Clinical Biochemistry 49 (2016) 692–698

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Analysis of the diagnostic efficiency of serum oxidative stress parameters in patients with breast cancer at various clinical stages

Jia-Fu Feng [a,*], Ling Lu [b], Chun-Mei Dai [a], Dong Wang [c], Yun-Hong Yang [d], Yu-Wei Yang [a], Yun-Shuang Liu [a]

[a] Department of Laboratory Medicine, Mianyang Central Hospital, Sichuan 621000, China
[b] Department of Obstetrics and Gynecology, Mianyang Central Hospital, Sichuan 621000, China
[c] Department of Oncology, Mianyang Central Hospital, Sichuan 621000, China
[d] Department of Pathology, Mianyang Central Hospital, Sichuan 621000, China

ARTICLE INFO

Article history:
Received 12 August 2015
Received in revised form 13 February 2016
Accepted 15 February 2016
Available online 18 February 2016

Keywords:
Breast neoplasms
Oxidative stress
Total oxidant status
Total antioxidant status

ABSTRACT

Background: Reactive oxygen species (ROS) are balanced through enzymatic mechanisms and exogenous antioxidants; imbalance results in oxidative stress (OxS). It is known that OxS plays an important role in the occurrence, development, and metastasis of breast cancer. The present study aimed to assess serum total oxidant status (TOS), total antioxidant status (TAS), and oxidant stress index (OSI) in patients at different clinical stages of breast cancer and to evaluate their diagnostic accuracy.

Methods: Serum TOS, TAS, and OSI were determined in 91 patients with breast cancer at different stages, 51 patients with benign breast tumors, and 35 healthy adults.

Results: Significant differences in serum TOS ($F = 104.384$, $p = 0.000$), TAS ($F = 18.247$, $p = 0.000$), and OSI ($F = 62.598$, $p = 0.000$) were observed among the 3 groups (benign breast tumor patients, breast cancer patients, and healthy women). Of the enrolled breast cancer patients, significant differences were also observed among different tumor stages, with TOS and OSI gradually increasing as the disease progressed, while TAS diminished. Receiver operating characteristic curve analysis revealed that the area under the ROC curve for OSI (AUC_{OSI}) was significantly higher than AUC_{TAS} ($z = 2.344$, $p = 0.019$) in distinguishing breast cancer from control groups (including disease control and the healthy control). The AUC_{OSI} ($z = 4.700$, $p = 0.001$) or AUC_{TOS} ($z = 4.700$, $p = 0.001$) was significantly higher than AUC_{TAS} in distinguishing breast cancer from the healthy control. The AUC_{OSI} ($z = 5.907$, $p = 0.000$) or AUC_{TOS} ($z = 5.667$, $p = 0.000$) was significantly higher than AUC_{TAS} in distinguishing benign breast tumors from the healthy control.

Conclusion: Oxidative stress parameters might serve as important indexes for monitoring breast cancer occurrence and progression. The combined evaluation of TOS, TAS, and OSI could be more beneficial for clinical assessment.

1. Introduction

According to the World Health Organization, breast cancer is one of the most common cancers in women, accounting for 16% of all female cancers, and its incidence is growing annually at a 2% rate [1]. Global cancer statistics for 2014 show that the morbidity and mortality of breast cancer account for 29% and 15% of those from all female tumors, respectively [2]. Breast cancer, therefore, is a serious concern for women's health and quality of life. However, approximately two-thirds of properly treated patients with early detected breast cancer can survive more than 20 years [3]. Thus, early detection is the basis of better prognosis and survival for breast cancer patients. A lot of tumor markers were associated with breast cancer, such as carcinoembryonic antigen, CA15-3, estradiol and progesterone receptors. But these markers have low performance for early diagnosis of breast cancer [4].

It is agreed on that intra-cellular oxidative damage is a general mechanism for cell and tissue injuries in vivo of cancer patients. Intracellular oxidative damage is mainly caused by oxidant, including free radicals and reactive oxygen species. The oxidant can react with unsaturated bonds of lipids in cell membrane and cause protein denaturation and damage in nucleic acid. In physiological conditions, antioxidants (enzymatic and non-enzymatic substances) can prevent and repair the damage of oxidant. Oxidative stress (OxS) appears through increasing oxidant generation and/or decreasing antioxidant levels in the target cells and tissues. In recent years, despite the recurrence and development of breast cancer induced by oxidative stress has gathered the attention of many researchers [5–9], most published

* Corresponding author.
E-mail addresses: jiafufeng@aliyun.com (J.-F. Feng), linglu612@163.com (L. Lu), 313314793@qq.com (C.-M. Dai), mychml2007@aliyun.com (D. Wang), yunhongyang625@yahoo.cn (Y.-H. Yang), yyw318@vip.sian.com (Y.-W. Yang), yunshuang1964@sina.cn (Y.-S. Liu).

http://dx.doi.org/10.1016/j.clinbiochem.2016.02.005
0009-9120/

reports have remained attentions on a single or several oxidant/antioxidant substances observed between plasma fluorescent oxidation products and breast cancer risk in proximate or distant samples [10]. Panis C, et al. found that OxS parameters were evaluated by plasmatic lipoperoxidation, carbonyl content, thiobarbituric reactive substances, nitric oxide levels, total radical antioxidant parameter, superoxide dismutase, catalase activities and GSH levels [11].

The lack of studies on overall OxS status is not encouraging for fully elucidating the relationship between breast cancer pathogenesis and overall serum OxS parameters [11–13]. The present study, therefore, aimed to explore the relationship between serum OxS status and breast cancer occurrence and development. We compared serum total oxidant status (TOS), total antioxidant status (TAS), and oxidant stress index (OSI) in patients with breast cancer at different stages, patients with benign breast tumors, and healthy women. We also compared the obtained serum data among enrolled breast cancer patients at different clinical stages. This information should help in assessing the diagnostic accuracy of OxS for breast cancer.

2. Materials and methods

2.1. Subjects

This was a prospective diagnostic study. Ninety-one breast cancer patients (group A) and 51 benign breast tumor patients (group B), based on their presenting symptoms, from the Mianyang Central Hospital, Sichuan Province, China, as well as 35 healthy women (group C) from the same region. The patients were selected consecutively according to the inclusion and exclusion criteria from newly diagnosed patients treated at the hospital. The inclusion criteria included being female with no smoking history and no medication use in the month prior to enrollment or during the study period, including oral contraceptives or supplements of any kind. The exclusion criteria included any other diseases such as diabetes mellitus, hypoglycemia, gout, protein-energy malnutrition, vitamin A/D deficiency, thyroid disease, osteoporosis, rheumatoid arthritis, and liver diseases. For the healthy controls, the same inclusion and exclusion criteria were applied. The healthy population was selected from subjects in the health examination center ($n = 28$) and the clinical laboratory staff ($n = 7$) at Mianyang Central Hospital, Sichuan, China. No age difference was observed among the participants. All cases were diagnostically confirmed based on the patients' clinical signs and percutaneous image-guided core biopsy results [14]. Breast cancer patients were divided into 4 subgroups according to their tumor–node–metastasis (TNM) stage [3]. There were 19 cases of stage I, 32 of stage II, 28 of stage III, and 12 of stage IV. Clinical information for all subjects is shown in Table 1.

This study was approved by the Medical Ethics Committee of Mianyang Central Hospital, Sichuan Province, China, and written informed consent was obtained from all subjects.

2.2. Blood sampling

The patients received no kind of chemotherapy or radiotherapy before blood samples were collected. Participants were asked to fast for at least 12 h, and 5 mL of venous blood was collected from each into BD Vacutainer® Serum Tubes (standard tubes with no preservatives, Becton Dickinson Company, USA). Serum was separated by centrifugation at 3000 rpm for 15 min within 2 h after sample collection. The samples were then stored at −30 °C until analysis, which was performed within 48 h. All samples were collected from patients 48 h after diagnosis, prior to surgery and/or any anticancer therapy.

2.3. Measurements of Her-2, Ki-67, estradiol and progesterone receptors

Serum Her-2 levels were measured on a fully automated ADVIA Centaur XP system with a two-site sandwich immunoassay using direct, chemiluminescent technology, and supporting kits were purchased from the Siemens Healthcare Diagnostics Inc. (Tarrytown, NY, USA).

Immunohistochemistry was performed for estimating Ki-67, estradiol receptor (ER) and progesterone receptor (PR) using an EnVision two-step protocol. The primary antibodies used were: Ki-67 antibody (mouse monoclonal IgG1: MIB1), ER antibody (rabbit monoclonal IgG: SP1) and PR antibody (rabbit monoclonal IgG: SP2), which were bought from Fuzhou Maixin Biotech. Co., Ltd. (Fujian, China). Known positive and negative control specimen accompanied each staining-run in order to monitor reliability of the results. Ki-67 positivity was expressed as a percentage, and the calculation method is as follows: positive staining was localized in the nucleus, the number was counted according to the percentage of the positive cells in the field, the percentage of positive cell in any five high-power fields were assessed, and the average was defined as the percentage of positive cells of the patient. Judgment standard of positivity in ER and PR: positive staining was localized in the nucleus. Negative was defined when stained cell number was <10% and positive was defined when stained cell number was ≥10% in each slice.

Table 1
Demographical data of the subjects.

	Group A (n = 91)	Group B (n = 51)	Group C (n = 35)	F/χ^2, p
Age (years)				
Mean ± SD	47.4 ± 8.3	46.4 ± 8.6	45.1 ± 7.2	1.060, 0.349
Quetelet index	25.4 ± 4.5	24.5 ± 4.4	24.7 ± 3.6	0.805, 0.449
Education				1.566, 0.457
High school or lower	18	11	4	
College or above	73	40	31	
Residency				0.163, 0.922
City	51	29	21	
Countryside	40	22	14	
Menopausal status				0.748, 0.945
Pre-menopause	29	18	12	
Peri-menopause	18	8	5	
Post-menopause	44	25	18	
Clinical stage				-
Stage I	19			
Stage II	32			
Stage III	28			
Stage IV	12			
Family history				1.392, 0.846
Yes	13	5	4	
No	63	39	27	
Missing or unknown	15	7	4	
Estradiol receptor				2.733, 0.098
Positive	37	14		
Negative	43	31		
Not determined	11	6	35	
Progesterone receptor				1.641, 0.200
Positive	32	15		
Negative	40	31		
Not determined	9	5	35	
Her-2 (μg/L)				
Median (range)	51.81 (0.71–149.26)	7.05 (0.79–14.03)	4.83 (0.83–9.01)	55.314, 0.000
Positive (>15.0)	61	0	0	
Negative	30	51	35	
Ki-67 (%)				
Median (range)	31.8 (5.5–79.6)	4.6 (1.0–12.9)		−7.320, 0.000[a]
Positive (>10%)	51	3		
Negative	15	27		
Not determined	25	21	35	

Note: group A, patients with breast cancer; group B, patients with benign breast tumors; group C, healthy control. Age and Quetelet index were expressed as means ± standard deviation (SD), and Quetelet index (kg/height^2) was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

[a] Independent-samples T test was performed.