Serum cyfra21-1 associated with biological aggressiveness and poor prognosis in male patients with urothelial carcinoma of bladder.

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Research article

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Abstract

Background : To assess the prognostic value of preoperative serum cyfra21-1 in male patients with urothelial carcinoma of bladder treated with radical cystectomy.

Methods: Patients underwent radical cystectomy from 2009-2018 at our center were retrospectively analyzed and 267 male patients met our criterions. The median follow-up was 34 months. The serum level of cyfra21-1 was measured using enzyme linked immunosorbent assay. Patients were divided into two groups (cyfra21-1≤3.30ng/ml and cyfra21-1>3.30 ng/ml). Clinical significance of cyfra21-1 level was assessed.

Results: Of the 267 patients, 110 (41.2%) had normal cyfra21-1, while 157 (58.8%) had elevated serum cyfra21-1. The prevalence of lymph node involvement, locally advanced stage (≥ pT3), tumor stages, tumor size and papillary were significantly higher in patients with elevated cyfra21-1 than in those with normal cyfra21-1. Patients with high cyfra21-1 showed worse Disease free survival and Overall survival than those with low cyfra21-1 (P = 0.001 and 0.007, respectively). In multivariate analysis, High cyfra21-1, lymph node involvement, lymphovascular invasion and papillary were independent predictors of worse Disease free survival (P = 0.036, <0.001, 0.002, 0.014 respectively). High cyfra21-1, lymph node involvement and lymphovascular invasion were also confirmed as independent predictors of worse Overall survival (P = 0.038, 0.010, 0.005, respectively.)

Conclusions: Elevated cyfra21-1 was associated with greater biological aggressiveness and worse prognosis than normal cyfra21-1.

Background

Bladder cancer (BC), the second common malignant tumors in the urogenital system, ranks 10th in terms of incidence worldwide, with an estimated 549,000 new cases and 200,000 deaths in 2018 according to the latest global cancer statistics. There is a strong prevalence in males and the elderly. The global incidence rate in men of 9.6 per 100,000 is 4 times higher than in women and it accounts for 2.8% of all cancer-related deaths in males[1]. Certain occupational exposures to chemical, water contaminants, and smoking have long been identified as risk factors for bladder cancer[2]. There are three types of bladder cancer that begin in cells in the lining of the bladder: transitional cell carcinoma, squamous cell carcinoma, and adenocarcinoma. Transitional cell carcinoma constitutes the majority of BC. In clinical practice BC is divided into two main subsets: non-muscle-invasive BC (NMIBC) and muscle-invasive BC (MIBC). Approximately 25% of urothelial carcinomas are diagnosed as MIBC, which predicts a significantly worse prognosis than NMIBC[3]. Surgical treatment of NMIBC and MIBC tumors are completely different, with initial transurethral resection of bladder tumor (TURBT) being the first diagnostic and therapeutic procedure for NMIBC and radical cystectomy (RC) being the standard treatment for MIBC patients and patients with very high risk NMIBC [4]. Despite radical cystectomy, 5-year survival rate still remains low as 58%[4]. Urinary biomarkers for bladder cancer have a long history, and

several biomarkers have been investigated as predictors of oncologic outcomes, such as nuclear matrix protein 22 (NMP22), UroVysion, bladder tumor antigen and cytokeratin[5]. Numerous reports have been investigated regarding urinary biomarkers for bladder cancer. However, none of the current guidelines recommends the routine use because of their insufficient accuracy[6]. Therefore, identifying patients with unfavorable oncological outcomes, or combined with other clinicopathological characteristics, is important for designing treatment strategies for patients with BC.

Cytokeratin 19 fragment (cyfra21-1), first recognized in 1993, is a soluble fragment of cytokeratin subunit 19. High levels of cyfra21-1 have been detected in several neoplastic diseases, such as lung cancer, oral/oropharyngeal squamous cell carcinoma, gastric, ovarian, breast, and prostate cancers[7]. Previous studies evaluated the level of cyfra21-1in urine and serum, the results of the different studies have been variable, and pay more attention to the diagnosis in urothelial carcinoma of bladder[8]. In the present study, we explored whether the preoperative detection of cyfra21-1in patient serum after RC can be used to predict outcomes.

Methods

We retrospectively reviewed all of the male patients with urothelial carcinoma of bladder (UCB) treated with RC in Sun Yat-sen university cancer center (SYSUCC) from September 2009 to October 2018. During this period, shemale patients were not measured the serum cyfra21-1, and there were 267 male patients been researched which cyfra21-1 levels in serum were available. Patients who had synchronous malignancy (including upper urinary tract cancer), other bladder tumors as the main histology (squamous cell, small cell, etc.), distant or nodal metastasis at diagnosis and history of neoadjuvant chemotherapy were excluded from the study. Indications for RC in the presented cohort were muscle invasive disease, T1 tumors at high risk of progression (i.e., high grade, multifocality, carcinoma in situ (CIS), and tumor size, etc.), T1 patients failing intravesical therapy. RC was followed according to the standard procedure follow the EAU guideline, which dissection of the bladder with the terminal ureter and bilateral lymph and prostate were removed in male [9]. Bladder specimen after RC was assessed according to standard histopathological procedures. Only the patients with UCB were allowed for final analysis. Any other histological types or mixed-type tumors from the bladder specimen were excluded of our analysis. After surgery, we decide whether the patients need another adjuvant treatment based on the patient's pathological stage. Tumors were staged according to 2017 the American Joint Committee on Cancer/TNM Staging system for urethral carcinoma. We reviewed the baseline characteristic of the patients, including age, smoking; and pathological status including tumor grade, histologic variants (HV), pT, pN, tumor size, number of the tumor, lymphovascular invasion (LVI), papillary, CIS, necrosis in tumor, TNM stage, surgical margin status(SMS) and adjuvant chemotherapy(AC).

Adjuvant Chemotherapy (AC) in the present study were gemcitabine and cisplatin (GC). Blood samples were drawn within two months before RC. Our regular preoperative blood evaluation included cyfra21-1. We defined the cyfra21-1 $\leq$ 3.30 ng/ml (the up limit reference range that recommended by Roche

Diagnostics GmbH) as an normal level. Then we divided into two group according to the level of cyfra21-1 in serum.

The serum level of cyfra21-1 was measured using enzyme linked immunosorbent assay (ELISA). Cyfra21-1 measurement was automatically performed using a chemiluminescent immunoassay. This test was performed in the Department of Laboratory Medicine at Sun Yat-sen university cancer center (SYSUCC). Before testing, Twenty-five microliter aliquots of the samples and 100 μl of the enzyme conjugate were added to the appropriate wells and covered with a plate sealer at room temperature (20 ~ 27 °C). Then the plate was incubated for 1 hour at 37 °C.400 μl of each cleaning solution was added for 10 seconds and drained blotted after 5 washes with buffer. Each well added one hundred microliters of freshly prepared chemiluminescent substrate and laid up at room temperature in the dark for 5 minutes. The luminescence values of each microplate were measured immediately using a luminometer.

Follow-up was carried out by telephone interview and postoperative follow-up review of record. Follow-up schedule after RC was performed according to generally valid guidelines[9]. The last follow-up was completed in January 15, 2020. The disease free survival (DFS) was one point in our study. And the overall survival (OS) was the other point in our study. We defined DFS as the interval between surgery and relapse, and defined OS as the interval between surgery and last follow-up or death.

Variables were presented as means and standard deviations, and frequencies and percentages, respectively. The variables of the 2 groups were compared using the $\chi 2$ test or Mann-Whitney U test, as appropriate. The Spearman Rank correlation test was used to examine relationships between various parameters and clinical characteristics. OS and DFS after surgery were measured by using of Kaplan-Meier curves and the log-rank test.

Univariate Cox regression analyses were done to compare all the variables and significant prognostic factors identified from the univariate analysis were entered into the multivariate Cox regression analysis of survival to test for independence. $P < 0.2$ will be taken into next-step multivariable analysis. Hazard ratios (HR) estimated from the Cox analysis were reported as relative risks with corresponding 95% confidence intervals (CI). All statistical analyses were performed using SPSS21.0 software (IBM, Armonk, NY) and MedCalc (MedCalc Software, Ostend, Belgium). All tests were two-sided and a P value < 0.05 was considered statistically significant.

Results

There were 267 male patients assessed in our study. The median age was 63 years (range, 30-85 years). And the median follow-up was 34 months (range, 2-113 months). Patient characteristic are shown in Table 1. Overall, 110 (41.2%) had normal cyfra21-1, while 157 (58.8%) were found to have evaluated cyfra21-1.

Patients with high cyfra21-1 had higher locally advanced stage ($P = 0.001$) and tumor size ($P = 0.019$) than those with low cyfra21-1. lymph node involvement ($P = 0.016$) and tumor stage ($P<0.001$) status

was higher in patients with high cyfra21-1 than in those with low cyfra21-1. Besides, the group with elevated cyfra21-1 presented a higher risk of papillary (p=0.006).There was no significant difference between patients with high cyfra21-1 and those low cyfra21-1 in terms of the proportions of AC and SMS (P = 0.557 and P = 0.402, respectively).We did not found a significant difference in LVI (P=0.080) and HV (p=0.055)

In terms of prognostic values, During the follow-up, 109 patients (40.8%) relapsed of UCB and 87 patients (32.6%) died of UCB. Kaplan–Meier analyses showed that patients with elevated preoperative cyfra21-1 had worse DFS and OS than those with normal cyfra21-1 (P = 0.001 and 0.007, respectively; see Fig. 1 and Fig. 2). Multivariate analysis adjusting for the effects of clinicopathological features revealed that cyfra21-1 (hazard ratio (HR): 1.588; 95% confidence interval (CI): 1.032-2.444; P = 0.036), lymph node involvement, LVI and papillary were independent predictors of worse DFS (Table 2). The same analysis also confirmed that cyfra2-1(HR: 1.660; 95%CI: 1.029-2.680; P = 0.038), lymph node involvement and LVI were independent predictors of worse OS (Table 3).

Discussions

Despite improvements in surveillance and clinical treatment strategies, the prognosis of BC treated with RC still remains undesirable[4]. It is critical to identify those patients with a poor prognosis in advance to implement a timely intervention. Cytokeratin 19 is expressed in simple epithelia and their malignant counterparts. Cyfra21-1 is released from malignant epithelial cells into human serum, tissue fluid, urine and saliva by a cleaving enzyme, caspase-3 during apoptosis[10]. Detecting the level of serum and urinary cyfra21-1 for bladder cancer has a long history.

As far as we know, this is the first large-scale study which showed that serum cyfra21-1 level correlates with prognosis of UCB treated with RC. In our study, we found more than 50% of the patients with an elevated serum cyfra21-1. The muscle-invasive bladder cancer would more likely have an elevated cyfra21-1. Although there were many researches had validated the high level of cyfra21-1 would have worse outcomes to other cancer, these was no study confirmed the elevated cyfra21-1 level would associate with a poor outcome of UCB patients treated with RC. There are many research investigated the urine cyfra21-1 is a useful tumor marker for bladder cancer[11–13]. And there are many research investigated that cyfra21-1 level may be a diagnostic biomarker for diagnosing bladder cancer[14, 15]. In a retrospective single center study including 85 patients examined Serum cyfra21-1 seems to be a marker of advanced and high-grade urothelial carcinoma of the bladder. It is useful for monitoring this disease and for predicting the prognosis. Of the 85 patients, 5 underwent radical surgery, while the others underwent staging by biopsy plus transurethral resection (TUR) and radiological evaluation.it identified that positive for cyfra21-1 had significantly worse disease-specific survival (p = 0.0001, log rank test) [16].Other study examined the serum cyfra21-1 is a useful marker to monitor the clinical course of bladder cancer and to provide prognostic information included 117 patients with bladder cancer. Treatments for bladder cancer consist of transurethral resection of the tumor, total cystectomy, intra-arterial infusion chemotherapy, and Systemic chemotherapy[17].In these study, the patients were not

completely treated with radical cystectomy. And staging the tumor maybe more correct after RC. It also was not able to show that elevated cyfra21-1 levels was an independent prognostic factors for OS and DFS of patients with UCB. There was still need more works and sample to validate. In our study included 267 patients with UCB, we explored to analysis the relationship between serum cyfra21-1 and the prognostic value of patients with UCB treated with RC. The results showed that the patients with an elevated serum cyfra21-1 level would have a poor outcome and was an independent prognostic indicator for OS and DFS of patients with UCB. Besides, we also found that lymph node involvement, LVI and papillary were independent predictors of worse DFS. And we found that lymph node involvement and LVI were independent prognostic factors for OS of patients with UCB.

Our study validated that the higher preoperative serum cyfra21-1 is associated to lymph node metastasis, T stage, TNM stage, tumor size and papillary. We excluded the adjuvant chemotherapy out of the Multivariate analysis. The reason is that adjuvant chemotherapy only was used to the patient with locally advanced UCB.

Although, we excluded some factors would interfere with our results, some limitation exist in our study. Firstly, our results originate from retrospective data, and a small sample size that could cause some bias in our study. No prospective randomized trials were performed in UCB treated with RC. So we will enlarge the sample case and conduct prospective studies to validate our results. Second, our follow-up time is not very long, some results have not been achieved the median survival time, we will continue to observe these patients to obtain a more reliable result. Lastly, our results need more research to validation.

In conclusion, these was no study confirmed the elevated cyfra21-1 level would associate with a poor outcome of UCB patients treated with RC in present study. our data revealed that preoperative serum cyfra21-1 level was an independent prognostic factor for patients with UCB treated with RC. High cyfra21-1 in serum associated with tumor biological aggressiveness. Maybe it means that changing the serum cyfra21-1 level could affect the cancer cell invasive in some etiological mechanisms, decreased serum cyfra21-1 level may be a new treatment to improve the prognosis of patients. Based on our results, multi-institutional, international, prospective collaborative studies are necessary to improve patient management.

Conclusions

our data revealed that preoperative serum cyfra21-1 level was an independent prognostic factor for patients with UCB treated with RC. Measuring the cyfra21-1 level might be a simple way for predict the patients who had a poor outcome and need a further management.

Abbreviations

HV, histologic variants; LVI, lymphovascular invasion; CIS, carcinoma in situ; SMS, surgical margins status; RC, radical cystectomy; DFS, disease-free survival; OS, overall survival; UCB, urothelial carcinoma

of bladder

Declarations

Acknowledgments

Not applicable.

Authors' contributions

HTL, YLY, ZL, ZKM, KHX and LT were responsible for data collection and analysis, interpretation of the results, and writing the manuscript. ZKQ and HTL were responsible for conducting the study design, data analysis and interpretation. All authors have read and approved the final manuscript.

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Ethics approval and consent to participate

Due to the retrospective nature of this study, ethics approval by Institutional Review Board of Sun Yat-sen University First Affiliated Hospital was obtained (GZR2018-053) and the data were used confidentially for research work. Informed consent was written by every patient when they referred to hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Clinicopathological characteristics of patients with urothelial carcinoma and Serum cyfra21-1 obtained by radical cystectomy

	All patients		Serum cyfra21-1		
Characteristic	Number	%	≤3.30	>3.30	*P-value*
Total	267	100	110	157	
Age: median	63		61	64	**0.002**
Grade					0.109
			8		
			102		
			40.27		
			520		
Low	13	4.9	8	5	
High	254	95.1	102	152	
T stage					**0.001**
Ta/tis/1	79	19.6	45	34	
T2	57	21.3	25	32	
T3	88	33	30	58	
T4	43	16.1	10	33	
TNM stage					**<0.001**
0a/0is/I	76	28.5	45	31	
II	48	18.0	22	26	
III	140	52.4	43	97	
IV	3	1.1	0	3	
N stage					**0.016**
N0	186	69.7	85	101	
				39.68	
N1/2/3	81	30.3	25	56	
				43.50	
HV					0.055
No	220	82.4	96	124	
			62.83	58.36	
Yes	47	17.6	14	33	
			11.95	22.67	
Tumor Size					**0.019**
≤3cm	82	30.7	42	40	
			32.74	28.28	
>3cm	185	69.3	68	117	
			34.07	39.02	
Smoking					0.457
No	107	40.1	45	62	
			66.37	65.57	
Yes	160	59.9	65	95	
			26.99	29	
CIS					0.396
No	250	93.6	102	148	
			99.56	99.64	
Yes	17	6.4	8	9	
			0.44	0.36	
Number of tumor					0.545
1	231	86.5	95	136	
			51.77	50.54	

≥2	36	13.5	15	21	
			41.15	43.56	
Papillary					**0.006**
No	200	74.9	73	127	
				99.64	
Yes	67	25.1	37	30	
			0.44	0.36	
Necrosis in tumor					0.539
No	254	95.1	105	149	
			51.77	50.54	
Yes	13	4.9	5	8	
			41.15	43.56	
LVI invasion					0.080
No	138	51.7	63	75	
				1670	
Yes	129	48.3	47	82	
				6	
SMS					0.402
Negative	240	89.9	100	140	
				1670	
Positive	27	10.1	10	17	
				6	
Adjuvant Chemotherapy					
No	199	74.5	82	117	0.557
Yes	68	25.5	28	40	

Note: Result of chi-squared comparisons between serum cyfra21-1≤3.3mg/L and >3.3mg/L cohorts. Bold text indicates a statistically significant difference.

HV, histologic variants; LVI, lymphovascular invasion; CIS, carcinoma in situ; SMS, surgical margins status.

Table 2 Univariate and multivariate analysis of clinicopathologic factors for Disease free survival (DFS)

Characteristics	Univariate HR（95%CI）	*P*	Multivariate HR（95%CI）	*P*
Cyfra21-1				
≤3.30(Ref)	1		1	
>3.30	2.066(1.317-3.055)	**0.001**	1.588 (1.032-2.444)	**0.036**
Histologic variant				
No (Ref)	1			
Yes	1.427(0.907-2.247)	0.124	0.894(0.553-1.446)	0.649
Tumor stage				
0a/0is/I (Ref)	1		1	
II,III,IV	6.259 (3.046-12.862)	**<0.001**	4.068(0.956-17.309)	0.058
T stage				
Ta/tis/1 (Ref)	1		1	
T2-4	4.621 (2.477-8.619)	**<0.001**	0.938(0.287-3.069)	0.916
Grade				
Low (Ref)	1			
High	22.915(0.902-582.407)	0.058	315630.081(0-5.418×10^{218})	0.960
N stage				
N0 (Ref)	1		1	
N1-3	3.951 (1.164-1.602)	**<0.001**	2.156(1.402-3.317)	**<0.001**
Tumor Size				
≤3cm (Ref)	1		1	
>3cm	1.380 (0.892-2.133)	0.148	1.118(0.705-1.774)	0.635
Necrosis in tumor				
No (Ref)	1			
Yes	0.673(0.274-1.653)	0.387		
Lymphovascular invasion				
No (Ref)	1		1	
Yes	3.126 (2.081-4.694)	**<0.001**	2.049(1.313-3.199)	**0.002**
Smoking				
No (Ref)	1		1	
Yes	0.736(0.505-1.073)	0.111	0.722(0.490-1.064)	0.100
Carcinoma in situ				
No (Ref)	1			
Yes	0.974 (0.394-2.406)	0.954		
Number of tumor				
1(Ref)	1			

≥2	0.778 (0.427-1.418)	0.412		
Papillary				
No (Ref)	1		1	
Yes	0.536 (0.319-0.900)	**0.018**	2.088(1.158-3.763)	**0.014**
Age				
≤65(Ref)	1			
>65	1.095(0.750-1.599)	0.638		
Surgical margin status				
Negative (Ref)	1			
Positive	0.936(0.501-1.748)	0.834		

Note: Bold text indicates a statistically significant association. Abbreviations: CI, confidence interval; HR, hazard ratio.

Table 3 Univariate and multivariate analysis of clinicopathologic factors for Overall survival (OS)

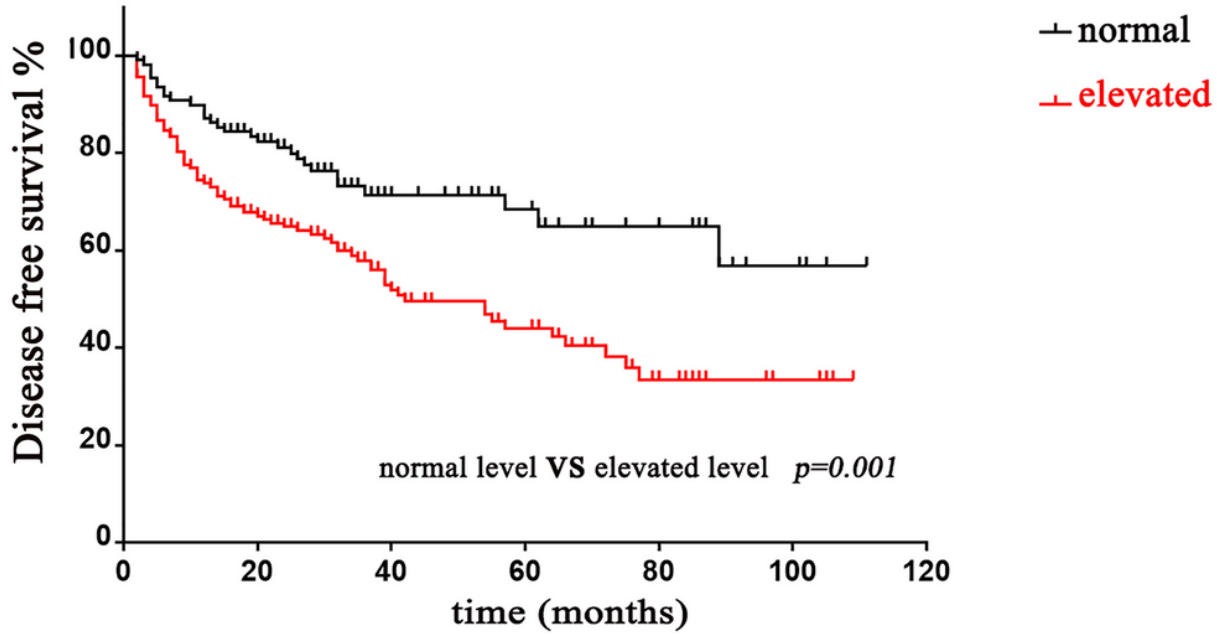
	Univariate		Multivariate	
Characteristics	HR（95%CI）	*P*	HR（95%CI）	*P*
Cyfra21-1				
≤3.30(Ref)	1			
>3.30	1.868(1.173-2.974)	**0.008**	1.660 (1.029-2.680)	**0.038**
Histologic variant				
No (Ref)	1			
Yes	1.767(1.088-2.869)	**0.021**	1.195(0.728-1.961)	0.482
Tumor stage				
0a/0is/I (Ref)	1			
II,III,IV	18.892(4.670-77.165)	**<0.001**	$0.001(0\text{-}4.606\times10^{63})$	0.926
T stage				
Ta/tis/1 (Ref)	1			
T2-4	19.715 (4.850-80.134)	**<0.001**	$12912.366\ (0\text{-}8.932\times10^{70})$	0.904
Grade				
Low (Ref)	1			
High	23.00(0.660-801.811)	0.084	$9332131.720(0\text{-}4.664\times10^{103})$	0.888
N stage				
N0 (Ref)	1			
N1-3	3.790 (2.468-5.820)	**<0.001**	1.833(1.154-2.912)	**0.010**
Tumor Size				
≤3cm (Ref)	1			
>3cm	1.103(0.684-1.780)	0.688		
Necrosis in tumor				
No(Ref)	1			
Yes	0.492(0.155-1.559)	0.228		
Lymphovascular invasion				
No (Ref)	1			
Yes	3.337 (2.105-5.291)	**<0.001**	2.046(1.244-3.366)	**0.005**
Smoking				
No(Ref)	1			
Yes	0.722 (0.474-1.100)	0.129	0.726(0.471-1.120)	0.148
Carcinoma in situ				
No (Ref)	1			
Yes	1.030 (0.320-3.310)	0.960		
Number of tumor				
1(Ref)	1			

≥2	0.770 (0.427-1.418)	0.459		
Papillary				
No (Ref)	1			
Yes	0.302(0.146-0.625)	**0.001**	1.086(0.507-2.327)	0.831
Age				
≤65(Ref)	1			
>65	1.314 (0.862-2.003)	0.205		
Surgical margin status				
Negative (Ref)	1			
Positive	0.966 (0.498-1.876)	0.919		

Note: Bold text indicates a statistically significant association.

Figures

Figure 1. Survival analyses using Kaplan–Meier methods for normal level and elevated level. Disease-free survival.

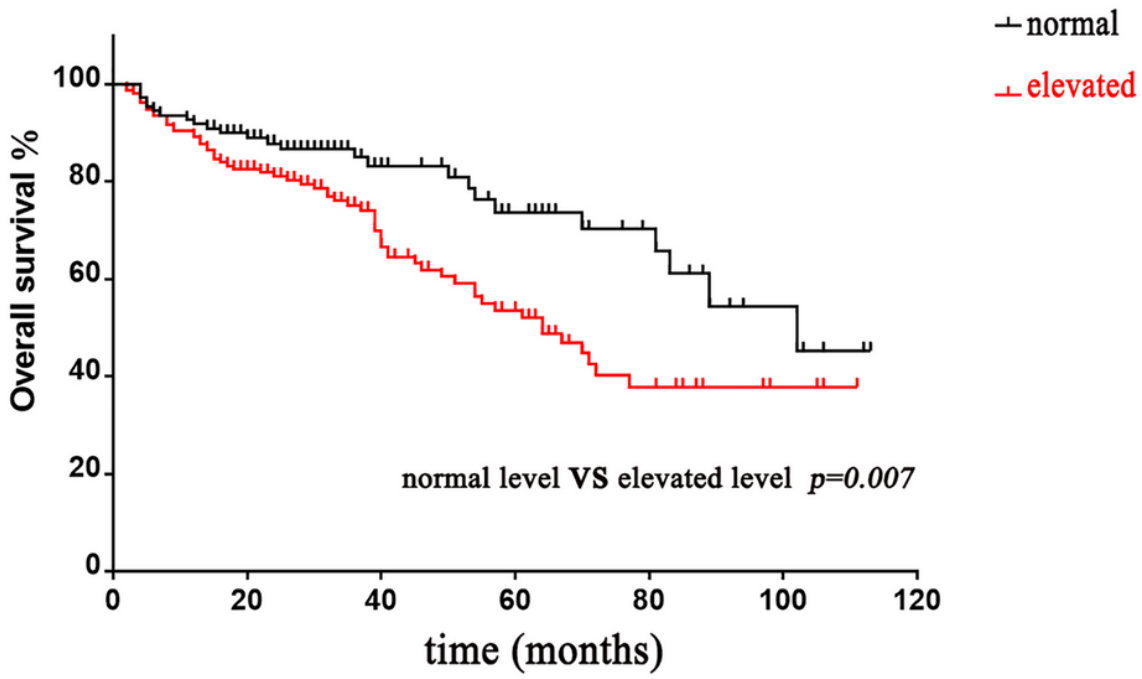


Number of risk

normal	110	91	83	82	81	80	80
elevated	157	106	89	83	78	78	78

Figure 1

Figure 2. Survival analyses using Kaplan–Meier methods for normal level and elevated level. Overall survival.



Number at risk							
normal	110	98	94	90	89	86	85
elevated	157	130	113	103	95	95	95

Figure 2