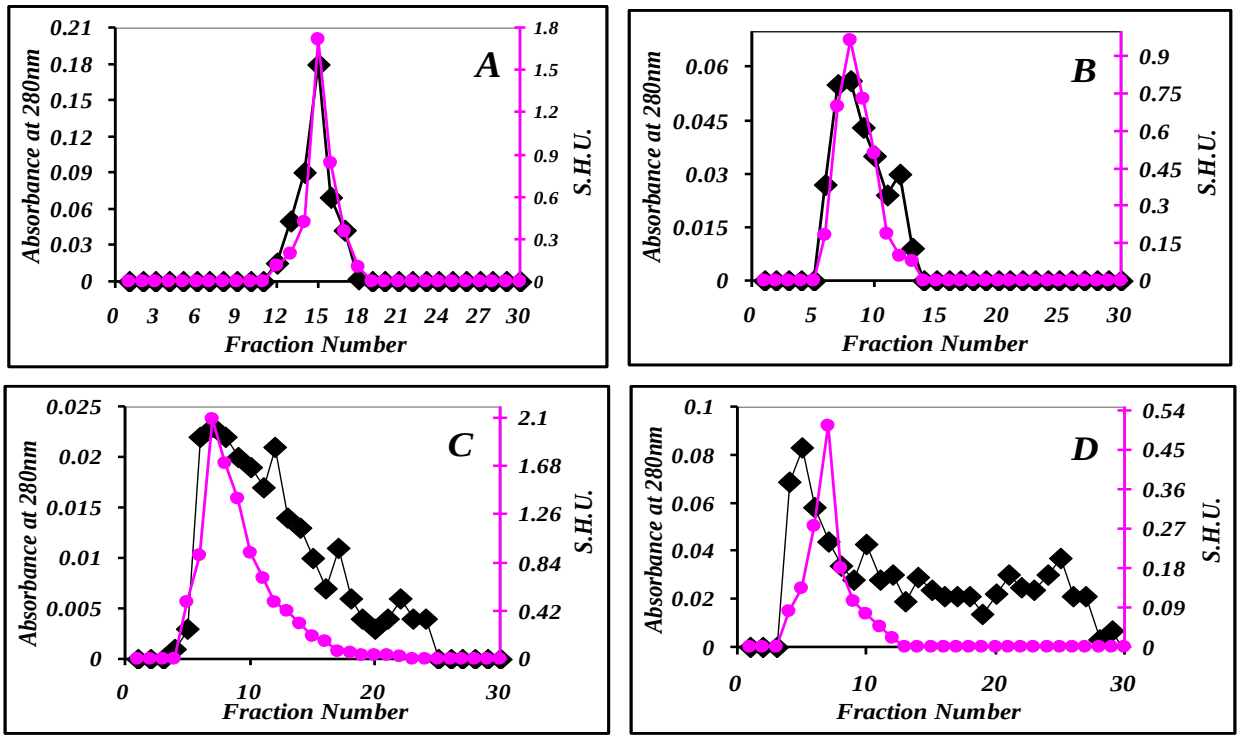

Fig-6: Affinity Chromatogram of ManBL from A: Malignant Kidney Tumor Patients, B: Benign Kidney Tumor Patients, C: Non Tumoral Kidney Diseases Patients, and D: Healthy individuals. Using Sepharose 6B // Mannose Column (1.6×1.3) at Flow Rate 30 ml / hour. The volume of each fraction was 1 ml. Tris Buffer of 20 mM and 7.4 pH contained 60 mM $CaCl_2$, used as a Washing Solution. The Elution step was carried out using the Tris Buffer (20 mM, pH 7.4) contained 5 mM EDTA.

The purification folds and the yield of ManBL from malignant kidney tumors, benign kidney tumors, non tumoral kidney diseases, and healthy individuals were139 with 26.4, 185 with 24.2 %, 45 with 6.4 %, and 158 with 18.3 %, respectively, (Table-7).

The analysis of PAGE electrophoresis pattern of purified lectins from patients and the control group highlighted the appearance of ManBL band in sera of patients with malignant kidney tumors, benign kidney tumors,

and non tumoral kidney diseases, in addition to the healthy subjects. The band seemed to be identical and independent on the source of the sample, suggesting unchanged expression of ManBL during malignant transformation of the kidney. Such observation confirmed the results of affinity chromatography of the intended lectin (Fig-7).

Table-7: Results of the Purification Protocol of Lectins from Sera of Patients with Benign Kidney Tumors, Non Tumoral Kidney Diseases, and Healthy Individuals

Purification step	Total volume (ml) and Total protein (mg)	Total activity (HU)	SHU (HA/ mg of protein)	Purification (fold)	Yield %
MKT Serum	3 45	44.325	0.985	1	100
ManBL	7 0.085	11.688	136.862	139	26.4
BKT Serum	3 45	4.353	0.097	1	100
ManBL	7 0.059	1.054	17.864	185	24.2
NTKD Serum	3 45	17.059	0.379	1	100
ManBL	8 0.064	1.093	17.078	45	6.4
HI Serum	3 45	4.706	0.105	1	100
ManBL	9 0.052	0.860	16.539	158	18.3

The abbreviations: MKT, BKT, NTKD, and HI: Malignant Kidney Tumor, Benign Kidney Tumor, Non Tumoral Kidney Diseases, and Healthy Individual sera

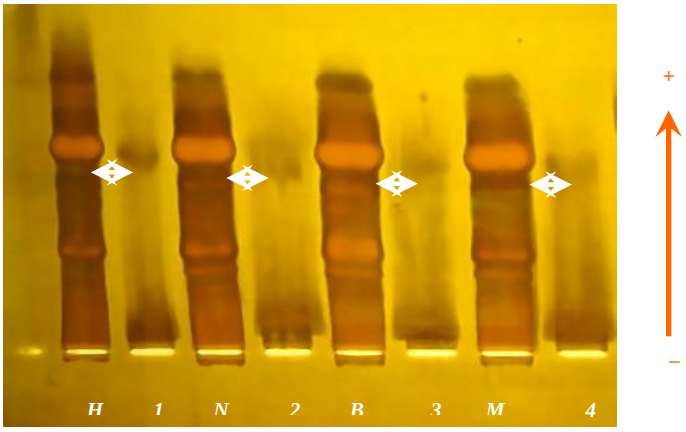

Fig-7: Conventional Polyacrylamide Gel Electrophoresis (PAGE) 7.5% for Proteins. Tris - glycin buffer (0.075 M, pH 8.9) used as the electrodes buffer solution. Prelctrophoresis conditions were 50 mA as a constant current for 30 minutes, with voltage of 15 v/cm, and at 4ºC. Electrophoresis was carried out for10 minutes at 20mA, then the process was continued for 3.5 hours at 4ºC by using 40 mA as a constant current and voltage of 15 v/cm. The gel was stained for protein with silver. The crude and purified samples that applied were:

H: crude Healthy Individuals Sera, **1:** purified ManBL from Healthy Individuals Sera, **NT:** crude Non Tumoral Kidney Affections Sera, **2:** purified ManBL from Non Tumoral Kidney Affections Sera, **B:** crude Benign Kidney Tumors Sera, **3:** purified ManBL from Benign Kidney Tumors Sera, **M:**crude Malignant Kidney Tumors Sera, **4:** purified ManBL from Malignant Kidney Tumors Sera

The approximate molecular weights of purified ManBLs were determined using conventional PAGE. Five standard proteins with known molecular weighs (Lysozyme 13.6 kD, Chemotrypsinogene 25 kD, Ovalbumin 47 kD, Bovine Serum Albumin 67 kD, Lactate Dehydrogenase 140 kD)were used. The estimated molecular weight of ManBLs was 77.45.

ManBL (the key component of innate immune system) is the first element to recognize microorganisms and control infections when adaptive immune response is immature during childhood or when it is compromised by immunosuppressive drugs[31, 17].Exon 1 of the ManBL2gene, which located on chromosome 10, contains 3 functional single nucleotide polymorphism at codons 52-54 and 57 referred to variant alleles of D, B and C respectively. The impairment of polymerization causes low serum levels or impaired ManBL function. Most ManBL deficient individuals are apparently healthy, because they have alternative mechanisms for antimicrobial protection. However, ManBL deficiency can become a strong risk factor of developing infectious diseases in immunocompromised individuals such as organ transplant recipients[32]. Clinically serum ManBL was suggested a double-edged sword

function, meaning that low serum ManBL levels are indicative of increased risk of infections[33, 34], whereas; high serum ManBL levels is associated with transplant rejection[35, 36], with advanced renal failure predicting all-cause mortality[32], and with increased cell invasiveness of primary tumors to distant sites[4, 37].

3.6 *ManBL Activity toward Various Human Blood Groups*

To examine the sensitivity of purified ManBLs in the agglutination reactions, blood of A^+, B^+, and O^+ groups were used as the source of the erythrocyte samples. O^+ RBCs were indicated to exhibit the maximal agglutination, among the evaluated blood groups. This finding could be explained by differences in glycosylation of the surface proteins of red blood cells[38].Several studies tested the effect of blood sources on the hemagglutination process of purified lectins from various sources[39-41]; the result of the present study agreed with some and disagreed with the others.

3.7 *Inhibition of ManBL(s) Activity by EDTA*

The hemagglutination process was carried out for the purified ManBLs using human O^+ red blood cells trypsinized suspension in the presence of EDTA. ManBL (regardless of its origin) lost the hemagglutination activity, completely, in the presence of 1×10^{-5} M EDTA.

3.8 *Relevance of ManBL Activity with pH Changes*

The effect of pH on the activity of the four purified ManBLs was investigated. Fig-8 points out that maximal lectin activity was achieved at pH 7.4 regardless to the source of the examined ManBL, while the purified lectins were sensitive to acidic (pH 3) and to basic (pH 12) conditions, under which the activities were completely lost.

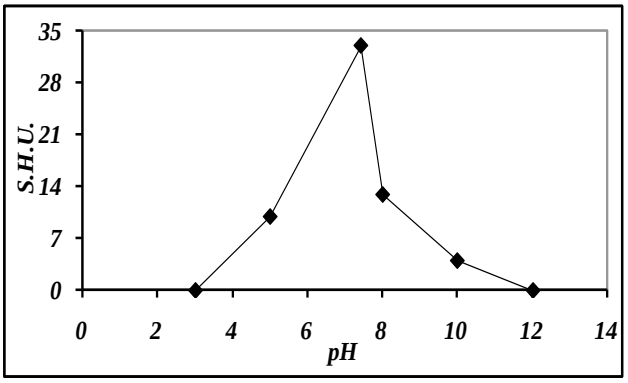

Fig-8: Effect of the pH on Purified ManBLs Hemagglutination Activity

3.9 *Thermal Stability of ManBL Activity*

To explore the effect of temperature on the hemagglutination activity of purified ManBLs, they were incubated at various temperatures (0°C, 30°C, 40°C, 50°C, 60°C, 80°C, and 100°C) for 1 hour; the mixtures were cooled until room temperature. The hemagglutination activity was carried out at 30°C[42].Thermal stability results revealed that purified lectins remained stable below 40ºC for one hour with no loss of hemagglutination activity, while; they loss about 40% of their hemagglutination activity at 50 ºC. Lectin activity disappeared when the denaturation was carried out at more than 60 ºC (Fig-9).

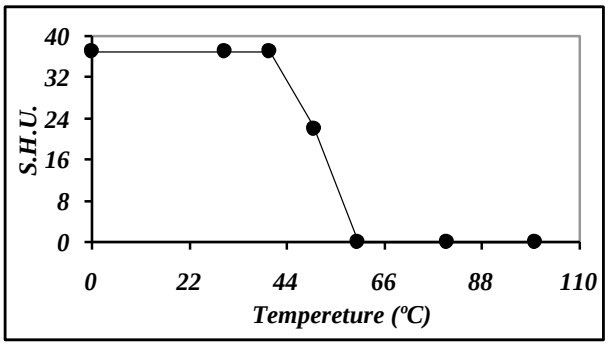

Fig-9: Thermal Denaturation of Purified ManBLs Hemagglutination Activity

The influence of temperature on the hemagglutination activity of purified ManBLs was also evaluated through the incubation of purified ManBLs with erythrocyte suspension at 0, 32, 37, 45, 60, and 75°C. 37°C seems to be more suitable among the examined temperatures for the agglutination process of purified ManBLs (Fig-10).

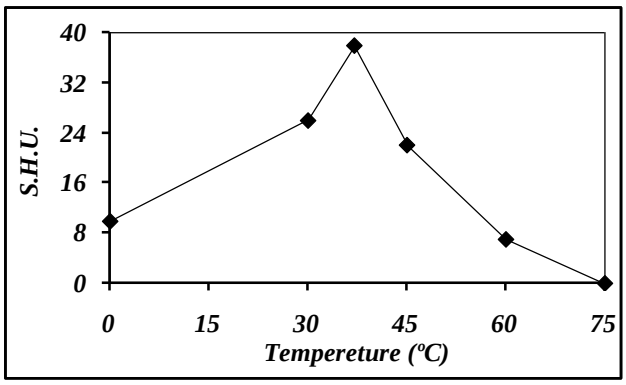

Fig-10: Temperature Effect on the Purified ManBLs Hemagglutination Activity

3.10 *The Carbohydrate Content in Purified Lectins*

Total carbohydrate content was found to be 13.5 % from the purified ManBL.

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