

## The Serum C-reactive Protein and Prooxidant-Antioxidant Balance in Patients with Esophageal Cancer Compared to Healthy Subjects

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### ABSTRACT

**Introduction:** The molecular mechanisms involved in pathogenesis of esophageal cancer have been the main concern of several studies. In this study, we aimed to investigate the changes of serum prooxidant-antioxidant balance (PAB) values as a redox index, as well as serum C-reactive protein (CRP) compared to healthy control group.

**Materials and Methods:** In a cross-sectional study, blood samples were drawn from 25 patients with esophageal cancer and 25 healthy subjects. Serum CRP and PAB value were measured in all samples according to relevant protocols.

**Results:** Serum CRP was significantly higher in our patients ( $14.3 \pm 3.2$  mg/L) compared to healthy control group ( $4.6 \pm 1.4$  mg/L), with a *P*-value of less than 0.001. The value of PAB in our patients ( $133.9 \pm 21.7$ ) was also higher than that of healthy subject ( $51.3 \pm 11.2$ ), indicating a redox perturbation in favor of oxidants.

**Conclusion:** There was a significant increase in both serum PAB value and CRP in patients with esophageal cancer compared to the control group, which indicated both oxidative stress and inflammatory response in patients with esophageal cancer, respectively.

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### Introduction

Esophageal cancer is a common cancer in Iran and world-wide with an increasing prevalence related to a number of risk factors (1-2). Due to its increasing incidence and prevalence, as well as the challenges in the treatment, it is very appealing to investigate about esophageal cancer on a molecular level from different points of view, such as finding diagnostic or prognostic biomarkers. Furthermore, a deeper understanding of

pathogenesis of this disease or finding new molecular targets for therapeutic approaches have been the aims of several other studies that have been performed on the molecular level (3-4). For instance, the role of reactive oxygen species (ROS) and their inflammatory effect is proved to be involved in esophageal carcinogenesis (2), and in the morbidity after esophageal cancer surgery as well (5).

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Reactive oxygen species are highly reactive compounds which can oxidize different kinds of cellular biomolecules such as proteins, lipids and DNA if their effect cannot be counteracted by cellular antioxidants that are supposed to maintain the redox balance and homeostasis, an essential condition for cell survival (6, 7). If this balance between oxidants and antioxidants perturbs in favor of oxidants, the state of oxidative stress occurs. It has been shown that oxidative stress is involved in pathogenesis of a wide variety of diseases such as different types of cancers (8-10), infections (11), autoimmune diseases (12), cardiovascular disorders (13, 14), neurologic diseases (15), and etc.

In addition, the role of oxidative stress in esophageal cancer has been investigated from different points of view (16). In a study by Sihvo *et al*, they proved that oxidative stress has a central role in malignant transformation in Barrett's esophagus and its progression toward esophageal cancer (17-18). The role of oxidative stress in the pathogenesis of reflux esophagitis and esophageal cancer have also been investigated and shown in animal model studies (19-20). In several other studies on animal and human models, it is shown that ROS produced by the metabolism of ethanol, which is a risk factor for esophageal cancer, or by chronic inflammation may mediate the carcinogenic process (19, 21, 22).

In this study, in order to further our knowledge about the relationship between oxidative stress and esophageal cancer, we aimed to investigate the changes of serum C-reactive protein (CRP) and prooxidant-antioxidant balance (PAB) as two inflammatory markers; even though the changes of CRP in patients with esophageal cancer have been reported in several other studies (23, 24), the investigation of serum PAB level as a new marker of oxidative stress was done for the first time in this study.

## Materials and Methods

Based on permission from the Ethical Committee of Research Council, Mashhad University of Medical Sciences and in a cross-sectional study, 5 ml of blood sample was collected from the brachial vein of 25 patients with esophageal cancer who had been admitted to the department of oncology, Imam Reza Hospital, Mashhad, Iran in 2012. The sampling was done only from new patients with this disease without any history of treatment for their condition, or any history of antioxidants consumption in recent two months prior to their admission that could affect the prooxidant-antioxidant balance. The samples for the control group were taken from 25 healthy

volunteers who were matched with our patients based on age and gender. The written informed consent was provided from all participants. Serum samples were collected from centrifugation of clotted blood samples at 2500 rpm for 10 minutes at room temperature. The samples were kept at -80 °C until laboratory measurements.

## Chemicals

C-Reactive Protein (human) EIA Kit was purchased from Cayman, USA (Item No. 10011236). TMB powder (3,3',5,5'-Tetramethylbenzidine, Fluka), per-oxidase enzyme (Applichem: 230 U/mg, A3791,0005, Darmstadt, Germany), chloramine T trihydrate (Applichem: A4331, Darmstadt, Germany), and hydrogen peroxide (30%) (Merck) were used for PAB assay. All other required reagents were purchased from Sigma-Aldrich, Germany.

## Measurement of serum CRP

The serum level of CRP was measured according to the protocol provided by the company (Cayman, USA) in which the immunometric assay is based on a double-antibody 'sandwich' technique.

## Measurement of serum PAB

The prooxidant-antioxidant balance was measured in serum samples of both patients and control group as described before by Alamdari *et al* (25). In summary, this method is based on the measurement of the balance between oxidants and antioxidants simultaneously, by using chromogen TMB that can be either oxidized to a color cation by oxidants, or reduced to a colorless compound by antioxidants; which finally provides a redox index. In order to provide standard solutions, varying proportions (0-100%) of 250  $\mu$ M hydrogen peroxide, as the oxidant, were mixed with 3 mM uric acid (in 10 mM NaOH), as the antioxidants. The absorbance of samples was read with an enzyme-linked immunosorbent assay reader at 450 nm. Afterward, the value of unknown samples was calculated using the standard curve.

## Statistical analysis

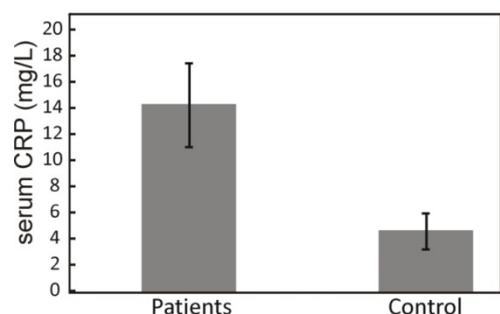
The data was analyzed statistically using *t*-test in Statistical Package for Social Sciences (SPSS version 16, Chicago, IL, USA). Since the results were normally distributed, they are presented as mean  $\pm$  SD. A *P*-value of less than 0.05 was considered as significant.

## Results

The average age of our patients was 62.7 $\pm$ 7.8 years, among them 19 and 6 were male and

female, respectively. The average age of the control group was  $58.3 \pm 9.3$  years. The selection of the gender in the healthy control was similar to our patients (20 males and 5 females).

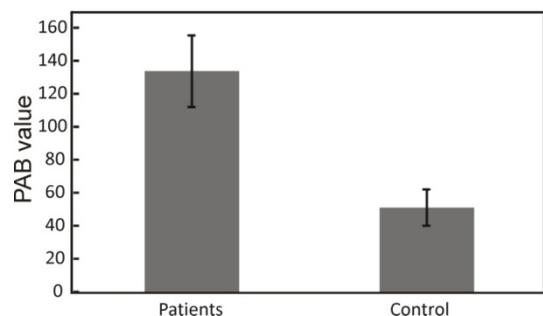
Quantitative CRP in serum sample of patients was  $14.3 \pm 3.2$  mg/L, while in the control group a value of  $4.6 \pm 1.4$  mg/L was obtained. Based on this data, a significant difference with a *P*-value of less than 0.001 was shown (Figure 1).



**Figure 1.** The significant difference of serum CRP in patients and control group. As shown, serum CRP of patients with esophageal cancer ( $14.3 \pm 3.2$  mg/L) was about 3-times more than our healthy subjects ( $4.6 \pm 1.4$  mg/L).

The PAB values of patients with esophageal cancer and control group were  $133.9 \pm 21.7$  and  $51.3 \pm 11.2$ , respectively (Figure 2), which showed a significant difference between the PAB value of our patients and the control group with a *P*-value of less than 0.001.

A meaningful correlation between serum CRP and PAB was not observed (data not shown).



**Figure 2.** The relationship between oxidative stress and esophageal cancer. The serum PAB value that is a redox index was higher in patients compared to the healthy subjects with a *P*-value of less than 0.001.

## Discussion

In this study, we compared the serum C-reactive protein and prooxidant-antioxidant balance, as two inflammatory markers, in patients of esophageal cancer with healthy subjects. Based on our results, patients had a significantly higher level of both CRP and PAB compared to the control group. Since the relationship between oxidative stress and esophageal cancer has been reported by several studies, we aimed to compare the serum PAB value in patients to the

value of healthy control group. PAB assay is a recently-introduced assay in which the balance between all oxidants and antioxidants can be measured simultaneously in an easy and cost-effective way (25). With respect to the standard curve that was used to measure PAB values, a higher PAB value means that oxidants are present at a greater concentration than are antioxidants. In this study, the PAB value in patients with esophageal cancer was about 2.6 times more than the control group. It means that there was a relationship between esophageal cancer and oxidative stress in our study.

Since the role of oxidative stress has been proven in a number of studies (26-28), the application of antioxidants as a part of therapeutic protocol in esophageal cancer has been suggested and subsequently investigated by several researchers. For example, in a recent study, Aiko *et al* showed that a diet containing several antioxidants may reduce oxidative stress following esophageal cancer surgery, which in turn may provide a tool for perioperative management to reduce morbidity after esophageal cancer surgery (5). In another study, the protective effect of an antioxidant dietary pattern against esophageal cancer was shown (29), which empower this hypothesis that oxidative stress has an important role in the molecular mechanism of this pathology.

Besides, the application of oxidative stress markers in monitoring patients with esophageal cancer has been proposed by a number of researchers through their investigations (30). Since PAB assay is an available and inexpensive test, its application in monitoring patients may be considered.

One limitation of this study was not grouping our patients based on the tumor stage. It was due to the number of patients that were recruited for this study. Since the correlation between tumor stage and oxidative stress can be hypothesized, the relationship between PAB value and tumor stage as well as tumor size, and tumor metastasis remains to be investigated in future studies. Besides, the correlation between PAB value and tumor prognosis needs further investigations.

Since the relationship between oxidative stress and esophageal cancer has been proven through this study as well as several others, this question also needs to be answered whether oxidative stress is the cause or result of this pathology?

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## Conflict of Interest

Authors declare there was not any conflicting interest.

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