

RESEARCH COMMUNICATION

Catalase, Carbonic Anhydrase and Other Biochemical Parameters in Esophageal Cancers in Turkey

Halit Demir¹, Zeynel Abidin Akkus², Aysegul Cebi³, Tahir Cakir⁴, Mustafa Izmirli⁵

Abstract

Purpose: The main aim of our investigation is to show possible changes in antioxidant balance and selected biochemical parameters in esophageal cancers. **Materials and Methods:** This study was performed for the determination of levels in 25 patients diagnosed as having cancer in the Yuzuncu Yil University (Medical Oncology) and 15 healthy volunteers at same department with negative cancer signs. **Results:** The activity levels of catalase of sick and health groups were 33.8 ± 4.31 (EU/gHg)⁻¹, 122.4 ± 31.7 (EU/gHg)⁻¹ respectively. The difference between the average levels was significant ($p < 0.001$). CA (carbonic anhydrase) activity was 2.19 ± 0.12 (EU/gHg)⁻¹, 2.46 ± 0.32 (EU/gHg)⁻¹ in groups at same order with no statistical significance ($p > 0.05$). The levels of albumin, globulin, ferritin, ALT, LDH, glucose, CRP, AST and uric acid were also determined in sick and healthy groups: 4.04 ± 0.24 , 4.04 ± 0.43 gr/dl ($p > 0.05$); 4.46 ± 0.82 , 3.17 ± 0.10 g/dl ($p > 0.05$); 175.4 ± 29.1 , 260.4 ± 15.45 g/dl ($p < 0.001$); 12.7 ± 1.13 , 19.5 ± 1.91 ng/ml ($p < 0.001$); 317.6 ± 13.8 , 298.0 ± 12.1 mg/dl ($p > 0.05$); 106.5 ± 12.1 , 89.7 ± 2.2 ($p > 0.05$), 5.94 ± 1.04 , 4.41 ± 0.28 U/L ($p > 0.05$); 19.4 ± 1.68 , 19.5 ± 1.91 mg/dl ($p > 0.05$); 30.0 ± 3.24 , 10.5 ± 0.64 mg/dl ($p < 0.01$) respectively. **Conclusions:** Consequently, in diagnosis of esophageal cancer, CA, CRP, uric acid and antioxidant examinations may be very important factors. Additional studies are needed to further address this important issue.

Keywords: Esophageal cancers - biochemical parameters - carbonic anhydrase - catalase

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Introduction

Esophageal cancer (EC) is a relatively rare and lethal disease in the world, being the seventh leading cause of cancer death worldwide. Incidence of esophageal carcinoma can be as high as 30-800 cases per 100,000 persons in particular areas of northern Iran, some areas of southern Russia, and northern China. The most common histologic types are squamous cell carcinoma (SCC) and adenocarcinoma (AC), which together constitute more than 90% of esophageal malignancies. Rarely, melanoma, sarcoma, small cell carcinoma, or lymphoma may arise in the esophagus (Tilanus, 1995; Wong et al., 2003).

Many recent studies have proved the role of oxidative stress in carcinogenesis (Wiseman and Halliwell, 1996; Monya et al., 2001). While oxidative stress arises as a consequence of a pathological event, a defense system promotes the regulation and expression of enzyme antioxidants such as superoxide dismutase (SOD), catalase and glutathione peroxidase, which constitute the principal cellular defense mechanisms against ROS (Jimenez et al., 2005). Catalase (CAT, H₂O₂: oxidoreductase, EC 1.11.1.6) is well known plasma antioxidant enzyme.

(Carbonic anhydrase) CA is a member of the alpha-family of carbonic anhydrases of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide to carbonic acid. Carbonic anhydrases are involved in several physiologic and biologic processes in humans. CA is not only a highly active enzyme but is also involved in cell-cell adhesion and cell proliferation (Sly and Hu, 1995).

C-reactive protein (CRP) is an acute-phase protein. The production of CRP is regulated by proinflammatory cytokines, in particular IL-6, IL-1 and TNF α , which serve as autocrine growth factors in neoplastic processes (Miki et al., 1989; Nozoe et al., 2001; Shimada et al., 2003; Gockel et al., 2006).

Lactate dehydrogenase (LD; EC 1.1.1.27) isoenzymes are formed by random combinations of two different subunits encoded by structurally distinct genes, LDHA and LDHB (Markert, 1963).

We aimed in this investigation to determine possible changes of catalase, carbonic anhydrase activities and some biochemical parameters (ALT, AST, uric acid, albumin, globulin, ferritin, LDH, glucose, CRP) in a series of esophageal cancers in a relatively high incidence area of Turkey.

¹Department of Chemistry, ²Department of Biology, Faculty of Science and Art, ⁴Department of Radiation Oncology, Yuzuncu Yil University, Van, ³Faculty of Health Sciences, Giresun University, Giresun, Turkey * For correspondence : halitdemir2005@yahoo.com

Table 1. Erythrocyte Activities of Catalase and Carbonic Anhydrase, Serum Levels of Albumin, Globulin, Ferritin, ALT, LDH, Glucose, CRP, AST and Uric Acid of the Esophageal Cancers and Healthy Groups

Parameters	Control n=15	Control	Patient	t	p
	Patient=25	M ±SD	M ±SD		
Albumin (g/dl)		4.04±0.24	4.04±0.43	0.011	0.991
Globulin (g/dl)		3.17±0.10	4.46±0.82	-1.208	0.235
Ferritin (g/dl)		260.40±015.45	175.35±29.09	2.149	0.038*
ALT (EU/I)		19.53±01.91	12.69±1.13	2.884	0.006*
LDH (U/L)		298.00±012.14	317.55±13.77	-0.970	0.960
Glucose (mg/dl)		89.70±02.18	106.45±12.14	-1.057	0.297
CRP (mg/dl)		4.41±0.28	5.94±1.04	-1.208	0.270
AST (EU/I)		19.53±01.91	19.40±1.68	0.051	0.960
Uric acid (mg/dl)		10.53±0.64	30.03±3.24	-4.605	0.000**
Catalase (EU/gHg) ⁻¹		122.37±031.70	33.80±4.31	3.546	0.001*
Carbonic anhydrase (EU/gHg) ⁻¹		2.46±0.32	2.19±.12	0.949	0.349

**p<0.01; *p<0.001

Materials and Methods

This study was performed on 25 patients with EC who underwent chemotherapy at the Department of Internal Medicine (Medical Oncology), Yuzuncu Yil University Faculty of Medicine and 15 healthy volunteers in 2005-2006. All patients signed informed consent forms in accordance with our institutional guidelines. Normal gastric mucosa served as a positive control. The study was approved by the local ethics committee. Venous blood samples were drawn into sterile vacuum tubes when oesophageal carcinoma was detected before surgery or anticancer therapy. The tubes were centrifuged at 3000 rpm and separated sera were stored at -80°C for future use.

Biochemical analysis of CAT activity in erythrocytes was performed with a method described by Aebi (1984) in the Biochemistry Laboratory of Chemistry Department, Faculty of Art and Science, Yuzuncu Yil University. Briefly, the supernatant (0.1 ml) was added to a quartz cuvette containing 2.95 ml of 19 mmol l⁻¹ H₂O₂ solution prepared in potassium phosphate buffer (0.05 M, pH 7.00). The change in absorbance was monitored at 240 nm for 5 min using a spectrophotometer (Shimadzu UV-1201, Japan).

CA activity was assayed by hydration of CO₂. Hydration of CO₂ was measured by the method of Rickli and Wilbur-Anderson with bromothymol blue as an indicator (Rickli et al., 1964).

The data were expressed as mean (M) ± standard deviation (SD) and analyzed using one-way repeated measures analysis of variance (ANOVA). Student t-test was used to test for differences among means for which

Table 2. Patients and Healthy Humans Characteristics

Characteristic	No. of Patients (25)	No. of Healthy human (15)
Age, years		
Median	56	50
Range	25-75	23-55
Sex		
Male	12	8
Female	13	7
Primary sites		
Esophagus	25	

one-way ANOVA indicated a significant (P<0.01, P<0.001 or P<0.05) F ratio.

Results

The catalase activities of patient and control groups were 33.8±4.31 (EU/gHg)⁻¹, 122.4±031.70 (EU/gHg)⁻¹ respectively. The difference between the average levels of groups was significantly important (p<0.001). CA (carbonic anhydrase) activities were 2.19±.12 (EU/gHg)⁻¹, 2.46±0.32 (EU/gHg)⁻¹ in groups at same order with not statistical importance (p>0.05).

The levels of albumin, globulin, ferritin, ALT, LDH, glucose, CRP, AST and uric acid were determined in sick and healthy groups; 4.04±0.24, 4.04±0.43 gr/dl (p>0.05); 4.46±0.82, 3.17±0.10g/dl (p>0.05); 175.35±29.09, 260.40±015.45g/dl (p<0.001); 12.69±1.13, 19.53±01.91 ng/ml (p<0.001); 317.55±13.77, 298.00±012.14 mg/dl (p>0.05); 106.45±12.14, 89.70±02.18 (p>0.05), 5.94±1.04, 4.41±0.28 U/L (p>0.05); 19.40±1.68, 19.53±01.91 mg/dl (p>0.05); 30.03±3.24, 10.53±0.64 mg/dl (p<0.01) respectively.

Discussion

Esophageal cancer is one of the most rapidly progressive and difficult to treat diseases. Esophageal carcinoma arises in the mucosa. Subsequently, it tends to invade the submucosa and the muscular layer and, eventually, contiguous structures such as the tracheobronchial tree, the aorta, or the recurrent laryngeal nerve (Chang et al., 2008; Türkyılmaz et al., 2009).

Both genetic and environmental factors may contribute to the development of esophageal adenocarcinoma (McManus et al., 2004). Among the environmental factors, long-term antacid therapy might promote esophageal adenocarcinoma by producing hypergastronemia and promoting bile acid-induced mutagenesis in a neutral pH environment (Stamp, 2006; Feagins et al., 2007; Hao et al., 2009). Lipid peroxides are formed by auto-oxidation of polyunsaturated fatty acids found primarily in cell membranes. An increase level of lipid peroxides in the tissue therefore reflects membrane damage. It is reported that water immersion restraint rats caused significant increase of gastric mucosal lipid peroxide which reflected

on gastric mucosal injury. The gastric mucosal injury is also known as the post-operative complication due to physical stress (Kawamura et al., 1992). In human studies, a positive association between mucosal free radicals levels and the grade of esophagitis has been described (Olyae et al., 1995; Wetscher et al., 1995). Antioxidant potential in all cases of gastrointestinal tract cancer has been unbalanced which has led to increase in reactive oxygen species action and enhancement of lipid peroxidation and cancer procoagulant generation. Catalase subsequently reacts with hydrogen peroxide which was produced by SOD, and decomposes it into water and molecular oxygen (Skrzydłowska et al., 2003; Hwang et al., 2007). These results indicated that lipid peroxidation may contribute to the development of DNA damage in the operation of esophageal cancer. CAT also decreased in the patients of esophageal cancer ($p < 0.001$) (Table 1).

Carbonic anhydrase (CA), a highly active carbonic anhydrase, is a novel tumor-associated protein. This tumor marker has been evaluated in several types of solid tumors (Zavada et al., 1993). No associations were found between control and CA of esophageal cancer (Table 1).

Ferritin is a ubiquitous protein that stores iron and releases it in a controlled fashion. The protein is produced by almost all living organisms, including bacteria, algae and higher plants, and animals. In humans, it acts as a buffer against iron deficiency and iron overload (Orino et al., 2001; De Zoysa and Lee, 2007). There was statistical difference related to plasma ferritin concentrations between esophageal patients and control subjects ($p < 0.001$) (Table 1).

Albumin is an umbrella term for a type of protein which is water soluble. Many plants and animals contain or secrete albumin. It is interesting to notice that even in patients with low albumin concentrations the supplementation may correct hypozincemia (Barve et al., 2008; Zhou et al., 2008). It was found that serum albumin activity was a little higher in patients with esophageal cancer compared to controls ($P > 0.05$).

Globulin is one of the two types of serum proteins, the other being albumin. Some globulins are produced in the liver, while others are made by the immune system. It is sometimes used synonymously with globular protein (Tekman and Öner, 1994). Globulin also increased in the patients of esophageal cancer ($P > 0.05$) (Table 1).

Many cancer cells use glucose as their primary fuel. Under the hypoxic conditions, in the centre of a tumor mass, there is often a region where glycolysis is the only source of ATP with lactic acid as the main end product. This is quite old news, going back to Warburg and the concept of using low blood glucose to suppress tumor growth (Mc Carty, 2001). There was not statistical significance between in esophageal cancer and controls ($P > 0.05$).

C-reactive protein (CRP) is an acute-phase reactant and a known indicator of the malignant potential of tumors. The synthesis is regulated by pro-inflammatory cytokines, which serve as growth factors in neoplastic processes. A correlation has been established between elevated serum CRP concentrations and malnutrition as well as impaired immunity in patients with oesophageal cancer (Nozoe et

al., 2001; Gockel et al., 2006). Acute-phase reactions of the organism have further been observed in tumor progression and tumour recurrence (Cooper and Stone, 1979). There were no associations between esophageal patients and control subjects ($P > 0.05$).

Uric acid (UA), which is the final product of purine nucleoside metabolism, is a strong peroxynitrite scavenger (Staub, 1999). Uric acid may be a marker of oxidative stress, and may have a potential therapeutic role as an antioxidant. On the other hand, like other strong reducing substances such as ascorbate, uric acid can also act as a pro-oxidant, particularly at elevated levels (Becker, 1993; Glantzounis et al., 2005; Uğur-Chousein, 2005). There was statistical difference related to plasma uric acid concentrations between esophageal patients and control subjects ($p < 0.01$) (Table 1).

Lactate dehydrogenase (LDH) catalyses the reversible transformation of pyruvate to lactate, having a principal position in the anaerobic cellular metabolism. Induction of LDH occurs during hypoxia and LDH transcription is directly regulated by the hypoxia-inducible factor 1 (HIF1). Serum LDH levels have been correlated with poor prognosis and resistance to chemotherapy and radiotherapy in various neoplastic diseases (Koukourakis et al., 2003). Expression of mammalian LDHA and LDHB is regulated during development and is tissue specific; therefore, alterations in the serum LD isoenzyme pattern serve as indicators of pathologic conditions and cancer development (Maekawa, 1988). No differences were observed for esophageal patients and control subjects ($P > 0.05$).

Alanine transaminase or ALT is a transaminase enzyme (EC 2.6.1.2). It is also called serum glutamic pyruvic transaminase (SGPT) or alanine aminotransferase (ALT). ALT is found in serum and in various bodily tissues, but is most commonly associated with the liver. It catalyzes the two parts of the alanine cycle. ALT is a sensitive indicator of liver cell injury and has been used to identify patients with liver disease for almost 50 years (Karmen et al., 1955; Riedler et al., 1975; Sevelius, 1995). There was statistical significance between in esophageal cancer and controls ($p < 0.001$) (Table 1).

An aspartate aminotransferase (AST) test measures the amount of this enzyme in the blood. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. AST formerly was called serum glutamic oxaloacetic transaminase (SGOT). The exact mechanism behind AST or AST/ALT ratios is unclear and is it not known how they induce esophageal cancer. One possible explanation is that aminotransferase levels partially reflect the amount of alcohol intake. Previous studies reported that smoking and alcohol usage increased the risk of esophageal cancer multiplicatively (Kimm et al., 2010). An important limitation for using AST as an indicator of liver toxicity is the fact that this enzyme is also present in the heart, skeletal muscle, kidney and brain. In contrast, serum alanine aminotransferase (ALT) activity level is considered the most frequently relied upon laboratory indicator of hepatotoxic effects (Ozer et al., 2008). It was found that serum AST level lower in patients with esophageal cancer compared to controls but

not statistical significant ($P>0.05$).

Consequently, in the diagnosis of the illness with cancer CA, CRP, uric acid and antioxidant examinations may be very important factor to be asked in terms esophageal cancer.

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