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Determination of Serum Survivin for Prognostic Role in Esophageal Cancer

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Abstract

***Background & Aims*:** In the recent years, the considerable interest in survivin has significantly increased, because of its possible detection role in esophageal cancer.

Materials and Methods: Twenty eight patients diagnosed with esophageal cancer and thirty three healthy controls were assessed for the purpose of the study. From March 2015 until September 2017, the subjects who had esophageal cancer were enrolled in the study. Blood samples were from subjects enrolled in a prospective cohort undergoing annual esophageal cancer testing. The concentrations of serum survivin were determined using Elisa method.

***Results*:** Serum levels of survivin in the esophageal patient group increased compared to the healthy controls [164.06±55.03 (mean±SD) vs. 119.37±48.25, ng/L, P<0.04].Elevated serum survivin had positive correlation with clinical parameters**.**

***Conclusion*:** The positive association between elevated serum survivin and esophageal cancer status could be due to the fact that this protein involves in the development of esophageal cancer**.** Determination of serum survivin could differentiate normal and esophageal cancer subjects and lead to lower numbers of excessive esophageal biopsies.

***Keywords*:** Cancer, esophageal, prognostic, survivin

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Introduction

Currently, many biomarkers are routinely used as serum markers for detection of esophageal cancer. Owing to the low sensitivity and specificity of detection of these biomarkers, additional serum markers must be established for early detection and diagnosis of esophageal cancer (1).Earlier study has suggested that survivin may be an objective in the therapy of esophageal cancer (2). It is now clear that survivin is an attractive nominee for targeted treatment in esophageal squamous cancer cells (3).Researchers suggested that survivin over expression has no correlation with stage, and grade of differentiation of esophageal cancer (4).It has been revealed that inhibition of survivin in esophageal adenocarcinoma cell lines showed increased apoptosis(5). Interestingly, overexpression of p53 decreases survivin transcription (6).A study reported that survivin is an upregulated inhibitor of apoptosis protein in esophageal cancer (7). Survivin, related to the inhibitor of apoptosis protein family, plays an important role in cellular metabolism. Data indicates that survivin leads to uncontrolled cell growth and resistance to apoptosis (8). It is important to emphasize that survivin mRNA expression of tumor tissues was higher than normal tissues in esophageal cancer(9).It has also been suggested that esophageal cancer is of high prevalence and poor prognosis(10).Evidence reveals that survivin highly expressed in esophageal cancer(11-13).According to several studies, survivin expression levels are associated with the prognosis of esophageal cancer patients (14-17).Data have demonstrated that survivin is over expressed in esophageal cancer(18,19). By considering the controversial reports of the above studies, the resolution of this phenomenon needs additional research. In our on-going research projects on human cancer mechanism,we undertook the present project.

Materials and Methods

All chemicals were of the highest purity available. Human survivin Elisa kit, deionized water, and coated Elisa plate were used.

Instruments:

Centrifuge (Clement 2000**,** Australia), water bath (Fanazmagostar Co WM22),Vortex mixer labnet, Dionizer (HastaranTeb Co), and Microplate reader with 450 ±10 nm filter (Rayto life and analytical science Model RT -2100 C, Hamburg, Germany) were used.

Patients and Sample Collection:

Twenty eight patients diagnosed with esophageal cancer and thirty three healthy controls, after getting evaluated, were selected for the current research. Basic physical examinations have been performed and medical history has been collected from both groups. The patients from both groups were between 52 and 61 years of age.

Serum samples were obtained spanning the years from March 2015 to September 2017 from women and men enrolled in undergoing annual esophageal cancer testing. The patient group had signs and symptoms of esophageal cancer including weight loss, painful and difficult swallowing. Healthy controls were defined as subjects who had been followed at least for 2 years on a study with no esophageal cancer diagnosis, with comparable age and gender. They were recruited from the health examination center of Shahid Rajaee Medical University Hospital. All patients were confirmed with esophageal cancer by experienced oncologist. Clinical data including age, clinical stage, and smoking history was obtained from the two groups. In group (I), healthy control group, n=20, and in group (II), patients with esophageal cancer, n=39 samples were screened; however, 11 patients were excluded, because they of not having sufficient quantity for analysis. A total of 28 patients diagnosed with esophageal cancer samples were analyzed for this study. After fasting, overnight 3 mL of blood was collected. All serum obtained from venous blood of patients, yielded to general routine tests at the clinic for annual esophageal cancer testing, according to ethic committee rules. Blood samples were centrifuged (serum) and frozen at −20°C and transported, in proper containers, to the laboratory of Biochemistry Department of Medical University of Babol. Sample preparation was carried out as quickly as possible. The inclusion Criteria in this project werepatients with esophageal cancer, and the exclusion criteria were (i)

patients with other cancer or malignant diseases, (ii) patients who received chemotherapy or radiation therapy, (iii) patients with chronic liver and kidney diseases.

We determined serum survivin in two groups via Elisamethod. We collected the data on the participants' age, gender, as well as the following clinical characteristics.Written consent was obtained from each subject. The study project and the written consent procedures were approved by the Ethic Committee of Babol University of Medical Sciences(no: MUBABOL.REC.1394.304).

Assay procedure:

Survivin concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) method according to the manufacturer's instructions.

Standards preparation

For standard preparation 120µL original standard (40,80,160,320 and or 640 ng/ml) was added to and mixed well with 120 µL standard diluents. After that standard solution well, 50µL standard was added to 50µL streptavidin–HRP.Human survivin ELISA Kit, E3904Hu components were labeled with biotin, streptavidin–HRP and survivin standard solution.

Preparation of washing solution:

The washing concentration was diluted (30x) with deionized water was used.

Deionized water was used for the preparation of all reagents. The survivin assay buffer and survivin antibodies were warmed to room temperature to thaw solution prior to use.

Sample well, 40 µL of serum sample was added to 10 µL of survivin antibodies and 50 µL streptavidin-HRP. It was then covered with seal plate membrane, shacked slowly to get mixed, and incubated at 37 ºC for 60 min. For color development, 50µL chromogen A and B was added to each well, then incubated for 10 min at 37C.After that, 50 µL stop solution was added to each well to stop the reaction(blue color changes into yellow). Finally, the absorbance was measured at 450 nm. According to standard concentrations and the corresponding OD levels, the linear regression equation of the standard cure was estimated. Then according to the OD level of each sample, survivin content was calculated.

Statistics:

Results were expressed as mean ±standard deviation (SD) in the study. Descriptive statistics and analysis were performed in SPSS16 for windows.

Results

As it is evident from figure 1, serum survivin content in the esophageal patient group increased compared to the healthy controls. Serum survivin values showed to be significantly elevated in patient group with esophageal cancer compared to those of the healthy control group, a finding which was paralleled with clinical characters for these patients.

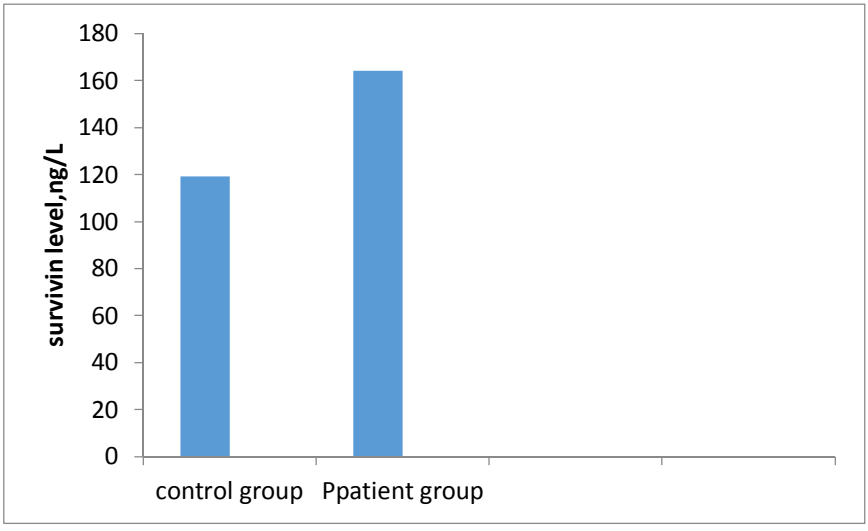

Figure 1. Serum survivin content in the esophageal patients group was increased compared to that in the controls

Discussion

When it comes to diagnosing esophageal cancer, The accuracy and speed of diagnosis with the help of biomarkers is important for the care of patients with esophageal cancer. The high accuracy of diagnostic tests helps doctors design a suitable treatment plan for esophageal cancer patients. Evidence proposes that esophageal cancer with some slow-growing organism may not result in a biochemical reaction and hence may not result in elevation of current markers in the serum, raising a concern regarding the ability of the tests in some positions. Therefore, there is high interest in the finding of new biomarkers to diagnose this kind of cancer faster. This information stimulates us to find marker responsive to the earlier stages of esophageal cancer, which are without difficulty detected in sera. The main findings of the current study are: firstly, serum survivin levels increased in esophageal cancer patients comparedwith healthy controls.

Secondly, survivin elevated levels have been associated withesophageal cancer clinical parameters. The elevation in serum survivin in esophageal cancer patients, which correlates with clinical parameters, may partly reflect survivin metabolism involving esophageal cancer (11,12). Studies indicate that serum surviving is produced from cancer cells (14,11). Our results do not agree with some investigators who reported that survivin is not a definitive indicator of esophageal cancer stage when analyzed in serum samples(4).It is difficult, however, to compare our results with other studies because they used different study designs, had different populations, investigated different sample types and utilized avariety of analysis methods to measure survivin.

Despite all these positive findings and recommendations some, limitations and methodological defects of our study should be reminded. Firstly, the sample size was relatively small. Our small sample size might have conducted the loss of power statistical examinations. Secondly, error may exist due to the differences in patient and control groups. Despite the fact that all patients and healthy control groups of our study were well specified with alike inclusion criteria, there may be other possible factors that were not taken into account that may have affected our findings. The biological role of survivin is still not very clear. Furthermore, molecular changes of survivin associated with tumor type, stage and its progression and intracellular processing of survivin should be investigated. We believe that this will contribute to the development of future research in the area of pathogenesis and management of esophageal cancer to improve the quality of life of patients with esophageal cancer.

Conclusion

Our study indicates that serum survivin shows markedly different in esophagealcancer patients compared to healthy controls. The serum survivin test has a low expense, is approachable, and has supplied favorable results for diagnosing esophageal cancer. Furthermore, survivin may have the possibility to be used as a credible marker for the recognition of esophageal cancer patients and leading to significantly lower numbers of excessive esophageal biopsies. Our findings might be useful for esophageal cancer diagnosis and therapy.

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Conflict of interest statement

The authors stated that there are no conflicts of interest.

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