Clinical significance of salivary, serum, nitric oxide, and arginase in breast cancer

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ABSTRACT

Breast cancer is the most commonly diagnosed cancer among females. A source of sampling for clinical diagnosis is saliva which has been utilized and it is a promising approach as collecting saliva is relatively easy and non-invasive. Over the past two decades, utilizing saliva as a biomarker, specifically for early cancer diagnosis has attracted much research interest. The aim was to alter the sample collection from blood to saliva for some components such as nitric oxide (NO) and arginase, in order to detect an easy, earlier and noninvasive diagnostic test as biomarkers and prognostic tools in patients with breast cancer**.** A total of 73 female volunteers were participated in this study, 25 healthy volunteers compared with 48 patients with breast cancer in order to estimate and compare both salivary and blood level components such as NO and arginase. The mean blood and salivary samples for both nitric oxide and arginase levels were significantly raised in patients with breast cancer when they compared with controls (P<0.001). In this study the changing of salivary levels of NO and arginase as compared with blood may be used as a non-invasive diagnostic tool alternative to serum testing component, which were significantly increased in patients with breast cancer in both blood and saliva and also, may be used as biomarkers and tumor progression tests in diagnosing of breast cancer.

Introduction

Carcinoma of Breast is the most frequently recognized cancer and the major cause of cancer death among women[1-3] in less advanced countries, with 882,900 cases diagnosed and 324,300 deaths in 2012, accounting for 25% of cancer cases and 15% of cancer deaths among women.[4] The recent article in the Journal displayed clearly that the adoption of widespread screening mammography; small breast cancers have raised in incidence over three times more than large cancers have reduced.[5] The biologic appearances of a tumor are now recognized to be more related to breast cancer prognosis than the tumor size.[6] Possibly modifiable risk factors include alcoholism, obesity, physical inactivity, and usage of menopausal hormone therapy. Reproductive such as the utilizing of oral contraceptives, endocrine factors, never having children, and along menstrual history are considered as risk factors of carcinoma of breast.[4] L-Arginine is metabolized to L-ornithine and urea by arginase, which is important in the metabolism of urea cycle as well as in the biochemical pathways that are crucial for cell proliferation.[7] Also, L-Arginine is metabolized by the inducible nitric oxide synthase (iNOS), arginase I, and arginase II. Arginase I and arginase II are encrypted by two discrete genes and are located in the cytoplasm and mitochondria, respectively.[8] ArginaseI is primarily participated in the detoxification of ammonia and urea creation, whereas arginase II is participated in biosynthetic functions, such as the synthesis of proline, ornithine, and glutamate. Polyamines are formed from ornithine, the second product of arginase metabolism.[7] The metabolism of L-Arginine can also be done by inducible nitric oxide synthase to produce nitric oxide and citrulline, which are important in vascular homeostasis and cytotoxic macrophages mechanisms. High levels of arginase

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activity have been described in patients with a number of malignancies including breast, gastric, colon, and lung cancers. Most reports have linked the raised arginase activity with the requisite for cells of malignant to produce polyamines to tolerate their rapid proliferation.[8] For many years it has been thought that the production of an extra of nitric oxide (NO) by inducible nitric oxide synthase (iNOS) has been documented as one of the most versatile players in various diseases.[9] It is participated in the tumors, pathogenesis and regulation of infectious diseases, chronic degenerative diseases and developments of autoimmune diseases. Because of its variety of reaction partners (DNA, low-molecular weight thiols, proteins, intermediates reactive oxygen and prosthetic groups), while its well-known production by three different nitric oxide synthases (NOS) and its activity is powerfully affected by NO levels.[10] NO and NOSs are abundant in malignant tumors and are recognized to apply both pro- and anti-tumor influences.[11] The analysis of saliva as a clinical diagnostic line for general diseases was mentioned just two decades ago, but great attention in the field has appeared recently because of its revolutionary prospective as a liquid biopsy.[12] The biomarker is distinct as an indicator of pathogenic progressions, normal biological or pharmacological responses to a therapeutic or other health precaution intervention. Several salivary components and gingival crevicular fluids have been characterized as biomarkers.[13] The salivary constitutes levels acting as biomarkers have been shown to be sensitive as serum levels.[14] The primary goals of the present study were to alter the sample collection from blood to saliva for some components such as NO and arginase, in order to detect an easy, earlier and noninvasive diagnostic test as biomarkers and prognostic tools in patients with breast cancer.

Materials and Methods

In this article, randomly assigned 73 women then collected both blood and salivary samples. Two groups of individual females were being included women with breast cancer patients and healthy groups. The first group included the breast cancer patient, involved 48 salivary and blood samples, with mean age 56±11 years who were selected from newly diagnosed breast cancer patients admitted to Nanakaly Hospital in Erbil city early diagnosed (with different stages) and the samples were collected before treatment while the second groups included the control group involved of 25 controls of healthy females with mean age 46±12 years of age. Before treatments, 5 mL of un-stimulated salivary samples would be taken in the morning within 3ml of blood from each participant, then centrifuged and stored in a disposal tube without anticoagulant and were be preserved in an ice-box then were be transferred to laboratory to assess the following substances: NO substrate using Nitric Oxide Colorimetric Assay Kit (BioVision, USA), according to the process provided by the manufacturer, while arginase enzyme levels were assayed enzymatically using commercial reagents [BioAssay Systems, QuantiChromTM Arginase Assay Kit (DARG-200), USA][15] using BioTekInstruments, Milan, Italy. The statistical analyses were run with SPSS version 17.0 for data analysis.[16]

Results

Of the 73 volunteer women enrolled, 48 women who had breast cancer, while 25 women were healthy individuals, both saliva and blood samples were collected from each volunteer. The salivary components levels of NO and arginase have been shown in Figure 1, while the results of the blood samples levels of the NO and arginase have been shown in Figure 2. The results showed that the levels of salivary levels of NO ($P<0.001$), salivary arginase enzyme ($P<0.001$), serum levels of NO ($P<0.001$), and serum arginase enzyme ($P<0.001$) were raised significantly in patient with breast cancer as compared with control groups (Figures 1 and 2).

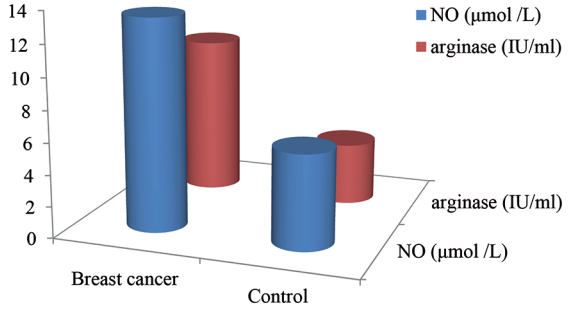

Figure 1. Salivary sample levels of nitric oxide (NO) and arginase in control and breast cancer patients' groups.

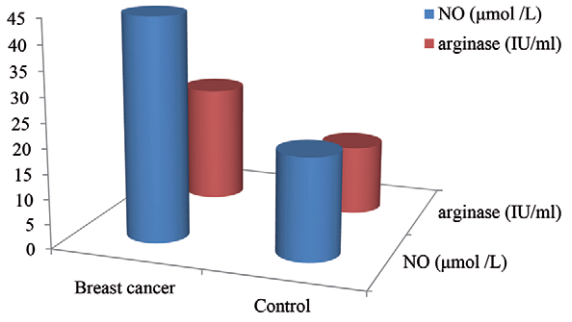

Figure 2. Blood sample levels of nitric oxide (NO) and arginase in control and breast cancer patients' groups.

Discussions

Cancer represents the top reason of morbidity and mortality worldwide, with nearly 14 million new cases and 8.2 million cancers associated with deaths in 2012, and this number is predicted to increase by about 70% over the next two decades conferring to the World Health Organization. The efficient and effective controlling of cancer patients depend on early diagnosis and/or the observing of treatment, something that is often hard to reach utilizing standard tissue biopsy techniques. Biological fluids hold great opportunities as a basis of noninvasive cancer biomarkers that can turn as replacement markers to biopsy-based sampling. The nature of noninvasive of these liquid biopsies eventually means that cancer diagnosing may be earlier and the capability to observe disease progression and/or treatment response characterizes as an example shift in the treatment of cancer patients.[17] There are certain body fluids which can be utilized for diagnosis such as serum, saliva, urine, cerebrospinal fluid *etc*.[18] So, saliva is termed as liquid biopsies that hold great clinical potential,[17] as diagnostics tools of their noninvasive nature allows for quick, easy of accessibility (transport and storage), safe handling, economical, and repeat sampling sorts,[19] that permit their utilizing in screening programs and for the close observing of treatment response and disease progression, letting for earlier intervention and dynamic treatment administration.[17,20] Also, it presents less risk of infection and accurate. With all these above revealed adding of saliva advantages can aid as diagnostic appliance as compared to serum,[21] while collection of blood is an invasive procedure and has a prospective risk of transmission disease through needle stick injuries. A great number of researchers are finding that saliva delivered an easily available, non-invasive diagnostic of disease and clinical situations.[22] The results of our study is significant elevated ($P<0.001$) for NO and arginase in both blood and saliva as shown in Figures 1 and 2 similar to reports that said saliva is titled as a mirror of the body, as it is reflected an ultra-filtrate of the blood and because its components alter under various pathological conditions,[12] which using of saliva in assessing as biomarkers for early analysis of cancer risk is potential.[14] NO, an extremely reactive free radical molecule is formed by activated macrophages and shows an important role in controlling the host protection mechanism against tumor cells. Numerous in vitro studies have also revealed that NO donors are cytotoxic to tumor cells causing to apoptosis, mainly including changes in mitochondrial permeability transition and relief of cytochrome c from the mitochondria.[23] Today, there is no simple, identical picture of the function of NO. Protective and toxic influences of NO are commonly seen in parallel.[7,10] It is remarkable inter- and intracellular signaling capacity sorts it extremely hard to expect the influence of NOS inhibitors and NO donors, which quiet hampers therapeutic applications.[10] NO is consistently noticed in the tumor microenvironment and has been established to promote tumorigenesis. NO has different influences on human breast cancer cells. At low level it stimulates proliferation by raising synthesis of some cells cycle protein[24] and in higher amounts it leads to apoptosis or cytostasis[25] by reducing the translation of certain cell cycle proteins.[24] Pervin *et al.* showed that the possible mechanisms and notorious cellular targets by which NO elevated proliferation of human breast cancer patient's cell lines MDA-MB-231 and MCF-7.[26] Actually NO utilizes distinct indicating pathways, which serves as an explanation to realize how NO influences tumor development. Some of these pathways, exclusively the capability of NO to mimic hypoxia at the level of hypoxia inducible factor 1α, as well as the part of macrophage polarization by apoptotic cells with additional alterations in the iNOS *versus* arginase ratio and activities.[27] It appears that in the tumor located, the immunosuppressive activity of myeloid-derived suppressor cell is antigen-nonspecific and is mainly regulated by the production of NO in combination with a great arginase activity. NO might inhibit T cells via a range of different mechanisms including inhibition of MHC class II gene expression, the blockade of phosphorylation and activation of Janus kinase 3, STAT 5 transcription factor, and stimulation of T-cell apoptosis. Arginase 1 activity is reasons of the reduction of arginine and translational obstruct of the chain of CD 3. This stops T cells from responding to several stimuli.[28] Elevate arginase activity[29] in combination with raised NO production by the myeloid-derived suppressor cell not only results in more distinct T-cell apoptosis but also causes to an elevated production of reactive oxygen species[9] with peroxynitrites and hydrogen peroxide.[28] In another site the researchers shows that macrophages are capable of stimulating tumor cell proliferation during the arginase pathway. As shown in an animal tumor model, the raise in NOS activity was shown at the stage of tumor rejection, whereas elevation in arginase activity was detected during tumor growth.[30] The activity of arginase enzyme in the peripheral blood mononuclear cells of 117 renal cell cancer patients was raised between 6-to-8-fold compared to normal controls,[31] this explaining is similar to our study which both NO and arginase were raised in both blood and saliva's samples (Figures 1 and 2) in patients with breast cancer.

Conclusions

In this study the salivary and blood levels of NO and arginase were significantly increased in patients

with breast cancer as compared control, and saliva's test was a mirror of blood test, so we can use saliva as a non-invasive, easy and earlier diagnostic tool alternative to serum testing component. Also, may be used as biomarkers and tumor progression tests.

References

1. Yousif AM, Ismail PA, Ismail NA. Steroid hormones, immunoglobulins and some biochemical parameters changes in patients with breast cancer. DJM 2016;10:1-8.
2. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
3. Chlebowski RT, Manson JE, Anderson GL, et al. Estrogen plus progestin and breast cancer incidence and mortality in the women's health initiative observational study. J Natl Cancer Inst 2013;105:526-35.
4. LY Yu, Tang J, Zhang C, et al. New immunotherapy strategies in breast cancer. Int J Environ Res Public Health 2017;14:1-18.
5. Lannin DR, Wang S. Are small breast cancers good because they are smallor small because they are good? N Engl J Med 2017;376:2286-91.
6. Welch HG, Prorok PC, O'Malley AJ, Kramer BS. Breast-cancer tumor size, over diagnosis, and mammography screening effectiveness. N Engl J Med 2016;375:1438-47.
7. Singh R, Pervin S, Karimi A, et al. Arginase activity in human breast cancer cell lines: N-hydroxy-L-arginine selectively inhibits cell proliferation and induces apoptosisin MDA-MB-468Cells1. Cancer Res 2000;60: 3305-12.
8. Rodriguez PC, Quiceno DG, Zabaleta J, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibitsT-cell receptor expression and antigen-specific T-cell responses. Cancer Res 2004;64: 5839-49.
9. Maarsingh H, Zaagsma J. Arginase and asthma: novel insights into nitric oxide homeostasis and airway hyperresponsiveness. Trends Pharmacol Sci 2003;24:450-55.
10. Bogdan C. Nitric oxide and the immune response. Nature Immunol 2001;2:907-16.
11. Fukumura D, Kashiwagi S, Jain RK. The role of nitric oxide in tumor progression. Nat Rev Cancer 2006;6: 521-34.
12. Rapado-González Ó, Majem B, Muinelo-Romay L, et al. Cancer salivary biomarkers for tumours distant to the oral cavity. Int J Mol Sci 2016;17:1531-52.
13. Abu Kasimi N, Zainal Ariffini SH, Hahni MA, et al. Stability of lactate dehydrogenase, aspartate aminotransferase, alkaline phosphatase and tartarateresistant acid phosphatase in human saliva and gingival creviculfluid in the presence of protease inhibitopr. Arch Biol Sci Belgrade 2013;65:1131-40.
14. Dhivyalakshmi M, Maheswari TNU. Expression of salivary biomarkers-alkaline phosphatase andlactate dehydrogenase in oral leukoplakia. Int J ChemTech Res 2014;6:3014-18.
15. Crombez EA, Cederbaum SD. Hyperargininemia due to liver arginase deficiency. Mol Genet Metab 2005;84:243-51.
16. Harris M, Taylor G. Medical statistics made easy. USA: Martin Duntiz; 2004.
17. Larrea E, Sole C, Manterola L, et al. New concepts in cancer biomarkers: circulating miRNAs in liquid biopsies. Int J Mol Sci 2016;17:627-69.
18. Dhivyalakshmi M, Maheswari TNU. Expression of salivary biomarkers-alkaline phosphatase andlactate dehydrogenase in oral Leukoplakia. IntJ ChemTech Res 2014;6:3014-18.
19. Chen D, Song N, Ni R, et al. Saliva as a sampling source for the detection of leukemic fusiontranscripts. J Transl Med 2014;12:321-25.
20. Shenoy SB, Shenoy P, Talwar A, et al. Evaluation of salivary enzymes in post-menopausal women with and without periodontitis. NUJHS 2014;4:88-91.
21. Pujari KN, Jadkar SP. Superoxide dismutase levels in leukemia's. Int J Med Sci 2011;2:96-100.
22. Joshi PS, Chougule M, Dudanakar M, Golgire S. Comparison between salivary and serum lactate dehydrogenase levels in patients with oral leukoplakia and oral squamous cell carcinoma - A pilot study. Int J Oral Max Path 2012;3:07-12.
23. Pervin S, Singh R, Freije WA, Chaudhuri G. MKP-1-Induced dephosphorylation of extracellular signal-regulated kinase is essential for triggering nitric oxide-induced apoptosis in human breast cancer cell lines:implications in breast cancer. Cancer Res 2003;63:8853-60.
24. Pervin SR, Chaudhuri G. Nitric oxide, Nω-hydroxy-l-arginine and breast cancer. Nitric Oxide 2008;19:103-6.
25. Lechner M, Lirk P, Rieder J. Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin. Semin Cancer Bio 2005;15:277-89.
26. Pervin S, Singh R, Hernandez E, Wu G, Chaudhuri G. Nitric oxidein physiologic concentrations targets the translational nachinery to increase the proliferation of human breast cancer cells: involvement of mammalian target of rapamycin/eIF4E pathway. Cancer Res 2007;67:289-99.
27. Weigert A, Brüne B. Nitric oxide, apoptosis and macrophage polarization during tumor progression. Nitric Oxide 2008;19:95-02.
28. Nagaraj S, Gabrilovich DI. Tumor escape mechanism governed by myeloid-derived suppressor cells. Cancer Res 2008;68:2561-63.
29. Rodriguez PC, Hernandez CP, Quiceno D, et al. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. JEM 2005;202:931-9.
30. Chang C-I, Liao JC, Kuo L. Macrophage arginase promotes tumor cell growth and suppresses nitric oxide-mediated tumor cytotoxicity. Cancer Res 2001;61:1100-6.
31. Ochoa AC, Zea AH, Hernandez C, Rodriguez PC. Arginase, prostaglandins, and myeloid-derived suppressor cells in renal cell carcinoma. Clin Cancer Res 2007;13:721s-6s.