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# Clinical Utility of Altered Expressions of P<sup>53</sup>, Vascular Endothelial Growth Factor and Survivin in Patients with Bladder Cancer

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**Abstract:** Background and Objectives: Bladder cancer is considered a major problem world wide. Aberrant P<sup>53</sup>, mutant vascular endothelial growth factor and deregulated expression of survivin were used as biochemical markers for investigation, prognosis and follow up. Aims: This study was conducted to assess the prognostic impact of altered expressions of these parameters in Erbil population with bladder cancer and correlated with other confounding factors age, gender and smoking effects, these factors also correlated with histopathologic characteristics such as grade and stage. Patients and Methods: This prospective study enrolled 50 newly diagnosed patients with bladder cancer, in addition 50 apparently healthy adults age – sex matched were also involved in this study. Serum parameters levels were measured using enzyme linked immunosorbent assay. Patients had these general criteria, newly discovered cases, no deep x-ray therapy, no chemotherapy, no hormonal therapy with histologic and cytologic confirmation of bladder cancer. Statistical Study: Data were analyzed using SPSS v. 18. Results: The mean serum P<sup>53</sup>, vascular endothelial growth factor and survivin levels in patient and control groups were P<sup>53</sup> = 1.682 ± 0.665, 1.192 ± 0.284, vascular endothelial growth factor = 5.296 ± 2.8, 2.000 ± 0.704 and survivin = 5.468 ± 0.715, 4.240 ± 0.656 respectively. The statistical analysis revealed that, serum parameters levels were significantly increased in patients as compared with control group p < 0.001. Conclusion: Data revealed for the first time the relation between altered expressions of interested parameters in combination pattern with the incidence of bladder cancer in Erbil population, which has not previously reported in this region. This study tested the hypothesis and supported the concept that higher serum levels of these parameters might be a pathogenic and prognostic factors and a markers of tumor aggressiveness in bladder cancer. Early diagnosis is necessary for maximizing the rate of therapy. The gold standard care for the investigating of bladder cancer is cystoscopy which detects tumors accurately but it is invasive, expensive, and represents a high burden to the patients, and also, small papillary and flat-growing cancer may be missed. Regular cystoscopic examinations performed for monitoring the patients, because the recurrent rate is high. Therefore, this study was conducted to investigate the possibilities for replacing cystoscopy with more accurate, safe, no expensive, noninvasive diagnostic test and to recognize a new approach for providing opportunities for early detection which is an important goal to speed up the therapy of patients.

**Keywords:** Bladder Cancer, P<sup>53</sup>, Vascular Endothelial Growth Factor, Survivin

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## 1. Introduction

Cystoscopy is the most important technique for investigating bladder cancer (BC). In addition voided urine cytology, which is highly specific, it is used in combination with cystoscopy and its sensitivity increased with increased the tumor grade and stage, and urine cytology has a low sensitivity, and fails to detect superficial, low-grade lesions, cystoscopy has recommended for follow up BC patients,

even those with low grade and stage.

Many researchers investigated possibilities for replacing cystoscopy with a more accurate noninvasive diagnostic test. Early diagnosis and treatment are important to speed up the rate of therapy. Elevation of biomarkers were a markers of tumor aggressiveness and may help to identify a subgroup of patients with stage one and two. In the light of these findings, it can be suggested that, change in the levels of these parameters could assist in the pathogenesis of BC, estimating

the extent of tumor aggressiveness, diagnosis, prognosis and follow-up, they provided primarily a potential etiologic insight, they could be a useful tool to predict and control the progression of BC and monitoring response to therapy. Several biomarkers were studied to improve the detection and follow-up. This study based on the need to develop safe, reliable noninvasive, no expensive technique for detecting BC. It is unlikely that a single marker can be possible to provide a complete prognostic picture. This is the reason for doing the researches to find a novel biomarkers that could either complementary for investigating methods or to replace them [1]. Although single marker may serve in selected cases, there is growing evidence that multiple markers used either individually or in combinations will be required for most applications.

Accordingly, the current study was focused on the estimation of altered expressions of three biomarkers. The study revealed alterations in the expressions of these biomarkers in patients with different grades and stages.

The purpose of this study was to investigate the relationship between the over expressions of the parameters with the incidence of BC and to examine this hypothesis, these parameters measured in serum by enzyme linked immunosorbent assay (ELISA). The hypothesis of this study that there were a significant alteration in the serum levels of focused parameters in patients with BC which are worthy to investigate, the aims of current study were to assess the serum level of P<sup>53</sup>, VEGF and survivin in bladder tumor as compared with control group, in order to see the bladder cancer effect on the mean serum levels of these parameters, to study the age –factor to find out the age effect on the prevalence of bladder cancer, in addition, to detect the size or length of the tumor effect on the mean serum levels of studied parameters, to investigate the grade, stage, smoking, gender effects on the mean serum levels of interested parameters and finally, measuring the correlation coefficient between all the studied parameters.

## 2. Patients and Methods

The present study was conducted to assess the prognostic impact of altered expression of *p53*, *VEGF* and *survivin* status (in protein levels) in Erbil patients with BC. This case control prospective study was performed at Hawler Medical University, College of Pharmacy in period between 15/4/2012 and 15/4/2013, in 50 patients with bladder cancer were diagnosed at Rizgary Teaching Hospital and Zheen International Hospital. This study were also included age – gender matched 50 apparently healthy normal individuals which were enrolled as a control group for comparing purpose. These were confirmed to be normal by biochemical and hematological examinations.

### 2.1. Study Design

This prospective study was involved 100 participants, 50 newly diagnosed patients with BC of both sexes (48 males and 2 females ) with a mean age of  $65 \pm 10.23$  years were

enrolled in this study after exclusion of the diseases by history, laboratory investigations and clinical examination, and 50 sex- age matched apparently healthy adults were randomly selected in the current study for comparing purpose as a control group, this group included 47 healthy males and 3 females with a mean age  $62 \pm 9.593$  years.

All patients have these general criteria, patients were newly diagnosed cases, with no deep X- ray therapy, no chemotherapy, no hormonal therapy with histologic or cytologic confirmation of BC. All patients have histopathological reports which proved their diagnosis. All patient group was subjected to detailed history taking, thorough clinical examination, abdominal and pelvic ultrasonography, chest X-ray, computed tomography, urine cytology and histopathological diagnosis of urinary bladder biopsies obtained by cystoscopy. All BC patients who enrolled into the present study exhibited no clinically malignant disease other than BC. All diagnosed patients with BC were confirmed by at least one relevant biopsy in addition to other relevant findings, including history, physical examination, laboratory data, and clinical course. All procedures were in accordance with the established ethical standards. Patients completed a questionnaire that related to family history, past medical history, and medication history.

A case sheets had been prepared for each patient including the following information: age, gender, chief complaint, investigations (biochemical, hematological, histopathological, cytological, urine cytology).

All clinicopathological data of the patients, including tumor type, grade, and stage, were collected from their pathological reports and clinical files. In addition, none of healthy volunteers had history of rheumatoid arthritis, inflammation or recent pregnancy, trauma, surgery (within 1 month) or menstruation (within 1 week) and cardiovascular diseases can also cause the induction the circulating level of VEGF [2-3]. They will be documented that these clinical conditions lead to increase production of VEGF [4]. None of the patients had any other significant diseases or malignancies except BC and only the newly diagnosed patients with no prior chemotherapeutic treatment were included in this study. Oral consent were taken from all the participants before participation in the current study.

### 2.2. Methods

#### 2.2.1. The Protocol of the Study

I- Tissue biopsies were sent to the histopathological laboratory to diagnose the BC, grade, stage and tumor size .

II- The fasting blood ( 10 cc ) samples were drawn from the vein of the participants (healthy adults and patients with BC) of both genders. The blood samples were left for 30 minutes for coagulation purpose, and then made centrifugation for 15 minutes at 2500-3500 revolution per minutes (rpm). The sera of the patients were separated and were divided into several parts and put them into several plastic plain tubes to do the biochemical tests of the current study. The sera of the patients were stored at ( -20 C ° ) till the day of the analysis within ( 1-4 months). Bladder tumors

were staged according to the 1997 TNM classification and assigned a grade according to the WHO classification. The tumors were divided into non-invasive (pTa or pTis) and invasive malignancies (pT1 or higher stage). Cytological findings were graded 0 (no atypical cells), 1 to 2 (low grade atypia) and 3 (high grade atypia).

### 2.2.2. Biochemical Determinations

P<sup>53</sup>, VEGF and survivin expressions (at protein levels) were estimated using ELISA technique, the method of detection of the studied parameters were measured according to the manufacture instructions.

P<sup>53</sup> ELISA Kit (The name of the manufacture is RayBiotech, Inc, Email: info@biotech.com, Web: www.raybiotech.com).

VEGF ELISA Kit (The name of the manufacture is United States Biological, Email: chemicals@usbio.net, Internet: www.usbio.net).

Human anti-Survivin antibody (ELISA KIT) (The name of the manufacture is CUSABIO BIOTEC CO., LTD. Email: cusabio@cusabio.com, www.cusabio.com).

### 2.3. Statistical Study

Data were analyzed using SPSS v. 18. The results were expressed as mean ± SD. T-test was conducted to compare the two means. Post-hoc test was conducted to show the significant difference between two of the three variables.

Multiple regression was used to show the association between each of the biomarkers (as dependent variables) and several independent variables. P ≤ 0.05 was considered statistically significant.

## 3. Results

The present study addressed, the role of mutant P<sup>53</sup>, VEGF aberrations and survivin altered expression (at the protein levels) in Erbil patients with BC, illustrated the clinical significance of these aberrations in biochemical markers.

### 3.1. Baseline Characteristics

The studied group comprised, 96% of patients were males, and about 4% were females. The mean age at diagnosis was 65 ± 10.23 years. The presenting clinicopathological features of the patients group was shown in (Table 1), 40% of the patient group were smoked and 60% were non-smoked, while 56% of the patients with in stage I (T<sub>1</sub>) and 44% of the patients with in stage II (T<sub>2</sub>), in addition, 26%, 40% and 34% of patients had grade I, II, III respectively. Moreover, the tumor size was 350.5208 and the mean serum levels of P<sup>53</sup>, VEGF and survivin were 1.682 ± 0.665, 5.296 ± 2.8 and 5.468 ± 0.715 respectively. While, (Table 2) shows the characteristics of the control group.

Table 1. The characteristics of the studied patients.

	Control	%	No.	Age	Tumor Size	Patients	Pvalue	P53 ng/ml	VEGF ng/ml	Survivin ng/ml
Number	50		50							
Smoking		40	20							
Non-Smoking		60	30							
Males		96	48							
Females		4	2							
Stage I		56	28					1.550	4.268	5.253
Stage II		44	22					1.850	6.667	5.800
Grade 1		26	13					1.346	3.715	4.678
Grade 2		40	20					1.610	5.145	5.164
Grade 3		34	17					2.024	3.510	6.006
Mean				65.12	350.52			1.682	5.296	5.468
Median				65.00	259.50			1.400	3.3500	4.900
±SD				10.23	374.44			0.665	2.8	0.715
P53 ng/ml	1.19 ± 0.28					1.68 ± 0.66	< 0.001			
VEGF ng/ml	2 ± 0.7					5.3 ± 2.8	< 0.001			
Survivin ng/ml	4.24 ± 0.66					5.47 ± 0.72	< 0.001			

Table 2. The characteristics of the control group.

Age	No	Mean	±Std.Deviation	Std.Error	Lower Limit	Upper Limit
P53						
<50	4	1	0.282843	0.160286	0.67751	1.32249
50-59	16	1.1	0.272554	0.0801431	0.938755	1.26124
60-69	20	1.23	0.205751	0.0716822	1.08578	1.37422
70-79	6	1.633	0.11547	0.130873	1.37002	1.89665
80+	4	0.9	0.141421	0.160286	0.57751	1.22249
Total	50	1.192	0.284195			
VEGF						
<50	4	1.65	0.353553	0.50302	0.637917	2.66208
50-59	16	2.1	0.630193	0.251516	1.59396	2.60604
60-69	20	1.95	0.791974	0.224963	1.49738	2.40262

Age	No	Mean	±Std.Deviation	Std.Error	Lower Limit	Upper Limit
70-79	6	2.567	0.7636396	0.410724	1.7403	3.39303
80+	4	1.45	0.636396	0.503032	0.437917	2.46208
Total	50	2.008	0.708237			
Survivin						
<50	4	4.55	0.353553	0.490153	3.56383	
50-59	16	4.15	0.611789	0.245077	3.65692	
60-69	20	4.2	0.765942	0.219203	3.75897	
70-79	6	4.6	0.818535	0.400208	3.7948	
80+	4	3.35	0.494975	0.490153	2.36383	
Total	50	4.192	0.701142			
Mean Age	50	62	9.593			
Gender Males	47					
Gender Females	3					
Smoking	22					
Non-Smoking	28					

### 3.2. Bladder Cancer – Effect on the Serum Levels of Studied Parameters

**Table 3.** Comparison between the patients and control groups regarding the mean serum levels of P53, VEGF and surviving.

	Group	N	Mean	SD	p value
P53 ng/ml	patients	50	1.682	.665	< 0.001
	Control	50	1.192	.284	
VEGF ng/ml	patients	50	5.296	2.800	< 0.001
	Control	50	2.000	.704	
Survivinng/ml	patients	50	5.468	.715	< 0.001
	Control	50	4.240	.656	

The mean serum levels of P<sup>53</sup> in patient and control groups were 1.682 ± 0.665, 1.192 ± 0.284 respectively (Table 3), in addition, the mean serum levels of VEGF in patient and control groups were 5.296 ± 2.800, 2.000 ± 0.704 respectively, moreover, the mean serum levels of survivin in patient and control were 5.468 ± 0.715, 4.240 ± 0.656 respectively.

**Table 4.** Comparison between different age categories regarding the serum levels of interested parameters in patients group.

Age	No	Mean	Std.Deviation	Std.Error	Lower Limit	Upper Limit	Coeff.OfVariation%	F-Ratio	p-Value Between groups & with groups
P53									
<50	4	1	0.282	0.160	0.677	1.322	28.28	4.431	0.18
50-59	16	1.1	0.272	0.080	0.938	1.261	24.78		
60-69	20	1.23	0.205	0.071	1.085	1.374	16.73		
70-79	6	1.633	0.115	0.130	1.370	1.896	7.07		
80+	4	0.9	0.141	0.160	0.577	1.222	15.71		
Total	50	1.192	0.284				23.84		
VEGF									
<50	4	1.65	0.353	0.503	0.637	2.662	21.43	0.95	0.457
50-59	16	2.1	0.630	0.251	1.593	2.606	30.01		
60-69	20	1.95	0.791	0.224	1.497	2.402	40.61		
70-79	6	2.567	0.763	0.410	1.740	3.393	29.76		
80+	4	1.45	0.636	0.503	0.437	2.462	43.89		
Total	50	2.008	0.708				35.27		
Survivin									
<50	4	4.55	0.353	0.490	3.563		7.77	1.14	0.366
50-59	16	4.15	0.611	0.245	3.656		14.74		
60-69	20	4.2	0.765	0.219	3.758		18.24		
70-79	6	4.6	0.818	0.400	3.794		17.79		
80+	4	3.35	0.494	0.490	2.363		14.78		
Total	50	4.192	0.701				16.73		

Laboratory studies have shown that there were significant elevations in serum levels of P<sup>53</sup>, VEGF and survivin as compared with control group P < 0.001, P < 0.001, P < 0.001 respectively, these findings were accordance with current hypothesis.

### 3.3. Age– Factor

The mean age of patients at diagnosis were 65 ± 10.23 (Table 1), in addition the (Table 4) was shown the comparison between different age categories regarding the mean serum levels of interested parameters in patients group.

The statistical studies was revealed that there were no statistical differences between the different age categories .

The regression coefficient study was shown regarding the effect of age on the mean serum levels of dependent variables P<sup>53</sup>, VEGF, survivin that, there was no significant effect of age on the mean serum levels of P<sup>53</sup> p = 0.119, VEGF p = 0.499, survivin p = 0.333 (Table 5).

Table 5. The effect of age on the mean serum levels of dependent studied parameters.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
P <sup>53</sup>	Constant	1.86	0.89		2.09	0.043	0.063	3.658
	Age	-0.01	0.009	-0.22	-1.591	0.119	-0.033	0.004
	TumorSize	0	0	0.241	1.59	0.12	0	0.001
VEGF	Constant	3.22	3.25		0.99	0.33	-3.34	9.79
	Age	-0.02	0.033	-0.08	-0.68	0.5	-0.09	0.04
	Tumor Size	0.002	0.001	0.32	2.43	0.02	0	0.004
survivin	Constant	3.93	1.36		2.9	0.009	1.09	6.77
	Age	0.012	0.012	0.2	0.99	0.33	-0.01	0.04
	Tumor Size	0	0	-0.04	-0.17	0.9	-0.001	0.001

3.4. Gender Factor

The statistical study was estimated that men were more susceptible to get bladder cancer than women (Table 1), 48 out of the 50 patients with BC were men (96 %) and only 2 patients werewomen ( 4%).

In addition, as shown in (Table 6) the regression coefficient were represented that there were no significant effects of gender on dependent variables P<sup>53</sup>, VEGF and survivin p = 0.369 , P= 0.260 ,P= 0. 917 respectively.

Table 6. The effect of gender on the mean serum levels of dependent variables.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
P53	(Constant)	1.29	.51		2.515	.014	.267	2.311
	Group(cases)	.51	.15	.396	3.500	.001	.219	.799
	Sex	.24	.27	.100	.904	.369	-.292	.777
VEGF	(Constant)	1.2	1.99		.602	.549	-2.776	5.177
	Group	3.34	.57	.571	5.917	< 0.001	2.215	4.469
	Sex	1.17	1.03	.106	1.135	.260	-.889	3.238
survivin	(Constant)	4.177	.725		5.762	.000	2.719	5.634
	Group	1.253	.202	.686	6.211	< 0.001	.848	1.659
	Sex	-.040	.380	-.012	-.105	.917	-.804	.724

3.5. Smoking –Factor

The statistical study was investigated the description of patients group regarding the smoking effect, as shown in (Table 1), 40% of the patients were smokers, while 60% of the patients were non- smokers.

In control group, (Table 2) shows that 44% of the control

group was smokers and 56% was non-smokers. In addition, (Table 7) was revealed that, there were a highly significant effects of smoking on the mean serum levels of VEGF and survivin, P < 0.001, while there was no significant effect of smoking on the mean serum levels of P<sup>53</sup>.

Table 7. The smoking effect on the mean serum levels of P<sup>53</sup>, VEGF and survivin.

Smoking	No	Mean	Std.Deviation	Std.Error	Lower Limit	Upper Limit	Coeff.or variation	F-Ratio	P
P53									
Yes	20	1.76	0.672309	0.129207	1.5763	1.9437	38.20%	1.96	0.1683
No	30	1.5266	0.5064	0.105497	1.3766	1.6766	33.17%		
Total	50	1.62	0.5834				36.02%		
VEGF									
Yes	20	6.9263	2.5234	0.4286	6.1126	7.7399	36.43%	38.21	0.001
No	30	3.54	1.3058	0.3411	2.8924	4.1875	36.89%		
Total	50	4.8530	2.4894				51.30%		
Survivin									
Yes	20	6.1444	1.0001	0.28709	5.5803	6.70855		12.43	0.001
No	30	4.9157	0.7918	0.19759	4.5275	5.30403			
Total	50	5.3107	1.0275						

3.6. Grade –Factor

The descriptive of the patients with BC at different grades I, II, III, regarding the number of patients in each group were shown in (Table 8), the mean value ± SD of P<sup>53</sup>, VEGF and survivin. The statistical analysis was shown that, mean serum levels of P<sup>53</sup> was increased significantly according to grade progression, moreover, there was a significant differences

between the different grades ( 1X3 , 2X3) ( p= .015).

In addition, the statistical analysis illustrated that the serum levels of VEGF was increased significantly according to grade progression, in addition, there were significant differences between the grades (1X3)(p= 0.10), furthermore, the statistical analysis was represented that, the serum levels of survivin was increased according to grade progression,

moreover, table 8 was revealed that, there were statistical significant differences between different grades ( 1x2 ,1x3 , 2x3 ) p = 0.05.

**Table 8.** Comparison between different grades and stages of bladder cancer regarding the serum levels of studied parameters.

	Grade	N	Mean	±SD	p value	Significance
P53 ng/ml	1.00	13	1.346	.288	.015	1 X 3
	2.00	20	1.610	.632		
	3.00	17	2.024	.772		
	Total	50	1.682	.665		2 X 3
VEGF ng/ml	1.00	13	3.715	2.227	.010	1 X 3
	2.00	20	5.145	1.841		
	3.00	17	6.769	3.510		
	Total	50	5.296	2.800		
Survivin ng/ml	1.00	13	4.678	.217	.050	1X2
	2.00	20	5.164	.271		1x3
	3.00	17	6.006	.679		2x3
	Total	50	5.283	.388		
P53 ng/ml	Stage	N	Mean	±SD	.114	p value
	1.00	T1/28	1.550	.596		
	2.00	T2/22	1.850	.722		
	Total	50	1.682	.665		
VEGF ng/ml	1.00	T1/28	4.268	2.047	.002	
	2.00	T2/22	6.667	3.116		
	Total	50	5.296	2.800		
Survivin ng/ml	1.00	T1/28	5.253	.614	.046	
	2.00	T2/22	5.800	.760		
	Total	50	5.468	.715		

**3.7. Stage- Factor**

The descriptive of the patients with BC at different Stage (T<sub>1</sub>,T<sub>2</sub>), regarding the number of patients in each group ,the mean value ± SD of P<sup>53</sup> ,VEGF and survivin were shown in (Table 8).

A statistical study was used to check the difference between T<sub>1</sub> and T<sub>2</sub> regarding the mean serum levels of interested parameters, ( Table 8 ) was shown that, regarding P<sup>53</sup>,there were no significant differences between (T<sub>1</sub>,T<sub>2</sub>),

p=0.114. Whereas, there were a statistical significant differences between (T<sub>1</sub>,T<sub>2</sub>) regarding the mean serum levels of VEGFp=.002 and survivin p= 0.046.

**3.8. Tumor Size – Factor**

There were no significant effects of tumor size on the mean serum levels of P<sup>53</sup> and survivin P = 0.12, P = 0.87 respectively. While,there was a significant effect of tumor size on the mean serum level of VEGF, P= 0.02 (Table 9).

**Table 9.** The effect of tumor size on the mean serum levels of studied parameters.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
P <sup>53</sup>	Constant	1.86	0.89		2.09	0.043	0.063	3.658
	Age	-0.014	0.009	-0.22	-1.591	0.119	-0.033	0.004
	TumorSize	0	0	0.241	1.59	0.12	0	0.001
VEGF	constant	3.221	3.25		0.991	0.328	-3.343	9.785
	Age	-0.023	0.033	-0.082	-0.682	0.499	-0.089	0.044
	Tumorsize	0.002	0.001	0.322	2.431	0.02	0	0.004
Survivin	constant	3.931	1.357		2.898	0.009	1.092	6.771
	Age	0.012	0.012	0.201	0.994	0.333	-0.013	0.038
	TumorSize	0	0	-0.037	-0.165	0.87	-0.001	0.001

**3.9. Correlation Coefficient**

There were no significant correlation between all studied parameters in control group (Table 10), while, (Table 11) was

shown that ,there were a positive significant correlation between P<sup>53</sup> with VEGF, survivin with P<sup>53</sup> and survivin with VEGF at the level p = 0.01 in patients group.

**Table 10.** The correlation coefficient between all studied parameters in control group.

		P53 ng/ml	VEGF ng/ml	Survivin ng/ml
P53 ng/ml	Pearson Correlation	1	.304	.125
	Sig. (2-tailed)		.139	.552
	N	25	25	25
VEGF ng/ml	Pearson Correlation	.304	1	.192
	Sig. (2-tailed)	.139		.357
	N	25	25	25
Survivin ng/ml	Pearson Correlation	.125	.192	1
	Sig. (2-tailed)	.552	.357	
	N	25	25	25

**Table 11.** The correlation coefficient between all studied parameters in patients group.

Correlations <sup>a</sup>		P53 ng/ml	VEGF ng/ml	Survivin ng/ml
P53 ng/ml	Pearson Correlation	1	.639**	-.050
	Sig. (2-tailed)		.000	.801
	N	50	50	50
VEGF ng/ml	Pearson Correlation	.639**	1	.225
	Sig. (2-tailed)	.000		.260
	N	50	50	50
Survivin ng/ml	Pearson Correlation	0.675**	0.734**	1
	Sig. (2-tailed)	.000	.001	
	N	50	50	50

\*\* . Correlation is significant at the 0.01 level (2-tailed).

a. Group = Cases

## 4. Discussion

### 4.1. General View

In this prospective case control study, a considerable attention has focused on the association between the variation in the serum levels of investigated parameters with the existence of BC. The hypothesis is that high levels of these parameters would be markers of a worse outcome. A combination of these markers ( $\geq 2$ ) has synergistic effects, the higher the number of altered biomarkers is, the higher the risk of disease progression. This study was designed to examine the association between the significant elevations of focused parameters with the prevalence of BC, moreover, the current study was investigated the relations between serum levels of focused parameters in both studied groups, taking into consideration other confounding factors age, gender and smoking factors as well as these parameter were correlated with grade, stage and tumor size.

The aim of the present study was to investigate the possible correlation and the impact of this pivotal cancer proteins on the prognosis of BC. Thus, the purpose of this study was to investigate the relationship between P<sup>53</sup>, VEGF and survivin overexpressions with the incidence of BC and to examine this hypothesis, the studied parameters were measured in serum using ELISA technique.

The ultimate aim of this approach was, to evaluate the impact of BC on serum levels of the interested parameters and to investigate the expression pattern of these parameters in order to determine their potential prognostic significances on the prevalence of BC and to investigate the possible correlation between the impact of these proteins on the prognosis, follow up and diagnosis of BC.

### 4.2. Effect of the Bladder Cancer on the Serum Levels of the Studied Parameters

This study was performed on the basis of the existing hypothesis that the patients with BC had significantly elevation in the mean serum levels of focused parameters as compared with the control group (Table 3)  $P < 0.001$ . Regarding the level of P<sup>53</sup> was compatible with the hypothesis and in concord with the results of many previous studies[5–7]. Recent studies have suggested an association between significant elevation of serum level of P<sup>53</sup> and risk of BC.

The explanation for increased the serum level of P<sup>53</sup> was that, mutant P<sup>53</sup> has usually increased half-life and is more easily detected [8] than the normal P<sup>53</sup> protein. Many reports have documented an alterations in the P<sup>53</sup> gene in BC [6, 9, 10] and it has been suggested that P<sup>53</sup> mutations might play an important role in BC progression [6].

The scientific researches were carefully reviewed and detected that the all publishers would agree that alterations in the p53 contributed to bladder tumor progression. Whereas the VEGF level, oxidative stress (OS) is linked to carcinogenesis [11], oxygen free radicals increase tumor cell production of the VEGF, moreover, OS can therefore lead to angiogenesis of the tumor. Oxygen radicals may also induce tumor cell migration, increasing the risk of invasion and metastasis [12]. Several reports have also demonstrated that the increased in the oxidant / antioxidant ratio are directly correlated with tumor progression, angiogenesis, and migration / invasion [13-17]. A number of studies have demonstrated a strong association between elevated tumor expression of VEGF and advanced disease in various cancers

[18]. Elevation in the serum levels of VEGF indicated to the angiogenesis.

The statistical analysis revealed that there were a highly significant differences between the levels of VEGF in patients group as compared with the control group  $p < 0.001$  (Table 3). So, this result was consistent with many previous researches [18-20]. This finding indicated that metastatic of BC resulting in a net increase in reactive oxygen species production, which leads to the induction of redox -sensitive protumorigenic and prometastatic genes such as VEGF. Systemic hypoxemia [21] induces elevations of serum VEGF levels. Hypoxia induce angiogenesis, which can occur when tumors outgrow their blood supply, is a likely explanation for the induction of angiogenesis in cancerous tissues. Tumor cells may produce many factors that promote angio- and lymphangiogenesis in order to obtain a sufficient nutritive supply as well as providing pathways for dissemination [22]. Regarding survivin, this study was designed to investigate the potential suitability of detected survivin in serum as a predictive molecular marker of BC and correlation of serum survivin level with tumor grade and stage. The statistical analysis revealed that there were highly significant differences between the level of survivin in patients group as compared with the control group  $p < 0.001$  (Table 3). Accordingly, this finding was consistent with current hypothesis. Survivin is a multifunctional protein that inhibits apoptosis, regulates cell division, and promotes angiogenesis. Survivin is common in tumor cells and represents an important target for diagnostic and prognostic purposes in BC. These aspects of survivin have also been investigated in the diagnosis and the prediction of tumor recurrence in patients with BC. Several studies on survivin expression confirmed the potential value of this protein as sensitive marker for early, noninvasive detection of BC [23-27]. In this study, the expression levels of survivin was significantly higher in patients with BC as compared with the control group ( $P < 0.001$ ) (Table 3), this finding was in harmony with previous results [28-30], which predict the role of survivin in the occurrence and development of BC. Previously, several groups have investigated the expression of survivin in bladder tissues- cells and introduced survivin as a very sensitive and specific marker for diagnosis and prognosis of BC [23,24, 31,32].

#### 4.3. Age – Factor

Age distribution was comparable with transitional cell carcinoma (TCC) distribution worldwide, patients with average age 62.6 years were affected with TCC that was in harmony with that of [33]. In addition, it was published that, cancer of the bladder affected mainly the elderly people with a median age at diagnosis of 65–70 years [34]. Moreover, [35] reported that the median age of BC at diagnosis is 70 years of age for men and women. Bladder cancer is rare in person age less than 40 years and typically nonaggressive and well differentiated [36]. These previous findings were concordant, with the result of the current study, the mean age of patients with BC at diagnosis was  $65 \pm 10.23$  years ( median 65 )

(Table 1). While (Table 4) was revealed the comparison between different age categories regarding the mean serum levels of interested parameters in patients group, this table was shown that there were no significant differences between all age categories regarding the mean serum level of studied parameters  $p > 0.05$ .

##### 4.3.1. P<sup>53</sup>

The investigation of the current study was consistent with the finding of [37] who reported that an insignificant relationship was observed between p<sup>53</sup> and age (Table 4).

##### 4.3.2. VEGF

As it was shown in (Table 4), the regression coefficient that, there was no significant correlation between the age factor and the mean serum levels of VEGF  $P = 0.499$ . The current result was concordant with finding of [37] who reported that no correlation was found between VEGF, and age. While, it was reported that, VEGF overexpression was more frequently observed in the old age group ( $\geq 60$  years old)  $P = 0.013$  [38].

##### 4.3.3. Survivin

According to the data published by [39] that, the prognostic impact of survivin was independent to the age of the patients, this result was in harmony with the finding of the current study as it was represented in (Table 4) regression coefficient, the mean serum levels of survivin was independent to the age of the patients  $P = 0.333$ . In concord with the result of the current study, [40, 41] also found that, no association between survivin expression and age - factor. Moreover, it was estimated that, the prognostic impact of survivin was independent of patient age [39].

#### 4.4. Gender – Factor

Men is considered the risk factor for incidence of BC [42,43]. Bladder cancer occurred more often in men than in women [33], this investigation was in harmony with the results of the current study. In the current study 96% of the patients were men and 4% of the patients were women (Table 1). In addition, the observation of the current study was concordant with previous findings [35,36] who reported that, men are 3 to 4 times more likely to develop BC than women.

##### 4.4.1. P<sup>53</sup>

There was no significant correlation between gender with the mean serum levels of P<sup>53</sup>  $P = 0.904$  (Table 6), and this finding was concordant with the result of [37] who reported that, an insignificant relationship was observed between p<sup>53</sup> and gender.

##### 4.4.2. VEGF

There was no significant correlation was represented between gender and VEGF  $p = 0.260$  (Table 6), this investigation was in harmony with the finding of [37].

##### 4.4.3. Survivin

There was no significant correlation was represented between gender and survivin  $p = 0.917$  (Table 6), this result



was concordant with the result of [40, 41] who published that, no association between survivin expression and gender - factor.

#### 4.5. Smoking –Factor

It is well known that OS and BC are closely related [44-46]. Cigarette smoking is considered as a risk factor for induction of OS and involved in DNA damage, and impaired anti oxidative defense mechanism [47]. Reactive oxygen species have multiple functions and are implicated in tumor initiation and progression [48,49].

##### 4.5.1. P<sup>53</sup>

The presence of P<sup>53</sup> mutations was also analyzed as a function of the smoking habits of the patients. The finding of the present study that, there was no significant correlation between P<sup>53</sup> and smoking P= 0.1683, as it was represented in (Table 7), this finding was consistent with previous finding [50].

The explanation for this finding which is in harmony with the concept that P<sup>53</sup> is considered as safe guard gene and it is the last gene which is affected by the oxidative damage.

##### 4.5.2. Vascular Endothelial Growth Factor

The data of the current study was exhibited that, there was a highly significant effect of smoking on the mean serum levels of VEGF P = 0.001 (Table 7).

##### 4.5.3. Survivin

Cigarette smoking is the common risk factor for induction OS and leading to DNA nitrogenous base oxidation, and destroyed the anti oxidative defense mechanism [47]. There was highly significant effect of smoking on the mean serum levels of survivin p= 0.0016 (Table 7).

To our knowledge, by reviewing the scientific researches, no data have published to reveal the association between the effect of cigarette smoking on the mean serum levels of survivin in patients with BC. Accordingly, there were no previous studies dealing with the effect of smoking on VEGF and survivin in BC. So, as there are no previous studies dealing with effect of smoking on the serum levels of VEGF and survivin, this finding is considered as the first attempt deals with this correlation.

#### 4.6. Grade –Factor

The descriptive of the patients with BC at different grades I, II, III, regarding the number of patients in each group, the mean value ± SD of interested parameters was represented in (Table 8).

##### 4.6.1. P<sup>53</sup>

The statistical analysis was shown that, the serum levels of P<sup>53</sup> was increased significantly according to grade progression, moreover, there was a significant differences between the different grades p= 0.015 (Table 8). It has been well documented that alterations in P<sup>53</sup> are also associated with overexpression of P<sup>53</sup> in advanced tumor grade. The research was undertaken to provide insights into the potential

role of P<sup>53</sup> for tumor metastasis and progression. It was illustrated that P<sup>53</sup> overexpression has been shown to correlate with tumor grade in primary bladder carcinomas [51], and it has been suggested that P<sup>53</sup> mutations might play an important role in BC progression [6]. The results of the current study were in harmony with the findings of [37,52] who published that, a statistical significance was noticed between P<sup>53</sup> with tumor grade P = 0.016. Higher grade tumors showed higher expression of P<sup>53</sup> protein than low grade tumors.

##### 4.6.2. Vascular Endothelial Growth Factor

The statistical analysis illustrated that, the serum levels of VEGF was increased significantly according to grade progression, moreover there were significant differences between the different grades P=0.10 (Table 8). In contrast to the current study [37] reported that, no correlation was found between VEGF with tumor grade.

##### 4.6.3. Survivin

The statistical analysis was exhibited that, the serum levels of survivin was increased according to grade progression, moreover, there were significant differences between different grades ( 1x2 , 1x3 , 2x3 ) p = 0.05 (Table 8) . In concord with the current result, [25,31,56] found a direct correlation between survivin overexpression and higher grade of tumors. Some groups reported a significant association of survivin expression with higher tumor grade in urinary samples or tissue specimens [23,28,29,53,54], these findings was agreeable with the result of the current study, and this reflect the role of survivin in differentiation of the tumor to its grade. Moreover, in contrast, [30,55] could not find a significant correlation between survivin with the grade of the tumors . While, the findings reported by [28,57,58] failed to reveal any significant association with tumor grade.

#### 4.7. Stage –Factor

A statistical study was used to check the difference between T<sub>1</sub> and T<sub>2</sub> regarding the mean serum values of the interested parameters.

##### 4.7.1. P<sup>53</sup>

The mean serum levels of P<sup>53</sup> were increased according to stage progression but this elevation was not reached the significant degree, so, there were no significant differences between (T<sub>1</sub>, T<sub>2</sub>), p > 0.05 (Table 8).

The explanation for this non-significant finding, might be due to small number size of population. In harmony with the result of the current study, [37] found that, an insignificant relationship was observed between P<sup>53</sup> with the stage, and lymph node metastasis. Overexpression of p<sup>53</sup> protein was found more frequently in tumors with high stage and was clearly associated with poor clinical outcome and it was also consistent with the our hypothesis that nuclear accumulation of the p<sup>53</sup> protein becomes more important and apparent in the late stages of bladder carcinogenesis.

As carefully reviewed the scientific researches and detected that the all investigators would agree that,

alterations in the P<sup>53</sup> contributed to bladder tumor progression. The result of the present study regarding p<sup>53</sup> protein overexpression in BC and its prognostic value was concordant with many previous studies [6,38,51,52,59] who illustrated that P<sup>53</sup> overexpression has been shown to correlate with tumor stage in BC, and it has been believed that P<sup>53</sup> alterations might play an essential role in BC progression [6]. In addition, it was stated that, over expression of P<sup>53</sup> protein was significantly associated with positive lymph nodes, advanced disease stage [59]. Moreover, it was reported that, P<sup>53</sup> might be a marker of depth of invasion or lymph node involvement [38]. It was investigated that P<sup>53</sup> alteration is a significant predictor of BC progression. Mutation of P<sup>53</sup> might accelerate carcinogenesis, especially by enhancement of cell proliferation, loss of apoptosis and by insufficient DNA repair [60]. It has now been widely accepted that the P<sup>53</sup> state plays a role in the progression of bladder tumors [61].

#### 4.7.2. Vascular Endothelial Growth Factor

There were a highly significant differences between (T<sub>1</sub>,T<sub>2</sub>) regarding the serum levels of VEGF p=0.002 (Table 8). Recent studies demonstrated a key role of angiogenesis, so, it was aimed to clarify the potential link between VEGF with disease progression and metastatic dissemination in patients with BC. The serum concentrations of VEGF were significantly increased according to stage progression, so, the data of the current study was consistent with the hypothesis that VEGF overexpression was strongly associated with late stage of tumor, increased incidence of BC, which was consistent with several previous studies [18,20,38,59,62]. This result supports the essential role of VEGF in regulating tumor angiogenesis. So, VEGF was also considered as an important factor for the prediction of the clinical course as well as it might be a marker of metastasis related to the depth of invasion or lymph node involvement [38]. While in contrast, [37] reported that, no correlation was found between VEGF, and stage progression and lymph node status.

#### 4.7.3. Survivin

Survivin expression might be considered as an unfavorable prognostic factor of BC. There were a significant differences between (T<sub>1</sub>,T<sub>2</sub>) regarding the mean serum levels of survivin p= 0.05 (Table 8). The statistical analysis was represented that, the serum levels of survivin was increased according to stage progression. In addition, some groups reported a significant association of survivin expression with higher tumor stage in urinary samples or tissue specimens [23,25,28,30,31,53,54,63] who cited that the higher expression levels of survivin showed a significant correlation P = 0.02 with tumor invasiveness (T<sub>1</sub>,T<sub>2</sub>) as well as associated with progressing pathologic stage, these findings were in concord with the result of the present study (Table 8). Additionally, survivin expression in the current study was in concord with finding reported by [41], who reported that, survivin was not expressed in normal bladder urothelium but was overexpressed in 67% of T1 tumors as well as [64] stated that, it is worthy of note that survivin mRNA

expression in the tumor was associated with lymph node metastasis, tumor stage.

In contrast with result of the current study, the finding published by [28,57,58] failed to reveal any association with tumor stage. Moreover, in harmony with the investigation of the current stud, [65] found that, expression of survivin and VEGF were significantly associated with TNM stage, T-stage and metastasis of nasopharyngeal carcinoma.

Accordingly, taken together, the finding of the present study and other published data on survivin expression in BC indicated that high survivin expression was a marker of tumor aggressiveness, so that, the lower level of serum survivin is the less invasive disease. This result also supported the search for survivin targeting strategies as novel approaches in the management of BC.

#### 4.8. Tumor Size –Factor

There were no significant effects of tumor size on the mean serum levels of P<sup>53</sup> and survivin, P= 0.12, p = 0.87 respectively (Table 9), while, there was a significant effect of tumor size on the mean serum level of VEGF, P= 0.02. In harmony with the result of the present study, [39,40] published that, the prognostic impact of survivin was independent of patient tumor size.

In contrast, [64] published that, it is worthy of note that survivin mRNA expression was associated with tumor size, depth of invasion, and decreased overall survival [64].

#### 4.9. Correlation Coefficient

The goal of this prospectively designed study was to assess the correlation between serum levels of P<sup>53</sup>, VEGF and survivin. The current study demonstrated that, the expression of survivin was consistent with the expression of VEGF in BC and both were closely correlated with poor prognosis of patients with BC, this findings were consistent with the results demonstrated by [65]. It was investigated that, there was a significant positive correlation between P<sup>53</sup> and VEGF in gastric cancer [66], this result was concordant with the finding of the current study at the level p= 0.001. The scientific researches were carefully reviewed and do not identified any study in BC dealing with correlation between P<sup>53</sup> and VEGF, so this study was the first attempt to deal with this type of correlation. In contrast to the result of the current study, [67] published that, there was a negative correlation between P<sup>53</sup> and VEGF in brain cancer. Whereas, [68] found out that, there was no correlation between P<sup>53</sup> and VEGF in patients with superficial BC. In addition, it was investigated that, there was a significant positive correlation between survivin and VEGF [69], these findings were in harmony with the finding of the current study at the level p= 0.001. Moreover, it was investigated that, there was a significant positive correlation between survivin and P<sup>53</sup> [70], this investigation was consistent with the result of the current study at the level p=0.001. While, [71] investigated different result that, there was no significant correlation between survivin and P<sup>53</sup> in patients with breast cancer. In addition, it

was concluded that the survivin gene expression is negatively regulated by P<sup>53</sup> in non-small cell lung carcinoma (NSCLC) [72], which can inhibit apoptosis and accelerate tumor proliferation to produce more aggressive carcinomas. It was suggested that survivin expression is negatively regulated by P<sup>53</sup> [73]. It was speculated that most patients with more advanced disease (T4 and nodal /systemic metastasis) may already have survivin overexpressed due to a later stage in the molecular pathogenesis process potentially expressing other molecular characteristics related with progression and metastasis. Whereas, the current study supported a survivin in combination with other biomarkers P<sup>53</sup> and VEGF to enhance prognostication and prediction. Promising candidate biomarkers that could potentially compose the prediction integrated panel would include those involved in cell cycle regulation (such as P<sup>53</sup>, VEGF) and cell proliferation in addition to survivin. The results of the current study and others have shown that structural and functional defects of these candidate biomarkers were common in BC and were associated with poor oncologic outcomes [24,54,74-76]. It was interesting to note that the majority of BC with significantly express mutant P<sup>53</sup> and high levels of VEGF and survivin proteins. This was not surprising, in view of current hypothesis that, current data demonstrated a highly significant association between P<sup>53</sup> nuclear accumulation with serum elevation levels of VEGF and survivin.

The strength of the present work lay in the fact that this was the first attempt that included the estimation of the P<sup>53</sup>, VEGF and survivin in combination manner. Furthermore, an interesting and novel finding in the present study was the correlation between aberrant expression of the P<sup>53</sup>, VEGF and survivin proteins and their relation with incidence of BC and the results were also correlated with age, gender and smoking effect as well as correlated with grade, stage of the tumors and tumor size. The identification of molecular markers that provide insight into the potential behavior or aggressiveness of tumors is a necessary step for the improvement of cancer treatment.

The limitation of this study that, because of the laboratory kits are very expensive, in addition, practical and economic constraints so, only a relatively small number size of population was enrolled in this study.

## 5. Conclusions

The current study revealed for the first time an expression of P<sup>53</sup>, VEGF, and survivin in combination patterns in patients with BC in Erbil city. P<sup>53</sup>, VEGF, and survivin could be used as prognostic biomarkers in BC. In addition, combinations of these markers ( $\geq 2$ ) have synergistic effects. The higher the number of altered biomarkers is, the higher the risk of disease progression. These findings suggest the possible involvement of alterations in the expression profile of P<sup>53</sup>, VEGF, and survivin in BC pathogenesis. Circulating VEGF seems to be a reliable marker of angiogenesis and tumor progression. The results of this study confirmed that the expression pattern of survivin in patients could have a

potential practical usefulness in BC diagnosis. Early diagnosis is necessary for maximizing the rate of therapy. The gold standard care for the investigating of BC is cystoscopy which detects tumors accurately but it is invasive, expensive, and represents a high burden to the patients, and also, small papillary and flat-growing cancer may be missed. Regular cystoscopic examinations performed for monitoring the patients, because the recurrent rate is high. Therefore, this study was conducted to investigate the possibilities for replacing cystoscopy with more accurate, safe, no expensive, noninvasive diagnostic test and to recognize a new approach for providing opportunities for early detection which is an important goal to speed up the therapy of patients.

Further study on the large population are needed to clarify these findings.

## Abbreviations

BC: Bladder cancer, ELISA: Enzyme linked immunosorbent assay, OS: Oxidative stress, VEGF: Vascular endothelial growth factor.

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