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Serum insulin-like growth factor binding protein-3 as a potential biomarker for diagnosis and prognosis of oesophageal squamous cell carcinoma

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ABSTRACT

Background: Insulin-like growth factor binding protein-3 (IGFBP3) has been reported to be related to the risk of some cancers. Here we focussed on serum IGFBP3 as a possible biomarker of diagnosis and prognosis for oesophageal squamous carcinoma (ESCC).

Methods: Enzyme-linked immunosorbent assay (ELISA) was used to measure the serum IGFBP3 level in the training cohort including 136 ESCC patients and 119 normal controls and the validation cohort with 55 ESCC patients and 42 normal controls. The receiver operating characteristics curve (ROC) was used to assess the diagnosis value. Cox proportional hazards model was applied to select factors for survival nomogram construction.

Results: Serum IGFBP3 levels were significantly lower in early-stage ESCC or ESCC patients than those in normal controls (p < .05). The specificity and sensitivity of serum IGFBP3 for the diagnosis of ESCC were 95.80% and 50.00%, respectively, with the area under the ROC curve (AUC) of 0.788 in the training cohort. Similar results were observed in the validation cohort (88.10%, 38.18%, and 0.710). Importantly, serum IGFBP3 could also differentiate early-stage ESCC from controls (95.80%, 52.54%, 0.777 and 88.10%, 36.36%, 0.695 in training and validation cohorts, respectively). Furthermore, Cox multivariate analysis revealed that serum IGFBP3 was an independent prognostic risk factor (HR = 2.599, p = .002). Lower serum IGFBP3 level was correlated with reduced overall survival (p < .05). Nomogram based on serum IGFBP3, TNM stage, and tumour size improved the prognostic prediction of ESCC with a concordance index of 0.715. **Conclusion:** We demonstrated that serum IGFBP3 was a potential biomarker of diagnosis and prognosis for ESCC. Meanwhile, the nomogram might help predict the prognosis of ESCC.

KEY MESSAGE

• Serum IGFBP3 showed early diagnostic value in oesophageal squamous cell carcinoma with independent cohort validation. Moreover, serum IGFBP3 was identified as an independent prognostic risk factor, which was used to construct a nomogram with improved prognosis ability in oesophageal squamous cell carcinoma.

Introduction

Oesophageal cancer ranks the seventh in global cancer incidence and the sixth in cancer mortality [1]. Worldwide ESCC is the most common type of oesophageal cancer, which is responsible for 90% of all oesophageal cancers [2]. At the time of diagnosis, patients often developed at advanced stage with lymph node metastasis, which results in a 5-years survival rate of 20%, approximately [3,4]. Extensive

lymphatic network of oesophagus and the lack of a protective serosa also lead to the poor prognosis of ESCC. Furthermore, in early stage, ESCC patients are often asymptomatic. This brings about early regional tumour progression, metastasis and delayed diagnosis [5]. ESCC has caused some economic and medical burden. Traditional diagnostic methods include imaging technology and endoscopic examination with biopsy. However, the biopsy is invasive and much painful and

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the accuracy of imaging technology such as computer tomography (CT) for differentiating lymph node metastasis and T staging is low only about 50%.[6]. On the other hand, the evaluation of the prognosis of ESCC is manly based on tumour node metastasis (TNM) stage, but it depends fully on the anatomical range of the carcinoma, which has been proved to be defective for survival analysis of ESCC patients [7]. Thus, convenient and accurate methods for early diagnosis and prognostic assessment of ESCC are urgently needed. Benefiting from technological advances, the development of non-invasive and minimally invasive biomarkers for the early diagnosis and prognosis of ESCC has attracted attention.

The evolutionarily conserved insulin-like growth factor (IGF) is essential for cells growth, development and metabolism [8]. IGFBPs designated as IGFBP1-IGFBP6 can modulate the bioavailability of IGF-I and IGF-II in cellular or circulation environment [9,10]. IGFBPs also regulated the cell cycle, and the function of IGFBPs in transcriptional regulation, DNA damage repair and apoptosis induction suggest that they are closely associated with tumour development, progression, and therapy resistance [11]. For example, IGFBP3 bound with retinoid X receptor (RXR) and increased retinoidinduced transcriptional activity, leading to apoptosis in prostate cancer cells [12]. Furthermore, it was reported that serum IGFBP1 as diagnostic biomarker detected early-stage upper gastrointestinal cancer and serum IGFBP2 may be a biomarker of severe dystrophy and muscle wasting associated with pancreatic ductal adenocarcinoma [13,14].

Researches suggested that active IGFBP3 can regulate cell apoptosis and proliferation in IGF-I dependent or independent pathways [15,16]. IGFBP3 as a tumour suppressor was reported to be down-regulated in some cancers. But some other publications showed its overexpression is associated with poor prognosis for several cancers including pancreatic endocrine neoplasms [11,17]. Meanwhile, epidemiological studies indicated that higher circulating IGF-I: IGFBP3 molar ratio or IGFBP3 level were an increased risk of breast cancer and colorectal cancer [18,19]. Researchers also showed that serum IGFBP3 of colorectal cancer patients was lower than that of normal controls and IGFBP3 as a serum biomarker for colorectal cancer diagnosis has better diagnostic ability than carcinoembryonic antigen (CEA). As well, circular IGFBP3 has the potential to be used as early indicator for colorectal cancer diagnosis [20,21]. Moreover, IGFBP3 as a risk factor for carcinogenesis has been demonstrated in many cancers [22,23]. However, the value of IGFBP3 as a biomarker of diagnosis and prognosis for ESCC remains unclear.

In our study, we investigated whether serum IGFBP3 possessed the potential as a diagnostic and prognostic marker for ESCC. In addition, we attempted to construct a nomogram involving serum IGFBP3 to help improve prediction of survival of ESCC patients.

Materials and methods

Study participants

A retrospective study was developed to access serum IGFBP3 as a potential biomarker for ESCC. Serum samples of patients were collected in the Cancer Hospital of Shantou University Medical College, from March 2013 to July 2014. Serum samples of healthy controls were from Physical Examination Centre in the same hospital, who was identified with health check-up and without suffering any neoplasm. Every patient included in this study conformed to the following standards: (1) ESCC diagnosis was confirmed by histopathological examination; (2) patients did not receive any anti-cancer therapy, or not diagnosed with other carcinomas except ESCC. Tumour stages were evaluated according to the eighth edition American Joint Committee on Cancer (AJCC) Cancer Staging Manual [24]. Early-stage was included stage I and stage II, and advanced stage was included stage III and stage IV. For the diagnostic assessment, ESCC patients and normal controls were randomly divided into a training cohort and a validation cohort in a ratio of approximately 3:1 through the random sampling procedure of SPSS software [25,26]. The peripheral blood samples were coagulated for 30 min at room temperature and centrifuged at 2500 g for 15 min, and serum samples were stored in the biobank of ultra-low temperature refrigerator (-80 °C).

Ethics statement

All participants in this study obtained written informed consent prior to the use of serum samples. This study complied with the Helsinki Declaration and was approved by the institutional ethics review board of Cancer Hospital of Shantou University Medical College, and the ethics approval number is SUMC2011XM-0066.

Enzyme-linked immunosorbent assay (ELISA) for IGFBP3

The concentration of serum IGFBP3 was measured by a commercial ELISA kit (CUSABIO, China) according to

the manufacturer's recommendations. Firstly, we added 100ul of standard and serum samples (a 100fold dilution) in antibody-coated 96-well microplates, which were covered with the adhesive strip and incubated for 2 h at 37 °C. Then we poured out the liquid of each well, added 100ul of biotin-antibody in each well and incubated the microplates for 1 h at 37 °C. After washing the plate with wash buffer, 100ul horseradish peroxidase-avidin was added to each well and incubated the plate for 1 h at room temperature. Then washing the plate, the microplates were added TMB substrate and incubated for 15-30 min in a dark environment. Stop solution was used to stop the colour formation. The plate microplate reader (Multiskan FC; Thermo Fisher Scientific) was carried out to measure the optical density at 450 nm. Concentrations of IGFBP3 standards for establishing a standard curve were 0, 0.78, 1.56, 3.12, 6.25, 12.5, 25, and 50 ng/mL, respectively. All measurements were repeated. Using the standard curve to obtain serum IGFBP3 concentrations, and actual concentration of serum IGFBP3 must multiplied by the dilution factor.

Statistical analysis

SPSS (version 19.0), GraphPad Prism software and R software, version 4.0.4 for windows were applied for statistical analyses. Student's t-test was implemented to measure the difference of serum IGFBP3 between early-stage ESCC, advanced-stage ESCC or ESCC patients and normal controls. The receiver operating characteristic (ROC) was developed to analyse the predicted probability value. ROC curve was drew to evaluate the optimum predicted probability, specificity and sensitivity of diagnosis and the area under the ROC curve (AUC) with the 95% confidence interval (CI). When the specificity was >90%, through the maximum sensitivity and minimised distance from the cut-off value to the upper left corner of ROC curve to assess cut-off value for diagnosing ESCC [27].

We used X-tile to classify the ESCC patients with higher or lower serum IGFBP3 and changed all continuous variables to categorical variables in prognostic cohort [28]. Pearson's Chi2 test was used to evaluate the connection between serum IGFBP3 and patient clinical characteristics. Patient survival time and significant difference were calculated by Kaplan-Meier method and log-rank test. We carried out univariate and multivariate Cox regression analysis to search independent risk factors of ESCC prognosis. 1-, 3-, 5years OS for ESCC patients were predicted by the constructed nomogram. Concordance index (C-index) was applied to analyse the distinction ability of the new model. The accuracy and benefit of the nomogram was evaluated by decision curve analysis (DCA). R packages included ggplot2, ggpubr, survminer and survival were used in analysis. Overall survival (OS) was defined as the time interval from the date of surgical resection to death or last follow-up. In every statistical test, when *P* values below 0.05 were considered significant, every *P* values were two-sided.

Results

Serum IGFBP3 levels in ESCC patients and normal controls

This study recruited 352 participants (Figure 1). The training cohort included 136 ESCC patients (59 early-stage patients) and 119 normal controls. 55 ESCC patients (22 early-stage patients) and 42 normal controls involved in the validation cohort. Clinical characters of two cohorts were shown in Table 1.

The serum IGFBP3 levels of ESCC patients and normal controls in two cohorts were tested by ELISA, as shown in Figure 2. Serum IGFBP3 levels (mean ± SD) of early-stage ESCC, advanced-stage ESCC or ESCC patients were significantly lower than normal controls training cohort (1096.573 ± 613.766 ng/ml, in 1119.625 ± 580.810 ng/ml or 1109.624 ± 593.200 ng/ml vs 1684.814 ± 354.828 ng/ml, p < .05). The results in validation cohort were consistent (1262.475 ± 476.643 ng/ ml, 1179.240 ± 526.914 ng/ml or 1212.533 ± 504.550 ng/ ml vs 1602.566 ± 490.362 ng/ml, *p* < .05). However, there is no significant different in comparison of serum levels of IGFBP3 between early-stage and advanced-stage ESCC patients (p > 0.05).

Diagnostic ability of IGFBP3 to differentiate ESCC and normal control

For diagnosis of ESCC, ROC curve analysis showed that the AUC of IGFBP3 to differentiate ESCC from normal controls was 0.788 (95%Cl, 0.733–0.844) in training cohort (Figure 3(A)). The sensitivity was 50.00% when the specificity was 95.80%. Results were confirmed in validation cohort, with the AUC of 0.710 (95%Cl, 0.608–0.812), specificity of 88.10% and sensitivity of 38.18% (Figure 3(B)). In both cohorts the cut-off value of 1026 ng/ml was consistent.

Early diagnostic performances were also accessed by ROC analysis. The AUC of serum IGFBP3 to discriminate early-stage ESCC patients from normal controls was 0.777 (95%Cl, 0.697–0.856), the specificity was 95.80% and the sensitivity was 52.54% in train cohort



Figure 1. Study overview of serum IGFBP3 in oesophageal squamous cell carcinoma.

Table 1.	Clinical	characteristics	of	samples	in	two	cohorts.
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	Training cohort				Validation cohort			
	ESC (n = 1	CC 136)	normal ESCC (<i>n</i> = 119) (<i>n</i> = 55)		normal (n = 42)			
Variables	NO	%	NO	%	NO	%	NO	%
Age								
Mean ± SD	58 ± 8		55 ± 11		58 ± 10		54 ± 7	
Range	41-83		36-83		26-80		45-70	
Gender								
Female	32	23.5	48	40.3	19	34.5	14	33.3
Male	104	76.5	67	56.3	36	65.5	24	57.1
Unknown			4	3.4			4	9.5
Smoke								
No	43	31.6			19	34.5		
Yes	93	68.4			36	65.5		
Alcohol drinking								
No	95	69.9			32	58.2		
Yes	41	30.1			23	41.8		
location of tumour								
Upper oesophagus	20	14.7			7	12.7		
Middle oesophagus	99	72.8			32	58.2		
Lower oesophagus	17	12.5			16	29.1		
Size of tumour								
<5 cm	98	72.1			36	65.5		
	38	27.9			19	34.5		
Histological grade								
High (Grade 1)	28	20.6			12	21.8		
Middle (Grade 2)	84	61.8			31	56.4		
Low (Grade 3)	15	11.0			4	7.3		
Unknown	9	6.6			8	14.5		
Depth of tumour invasion								
T1 + T2	25	18.4			18	32.7		
T3 + T4	111	81.6			37	67.3		
Lymph node metastasis								
NO	70	51.5			28	50.9		
N1 + N2 + N3	66	48.5			27	49.1		
TNM stage								
Early stage $(I + II)$	59	43.4			22	40		
Advanced stage (III + IVA)	77	56.6			33	60		

TNM: tumour node metastasis.



Figure 2. Serum IGFBP3 level. Median and interquartile range of serum IGFBP3 level in early-stage ESCC, advanced-stage ESCC, ESCC patients and normal controls in two cohorts (A: training cohort; B: validation cohort). Unpaired *t* test was applied to evaluate the IGFBP3 difference of early-stage ESCC, advanced-stage ESCC or ESCC patients and normal control in two cohorts.



Figure 3. ROC curve of IGFBP3 in ESCC diagnosis. ROC curves of serum IGFBP3 to distinguish ESCC patients and normal controls in two cohorts (A: training cohort; B: validation cohort), and diagnostic value of early-stage ESCC in two cohorts (C: training cohort; D: validation cohort).

(Figure 3(C)). With the same cut-off value from training cohort, the AUC was 0.695 (95%Cl, 0.561–0.828) and diagnostic specificity and sensitivity were 88.10% and 36.36%, respectively, in the validation cohort (Figure 3(D)). The predictive values and the likelihood ratios of serum IGFBP3 for improving the clinical interpretation of the diagnosis of early-stage ESCC and ESCC were also shown in Table 2.

Prognostic value of serum IGFBP3 in ESCC

We explored whether serum IGFBP3 could be applied for predicting prognosis. Among the 191 ESCC patients recruited in this study, 26 patients lacked complete follow-up data and were not included for prognostic analysis. Therefore, the prognostic cohort included 165 ESCC patients. Moreover, the maximum and median follow-up survival time for ESCC patients were 77 months and 55 months, respectively.

We assessed the association of serum IGFBP3 with clinical parameters using the Pearson's Chi2 test. Using X-tile, the cut-off value of IGFBP3 was defined as 1087.02 ng/ml. As displayed in Table 3, the correlated relationship between serum IGFBP3 levels and clinical features was not significant. Univariate Cox analysis indicated that smoking, depth of tumour invasion, TNM stage, tumour size, lymph node metastasis and serum IGFBP3 were prognostic factors of ESCC. Multivariate Cox analysis further suggested that TNM stage (p = .045, HR = 0.370, 95%CI: 0.140–0.980), tumour size (p = .003, HR = 0.415, 95%CI: 0.232–0.743) and serum IGFBP3 (p = .002, HR = 2.599, 95%CI:

Table 2.	Diagnostic	results	of	serum	IGFBP3	in	ESCC.

Group	AUC (95% CI)	Specificity	Sensitivity	NPV	PPV	NLR	PLR
Training cohort							
ESCC vs. normal controls	0.788 (0.733-0.844)	95.80%	50.00%	62.64%	93.15%	0.52	11.9
Early-stage ESCC vs. normal controls	0.777 (0.697–0.856)	95.80%	52.54%	80.28%	86.11%	0.5	12.51
Validation cohort							
ESCC vs. normal controls	0.710 (0.608-0.812)	88.10%	38.18%	52.11%	80.77%	0.7	3.21
Early-stage ESCC vs. normal controls	0.695 (0.561-0.828)	88.10%	36.36%	72.55%	61.54%	0.72	3.06

AUC: area under the curve; NPV: negative predictive value; PPV: positive predictive value; NLR: negative likelihood ratio; PLR: positive likelihood ratio; 95% CI: 95% confidence interval.

		IGFBP3	protein	
Variables	All cases	Low IGFBP3 level	High IGFBP3 level	p Value
Age				
<68	140	76	64	.368
	25	16	9	
Gender				
Female	43	27	16	.280
Male	122	65	57	
Smoke				
No	52	28	24	.737
Yes	113	64	49	
Alcohol drinking				
No	108	63	45	.359
Yes	57	29	28	
Location of tumour				
Upper oesophagus	24	13	11	.712
Middle oesophagus	114	62	52	
Lower oesophagus	27	17	10	
Size of tumour				
<5 cm	121	71	50	.210
	44	21	23	
Histological grade				
High (Grade 1)	35	19	16	.524
Middle (Grade 2)	97	51	46	
Low (Grade 3)	17	12	5	
Unknown	16	10	6	
Depth of tumour invasion				
T1 + T2	40	21	19	.634
T3 + T4	125	71	54	
Lymph node metastasis				
NO	92	49	43	.469
N1 + N2 + N3	73	43	30	
TNM stage				
Early stage $(I + II)$	76	40	36	.455
Advanced stage (III $+$ IVA)	89	52	37	

Table 3. Correlation of IGFBP3 with clinical features.

1.412–4.784) were independent factors to predict the prognosis of ESCC (Table 4). Furthermore, log-rank test and Kaplan–Meier revealed that the OS of ESCC patients with lower serum IGFBP3 level was shorter than those with higher serum IGFBP3 level, and the number of people alive at each time point in the both groups was also showed (p = .0057) (Figure 4(A)). In addition, we further drew an adjusted survival curve (covariates including size of tumour, smoking, TNM

stage, lymph node metastasis and depth of tumour invasion) for serum IGFBP3 and confirmed serum IGFBP3 level was related to patient survival time (Figure 4(B)).

Nomogram for OS of ESCC

For better evaluating prognosis of ESCC, we constructed a nomogram to forecast 1-, 3- and 5-years OS

	Univariate anal	Multivariate analyses		
Variables	HR (95% CI)	p Value	HR (95% CI)	p Value
Age ($\leq 68 \text{ vs} > 68$)	0.788 (0.384–1.617)	.516		
Gender (female vs male)	0.600 (0.292-1.231)	.163		
Smoke (no vs yes)	0.484 (0.243-0.965)	.039		
Alcohol drinking (no vs yes)	0.634 (0.366-1.096)	.103		
Location of tumour				
Upper oesophagus vs Lower oesophagus	0.291 (0.079-1.075)	.064		
Middle oesophagus vs Lower oesophagus	0.925 (0.449–1.906)	.832		
Size of tumour				
\leq 5cm vs > 5cm	0.346 (0.201-0.597)	<.001	0.415 (0.232-0.743)	.003
Histological grade				
G1 vs G3	0.493 (0.190-1.279)	.146		
G2 vs G3	0.683 (0.315-1.479)	.333		
Depth of tumour invasion				
T1 + T2 vs T3 + T4	0.417 (0.188-0.924)	.031		
Lymph node metastasis				
N0 vs N1 + N2 + N3	0.413 (0.236-0.723)	.002		
TNM stage				
early stage vs Advanced stage	0.311 (0.166-0.583)	<.001	0.370 (0.140-0.980)	.045
IGFBP3 (lower vs higher)	2.269 (1.245-4.135)	.007	2.599 (1.412-4.784)	.002

Table 4. Cox analysis of OS for ESCC.

HR: hazard ratio; 95% CI: 95% confidence interval; OS: overall survival.



Figure 4. Kaplan–Meier curves for OS. (A) survival curve for serum IGFBP3 with ESCC patients, Log-rank test was used to evaluate the significant difference. (B) adjusted survival curves for serum IGFBP3 with ESCC patients. The number of people alive at each time point in the high and low IGFBP3 groups was showed in "number at risk."



Figure 5. Nomogram based on serum IGFBP3, size of tumour and TNM stage to predict the 1 year, 3 years and 5 years of OS of ESCC patients.

based on TNM stage, size of tumour and serum IGFBP3 (Figure 5). In the nomogram, "points" were obtained according to different levels of individual predictors, and these points were added together to get "total points". We painted a straight line down from the "total points" line to intersect the lines of 1-, 3- and 5-years OS, where the intersection was the survival rates of 1-, 3- or 5-years. As shown in Table 5, the C-index of nomogram (0.715, 95%CI: 0.683-0.747) was higher than those of TNM stage (0.641, 95%CI: 0.611–0.671, *p* < .05), size of tumour (0.610, 95%CI: 0.577-0.643, p < .05) or serum IGFBP3 (0.604, 95%CI: 0.572–0.636, p < .05). The C-index curve contrasted with each single variable based on time distribution of three and five years and validated by bootstrap algorithm were plotted (Figure 6). The calibration curve revealed that the prediction accuracy of nomogram for OS was improved (Figure 7). Net benefit was displayed by DCA as shown in Figure 8, of which represented the nomogram to predict OS was better than those of other risk indicators. Then, we calculated the predicted total points according to nomogram and got the best cut-off value for OS (192.5) through Xtile. We divided ESCC patients into lower risk group or higher risk group according to the best cut-off value.

Table 5. C-index of IGFBP3, size of tumour, TNM stage and nomogram of prediction for OS.

actors	C-index (95% Cl)	p Value
For OS		
GFBP3	0.604 (0.572-0.636)	
Size of tumour	0.610 (0.577–0.643)	
FNM stage	0.641 (0.611–0.671)	
Nomogram	0.715 (0.683-0.747)	
Nomogram vs IGFBP3		<.001
Nomogram vs size of tumour		.004
Nomogram vs TNM stage		.015

nomogram: serum IGFBP3 + size of tumour + TNM stage.

Kaplan–Meier survival analysis and log-rank test confirmed that the OS of patients in the group of higher risk was shorter than those in the group of lower risk (p < .0001, Figure 9).

Discussion

The geographic distribution of ESCC varies widely. The region with the highest incidence extends from East Asia to Central Asia. With the difference in geographical distribution, ESCC is highly prevalent in Asia, while the pathological type of oesophageal cancer in Europe is mainly oesophageal adenocarcinoma [2]. Pathophysiological differences and changes in the oesophageal cancer microenvironment are barriers to



Figure 6. C-index curve to evaluate the predicted ability of the nomogram, C-index curve under the time distribution of 3 years (A) and five years (C) and internal verification by Bootstrap algorithm of three years (B) and five years (D).

developing biomarkers for early detection of oesophageal cancer [29]. These facts make it challenge to find promising early diagnostic markers for ESCC. Some blood-based biomarkers were clinically detected such as cytokeratin-19 fragment (CYFRA21-1), squamous cell carcinoma antigen (SCC-Ag) and carcinoembryonic antigen (CEA). However, these biomarkers lack evidence as early diagnostic biomarkers [30,31]. The endoscopic-biopsy approach is widely applied in clinic for the detection of oesophageal cancer and preinvasive lesions, but invasive experiences during examination and capacity of endoscopy and pathological professionals limit its extensive utilisation for the screening of asymptomatic populations. This method requires highly skilled endoscopists, which at present are not widely available in rural areas with high ESCC incidence most in need of effective screening. It's conceivable that a robust blood-based biomarker test that could be used in the early or asymptomatic stage of ESCC as the primary screening method would concentrate the population and further avoid unnecessary invasive procedure. Here, our study found serum IGFBP3 has potential diagnostic efficacy for early-stage ESCC and ESCC patients. We think that the non-invasive biomarker test of IGFBP3 is not meant to replace endoscopy, but to help identify patients who might harbour ESCC at an earlier stage.

Meanwhile, another dilemma is the prognostic assessment of ESCC. An accurate evaluation approach is the hinge to postoperative treatment selection and prognosis judgement of ESCC patients. At present, clinical staging mainly relies on the TNM stage system [32]. However, stage results may be affected by pathological assessment and tumour heterogeneity [33]. Studies showed that the combined application of some biomarkers and clinicopathological parameters were feasible to improve the accuracy of prognosis prediction in ESCC [34]. Through our study, we identified that serum IGFBP3 was an independent prognostic factor, and patients with the lower serum IGFBP3 indicated worse OS. Similar result was reported that the radiotherapy response of ESCC patients with



Figure 7. Calibration curve of nomogram to predict the overall survival rate of 1, 3 and 5 years (A–C). X-axis was the probability of 1, 3 or 5 years of OS predicted by nomogram. Y-axis was the actual OS of the patient included in the study.

elevated protein level of IGFBP3 was improved and overall survival was prolonged [35]. Besides, OS, eventfree survival and progression-free survival (PFS) of primary non-small cell lung cancer (NSCLC) patients with reduced IGFBP3 expression were shorter [36]. However, researches also indicated that serum IGFBP3 was not an independent prognostic indicator in multiple myeloma and breast cancer [37,38]. These results implied that serum IGFBP3 might have different prognostic significance in different carcinomas, and the prognostic potential of serum IGFBP3 in cancer should be further evaluated. In our study, the nomogram based on serum IGFBP3, size of tumour and TNM stage provided more precise prognostic prediction. The C-index of the nomogram was better than those three indicators. The decision curve and calibration curve indicated that the nomogram as a comprehensive evaluation model improved predictive ability for 1-year, 3-years and 5-years OS of ESCC patients.

Previous studies paid more attention to the correlation between IGFBP3 and the risk of oesophageal cancer. It is reported that, after adjusting the influence

of BMI, smoking and drinking, serum IGFBP3 level and molar differences in serum IGFBP3-IGF1 were inversely related to the risk of oesophageal cancer in an epidemiological research, and individuals with lower level of IGFBP3 were more possibly to develop oesophageal cancer [39,40]. These studies indicated IGFBP3 as a risk factor for oesophageal cancer. Our study revealed that serum IGFBP3 may be a biomarker for diagnosis and prognosis of ESCC. We found the serum IGFBP3 level was significantly lower in early-stage ESCC patients than those in normal controls. This is consistent with the research of YILMAZ et al, who carried out a small sample size study with 40 oesophageal carcinoma patients and 40 controls and did not analyse the diagnostic/prognostic value of IGFBP3 [41]. Compared to YILMAZ et al.'s research, the advantages of our study should be highlighted, which was designed in lager sample size with independent cohort validation. Several studies to support the lower level of serum IGFBP3 in ESCC should be noted. It is reported that, the methylation rate of IGFBP3 in oesophageal cancer patients, which was negatively correlated with the



Figure 8. Decision curve analysis of serum IGFBP3, size of tumour, TNM stage and nomogram, A-C shown the decision curve for 1 year, 3 years, and 5 years of OS.

positive expression of IGFBP3 in tissues, was higher than that in the normal controls in peripheral blood. And the positive rate of IGFBP3 in EC tissues was significantly lower than that in adjacent normal tissues [42]. These results suggest that methylation status of IGFBP3 may be related to the low expression of serum IGFBP3 in ESCC. In addition, serum IGFBP3 level was also lower in colorectal cancer compared with normal controls. Methylation of the IGFBP3 promoter and protease degradation such as matrix metalloproteinase-7 (MMP-7) for IGFBP3 were considered as the regulatory mechanism which may induce the low IGFBP3 level in colorectal cancer [20,43,44]. These evidences indicated that IGFBP3 may be regulated by multiple regulations. In the future, further studies are warranted to explore the mechanisms of the low expression of IGFBP3 in ESCC.

Some limitations existed in this study. First, although serum IGFBP3 showed diagnosis value for ESCC in both cohorts, the diagnostic performance of the validation cohort appeared to be inferior to that of the training cohort. This result may be due to the smaller sample size of patients and normal controls in the validation cohort. It seems inevitable that different sample sizes or different sample sources would lead to such bias. In addition to sample size difference between the two groups, the proportions of patients with different clinical features also varied. This might



Figure 9. Survival curve of risk stratification for OS according to prediction of nomogram. The low risk: Total points \leq 192.5 for OS, the high risk: Total points >192.5 for OS. Log-rank test was used to evaluate the significant difference.

also bring about the distinction in results for diagnostic performance to some degree. Actually, many studies on biomarker diagnosis evaluation showed obviously different results in different cohorts because of different sample sizes and different proportions of patients [45,46]. To overcome this problem, we believe that more different cohorts with large samples were required to be included to assess the diagnostic ability of serum biomarker. Moreover, as IGF-I bioactivity is partially regulated through IGFBPs, with ~80% bound to IGFBP3 [47,48], studies tended to investigate the associations of circulating IGF-I and IGFBP3 levels and analyse their roles simultaneously in cancers. However, our current study lacks examination of the serum level of IGF-I and its relationship with serum IGFBP3. IGF-I has been implicated in the development and progression of a variety of cancers. It was reported that IGF-I derived from adipose tissue improved cell growth and inhibited cell apoptosis of ESCC [49]. Therefore, in future study, it is an important point to combine detection of serum IGF-I and IGFBP3 and explore their biological functions.

Conclusion

We found evidence of serum IGFBP3 with potential diagnostic and prognostic value for ESCC. The differential expression level and the early diagnostic value of serum IGFBP3 confirmed in two cohorts, which indicated it might be applied as an early diagnostic biomarker. Moreover, our studies confirmed that serum IGFBP3 was an independent prognostic risk factor of ESCC and the nomogram containing serum IGFBP3 was constructed to facilitate the survival prediction of ESCC.

Author contributions

Yun Luo and Chao-Qun Hong: study design; data analysis; statistical analysis and drafting of the manuscript. Bin-Liang Huang, Tian-Yan Ding, Ling-Yu Chu and Biao Zhang: data analysis and interpretation and statistical analysis. Qi-Qi Qu, Xin-Hao Li, and Can-Tong Liu: data acquisition and analysis and study supervision. Yu-Hui Peng, Hai-Peng Guo, Yi-Wei Xu: critical revision of the manuscript; obtained funding and administrative, technical, or material support; equal contribution of this work. All authors have approved the final version and agreed to publish the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author, [Yi-Wei Xu]. The data are not publicly available due to restrictions e.g. their containing information that could compromise the privacy of research participants.

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