

Original

Serum Heat-shock Protein 70 Level for Predicting Postoperative Morbidity in Patients with Esophageal Cancer

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Summary

Background: Heat-shock protein 70 (HSP70) is important in host responses to various stresses. This study analyzed the correlation between preoperative serum concentrations of HSP70 and postoperative morbidity in patients with esophageal cancer.

Materials and Methods: Serum samples were obtained preoperatively from 31 patients with esophageal squamous cell carcinoma. Serum HSP70 concentrations were measured by enzyme-linked immunosorbent assay and the relationships between serum HSP70 level and postoperative morbidities were analyzed.

Results: The mean concentration of serum HSP70 was 9.71 ng/ml. When patients were dichotomized relative to this cutoff, no significant relationships between perioperative inflammatory markers and serum HSP70 level were observed. The incidence of postoperative complications was significantly lower in patients with HSP70 ≥ 9.71 ng/ml ($p=0.0281$).

Conclusion: Preoperative serum level of HSP70 was significantly correlated with postoperative morbidities in patients with esophageal cancer. Therefore, enhancing the HSP70 level prior to invasive surgery could be a feasible strategy to prevent morbidity.

Key Words: Esophageal cancer, esophagectomy, morbidity, HSP70

Introduction

Esophageal cancer is one of most common forms of cancer in the world and has proven to be one of the most difficult malignancies to cure. The primary curative treatment for esophageal cancer is esophagec-

tomy^{1,2}. However, esophagectomy is a complex and invasive surgical procedure, with relatively high morbidity and mortality rates despite advances in patient management and minimally invasive esophagectomy³. Therefore, predicting and preventing these life-threatening complications following esophagectomy is very important.

Heat-shock proteins (HSPs) are highly conserved proteins present in all kingdoms of organisms. These are expressed under stress conditions to protect cells from injuries, infection, oxidative damage, hypoxia, and thermal stress⁴. Any stress-induced protein denaturation is rectified by refolding and remodeling by HSPs.

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HSP70, the most extensively studied member of this family^{5,6}, has been shown to play an important role in numerous diseases including neoplasms⁷⁻⁹. HSP70 modifies the response to injury by increasing resistance to various inflammatory mediators, including oxidants and tissue necrosis factor (TNF)- α ¹⁰. HSP70 also inhibits nuclear factor-kappa B activation, altering protein and cytokine expression¹¹. In addition, immune cell HSP receptors bind HSPs released by necrotic cells and HSP-containing exosomes¹². By binding them, the production of TNF- α , IL-6, and chemokines decreases in some disease¹³⁻¹⁵. Moreover, HSP70 has been shown to protect against injury^{16,17}.

Recently, extracellular HSP70 has been measured in some diseases^{18,19}, but its levels have not, to our knowledge, been assessed in patients with esophageal cancer. We therefore measured serum HSP70 in patients with esophageal cancer and analyzed the correlation between these concentrations and postoperative morbidity.

Materials and Methods

Patients and serum

Serum samples were obtained a week before surgery from the peripheral veins of 31 patients with esophageal squamous cell carcinoma (29 men and two women) who underwent potentially curative surgery (transthoracic esophagectomy or thoracoscopic esophagectomy) at the First Department of Surgery, Dokkyo Medical University Hospital, between February 2010 and February 2016. We retrospectively reviewed the medical records of them. Patients ranged in age from 43 to 80 years, with a mean age of 63.4 years. Median follow-up time is 38.6 months. Tumor stage and disease grade were classified according to the seventh edition of the TNM classification of the International Union Against Cancer Criteria (UICC)²⁰. All distant metastatic lesions were in lymph nodes. Postoperative morbidities were defined as complications that seemed to be related to the surgical procedure. Complications were assessed in accordance with the Clavien-Dindo classification, and complications of grade II or above were regarded as significant. The study was approved by the Medical Ethics Committee of Dokkyo Medical University (approval number: R-20-6J), and all patients provided their written informed con-

sent.

Performed esophageal surgery

Esophageal surgery for this study cohort is performed by two surgeons. Surgeon 1 had been experienced about 300 cases of esophagectomy as a main operator, and surgeon 2 had about 150 cases as a main operator. Both of them had a certification of esophageal surgery from the Japan Esophageal Society. Surgeon 1 performed the surgery for 22 cases and Surgeon 2 performed for 9 cases.

Transthoracic esophagectomy was performed for 20 cases and thoracoscopic esophagectomy for 11 cases. Excision and reconstruction method is same for every patient. The subtotal esophagectomy and gastric conduit reconstruction via the posterior mediastinal route was performed and the esophagogastromy was made in the neck by end-to-side stapled anastomosis. Three-field (cervical, mediastinal, and upper abdominal) lymph node dissection was performed for all patients.

Quantification of serum HSP70

Blood samples were taken into clotting tubes for serum from patients with esophageal carcinoma before surgery. The blood samples were centrifuged at 3000 rpm for 15 min. Supernatants were stored at -80°C until use. Serum concentrations of HSP70 were measured using a quantitative sandwich enzyme immunoassay (HSP70 high sensitivity ELISA Kit ADI-EKS-715; Enzo Life Sciences, NY, USA). All samples were assayed in duplicate according to the manufacturer's instructions in a blinded fashion and averaged. The detection limit of the assay was set at 0.09 ng/ml.

Thereafter, the relationships between serum HSP70 level and perioperative inflammatory markers, and postoperative morbidities were analyzed. The primary endpoint of this study is the incidence of surgery-associated morbidity.

Statistical analysis

The chi-squared test and Fisher's exact test were used for statistical comparisons where appropriate. A *p*-value of less than 0.05 was defined as statistically significant. All statistical analyses were carried out using R software (version 3.3.1) (Free Software Foundation, GNU General Public License).

Table 1 Relationship between preoperative serum heat-shock protein 70 concentration and clinicopathological factors in patients with esophageal squamous cell carcinoma.

Parameter		N	Preoperative serum HSP70* concentration		p-Value
			Low (n = 14)	High (n = 17)	
Age, years	Mean ± SD	31	62.6 ± 9.7	64.0 ± 9.7	0.6863
Sex, n	Male	29	13	16	> 0.99
	Female	2	1	1	
Lesion location, n	Upper	5	3	2	0.9058
	Middle	17	8	9	
	Lower	9	3	6	
Depth of tumor invasion, n	T1	12	4	8	0.7713
	T2	3	1	2	
	T3	15	9	6	
	T4	1	0	1	
Lymph node metastasis, n	N0	14	7	7	0.8976
	N1	17	7	10	
Stage, n	0	2	1	1	0.9691
	I	8	3	5	
	II	5	3	2	
	III	5	3	3	
	IV	11	5	6	
Lymphatic invasion	No	11	5	6	0.7242
	Yes	20	9	11	
Blood vessel invasion, n	No	12	6	6	0.9524
	Yes	19	8	11	
Infiltrative growth pattern, n	a	7	2	5	0.7159
	b	16	9	7	
	c	8	3	5	

*: Heat-Shock Protein 70

Table 2 Relationship between preoperative serum heat-shock protein 70 concentration and postoperative morbidity

Surgery-associated morbidity	N	Preoperative serum HSP70* concentration		p-Value
		Low (n = 14)	High (n = 17)	
(-)	24	8	16	0.0281
(+)	7	6	1	

*: Heat-Shock Protein 70

Results

Serum concentrations of HSP70

The mean ± standard deviation serum concentration of HSP70 in the 31 esophageal cancer patients was 9.71 ± 5.89 ng/mL. Based on the mean concentration, the 31 patients were divided into two groups, with 14 patients having an HSP70 level below this cutoff (low HSP70 group) and 17 having a level above it (high HSP

70 group). When the correlations between serum HSP70 concentrations and the clinicopathological characteristics of these patients were assessed, no significant correlations were found (Table 1).

Relationship between serum HSP70 and perioperative inflammatory markers

When we analyzed the correlations between serum HSP70 concentration and perioperative indicators of inflammation, including maximum body temperature, white blood cell count, and C-reactive protein (CRP) concentration, no significant correlations between HSP70 and any of these markers were observed (data not shown).

Relationship between serum HSP70 and perioperative complications

Among the 31 patients assessed, seven (22.6%) had surgery-associated complications, including 6/14 pa-

Table 3 Postoperative morbidities experienced by patients with esophageal cancer undergoing esophagectomy

Morbidity	N	Preoperative serum HSP70* concentration		p-Value
		Low (n = 14)	High (n = 17)	
Pneumonia, n	No	30	14	> 0.9999
	Yes	1	0	
Anastomotic leakage, n	No	27	10	0.0318
	Yes	4	4	
Arrhythmia, n	No	30	14	> 0.9999
	Yes	1	0	
Hoarseness, n	No	27	10	0.0318
	Yes	4	4	

*: Heat-Shock Protein 70

Table 4 The relationship between preoperative parameters and postoperative morbidities

Parameter		Morbidities		p-value
		No (n = 24)	Yes (n = 7)	
Age, years	Mean ± SD	63.00 ± 10.57	64.57 ± 5.22	0.7091
Sex, n	Male	22	7	> 0.9999
	Female	2	0	
BMI, kg/m ²	Mean ± SD	22.28 ± 2.45	20.67 ± 5.58	0.2700
Stage	0	1	1	0.2989
	I	7	1	
	II	5	0	
	III	2	2	
	IV	8	3	
WBC, /mm ³	Mean ± SD	5737.50 ± 1520.24	6057.14 ± 1111.84	0.6105
Hb, g/dL	Mean ± SD	13.13 ± 1.76	12.93 ± 1.88	0.7995
AST, IU/L	Mean ± SD	24.92 ± 20.25	26.71 ± 9.71	0.8232
ALT, IU/L	Mean ± SD	21.17 ± 14.70	19.00 ± 10.13	0.7189
TP, g/dL	Mean ± SD	6.90 ± 0.48	6.71 ± 0.40	0.3555
Alb, g/dL	Mean ± SD	3.93 ± 0.33	3.74 ± 0.32	0.1816
Cre, mg/dL	Mean ± SD	0.76 ± 0.16	0.89 ± 0.20	0.1004
CRP, mg/dL	Mean ± SD	0.24 ± 0.24	0.20 ± 0.20	0.7104
HSP70*, ng/mL	Mean ± SD	10.88 ± 5.75	5.73 ± 4.76	0.0396

*: Heat-Shock Protein 70

tients (42.9%) in the low HSP70 group and 1/17 patients (5.9%) in the high HSP70 group ($p=0.0281$) (Table 2). The incidences of each type of complication in the two groups are shown in Table 3. Among them, the frequency of anastomotic leakage and hoarseness differed markedly between the two groups, occurring in four patients in the low HSP70 group but not at all in the high HSP70 group ($p=0.0318$). There was no surgery-related mortality. Additionally, we examined the relationship between preoperative parameters and

the occurrence of complications. According to that, there was no preoperative factor related to surgery-associated complication but HSP70 (Table 4).

Discussion

Esophageal cancer is one of the most difficult malignancies to cure. The prognosis remains unsatisfactory despite significant advances in surgical techniques and perioperative management⁽²¹⁾. Moreover, the difficulties of radical surgery increase perioperative morbidity

and mortality rates, negatively affecting short-term survival. Esophagectomy is a complex and invasive surgical procedure, with relatively high morbidity (40%) and mortality (5-10%) rates despite advances in patient management²². Therefore, it is important to identify markers to predict the risk of perioperative morbidity for the management of safe surgery.

Previously, we hypothesized that postoperative morbidity is associated with the reparative ability of the patient, and targeted HSP70 as possibly being involved in this process. Thus, we previously reported that serum level of antibody to HSP70 is associated with the incidence of postoperative morbidity²³. According to that investigation, anastomotic leakage was found to be the complication most strongly associated with a low level of serum anti-HSP70. Because *HSP70*, the most highly conserved gene of the HSP family, has been shown to modify responses to injury by increasing resistance to inflammatory mediators¹³, we thought that our findings were reasonable and anti-HSP70 might be a candidate predictive marker for postoperative morbidity of esophageal cancer. However, anti-HSP70 is an indirect marker and we were unable to record the level of HSP70 directly. Therefore, in this study, we measured serum HSP70 itself to provide stronger evidence.

We performed esophagectomy, lymph node dissection and esophageal reconstruction in the same manner for all patients even though two approaches (transthoracic and thoracoscopic) were used. There was no significant difference between surgical approach, preoperative HSP70 value, and postoperative complications (data not shown). Among the postoperative complications in our patients were pneumonia, anastomotic leakage, arrhythmia, and hoarseness. The postoperative complication rate was significantly lower in the high HSP70 group than the low HSP70 group for anastomotic leakage and hoarseness. Leakage of the esophagogastric anastomosis has been linked to ischemia of the gastric conduit and low blood oxygen level²⁴, as well as to reduced intra- and postoperative tissue blood flow, which can lead to cell damage.

Hoarseness occurs due to lymph node dissection around the recurrent nerve, especially on the left side. In many cases, technical matters such as pressure, traction, or diffusion of heat from surgical devices can

cause nerve cell damage²⁵. Our findings suggest that patients with a high serum level of HSP70 may recover better from cell damage after anastomosis and lymph node dissection around the recurrent nerve.

It must be coincidental that the number of anastomotic leakage/hoarseness and pneumonia/arrhythmia are completely same in this study. Hoarseness is derived from recurrent laryngeal nerve palsy and is deeply involved in aspiration pneumonia. On the other hand, arrhythmia affect anastomotic leakage due to microangiopathy of anastomotic site. However, ours are mismatched.

In experimental models of disease, HSP administration can prevent or arrest inflammatory damage, and in initial clinical trials in patients with chronic inflammatory diseases, HSP peptides have been shown to promote the production of anti-inflammatory cytokines, indicating that HSPs have immunoregulatory potential^{26,27}. Immunization with recombinant HSP70 has been shown to induce protective immunity against infectious diseases²⁸. These findings may support the concept that patients with esophageal cancer with a high level of serum HSP70 may be at a lower risk of perioperative morbidity. Our data of which there was no preoperative factor related to surgery-associated complication but HSP70 may support such concept, too.

Our results suggest that complication rates may be reduced by preoperatively enhancing HSP70 expression, for example, by the induction of preoperative hyperthermia, which has also been shown to have antitumor effects. Geranylgeranylacetone may also induce high levels of expression of HSP70, thereby reducing perioperative morbidity²⁹.

In conclusion, this study showed a significant relationship between the preoperative serum concentration of HSP70 and postoperative morbidities of patients with esophageal cancer. If the usefulness of HSP70 as a suppressor of postoperative morbidity is confirmed in animal models, for example, using hyperthermia or geranylgeranylacetone, enhancing the HSP70 level may be a feasible strategy for preventing postoperative morbidities.

Ethical standard statement

All procedures followed were in accordance with the

ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Conflict of interest

Masanobu Nakajima and co-authors declare no conflicts of interest.

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