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Redox status in saliva and serum of Iraqi patients in a pre-malignant and early malignant stage of breast tumors

Samar Ahmed Jabbar[1], and Hathama Razooki Hasan[2]

1 Iraqi center of cancer and medical genetics research, Mustansyriah university, Baghdad, Iraq.
2 Baghdad university, college of science, Baghdad, Iraq.

Abstract

Breast cancer, as is commonly assumed, one of the most diagnosed kind of cancer is a multifactorial disease, its exact cause is still unknown. It is considered the second killer cancer among women. The present study participants were Iraqi women patients in pre-malignant and malignant (n=59) women with breast tumors as well as age matched healthy Iraqi women (n=27). This study aimed to evaluate the redox status upon transformation from premalignant (benign) to malignant stage of breast cancer by evaluating the saliva-blood correlation to look for the possibility using saliva as alternative fluid for diagnosis of the tumor stages. This was achieved by measuring the [total oxidant status level (TOS), total antioxidant status level (TAS), and oxidative stress index level(OSI). (TOS) and (TAS) were determined in saliva and serum fluid samples from all participants using colorimetric methods, while the oxidative stress index (OSI) was calculated. The results obtained from the study showed that the P value ≤ 0.01 significantly increased the measured [TOS] level and the calculated [OSI] level in saliva and serum fluid samples in contrast significantly decreased the P value ≤ 0.01 in the [TAS] level in both types of fluids of the patient groups compared to that of the healthy control group. Similar results were obtained when the values of these parameters were compared in both samples (serum and saliva) samples were compared between the patient groups in both breast tumors (Pre-malignant& malignant).

Keywords: *Pre-malignant, malignant, total oxidant status, total antioxidant status and oxidative stress index.*

Introduction

Breast tumors are widely spread among women worldwide. They are classified into benign type (premalignant) and malignant (serious), the latter being more dangerous than the former type because of its ability to spread to other tissues and organs and is usually responsible for death [1]. In general, cancer cells are characterized by many biochemical features that lead to different alterations and aberrant metabolism that cause their transformation from normal cell to tumor and from one stage to another of malignancy. Cancer is known to occur as a result of interactions of different factors, such as environmental and genetic factors, and among the most reported reasons for the increased risk of cancer is oxidative stress [2].

Corresponding Address:
Samar Ahmed Jabbar
Iraqi center of cancer and medical genetics research, Mustansyriah university, Baghdad, Iraq.
Email: samer.ahmed@uomustansiriyah.edu.iq

Free radicals can be described as single molecules, or atoms, or molecular fragments that have unpaired electrons in their atomic or molecular orbitals. Free radicals are produced in all cells and have several benefits for the healthy body, such as avoiding inflammation, killing bacteria , regulation of smooth muscles that regulate internal organs and proper functioning of blood vessels; they even play a crucial role as second messengers, thus modulating signaling pathways to regulate cell function. On the contrary, in the case of their production in an uncontrolled way they are considered one of the main causes of the pathogenesis of different diseases [3]. The existence of all types of free radicals as reactive nitrogen species (RNS), reactive oxygen species (ROS) as well as reactive sulfur species (RSS), are well-being controlled existence are very vital to assist and sustain cellular homeostasis in perfectly functioning tissues [4]. To combat the damage resulting from its excess production in the cell, the body has developed different strategies to prevent its damage, such as repairing oxidative damage and antioxidant defense mecha-

nisms, which are the first preference for treating stress based on oxidative stress [5]. The antioxidant whose main function is to prevent oxidation in various contexts was defined by Gutteridge and Halliwell, 2007 as "any molecules that delay, prevent, or remove oxidative damage to a target molecule" [6].

The status of oxidative stress is described as a complicated process that occurs due to the imbalance between the free radical production and the activity of the different antioxidants mechanisms. The effect of presence of such status depends on the type of oxidants present, their composition, the site of free radicals production and their severity, the activity of the different antioxidants, and the efficiency of their repair systems. Today, this oxidative stress is known to play a vital role in the pathogenesis of many diseases [7, 8]. It can be expressed by the term oxidative stress index (OSI), which represents the measurement of total oxidative stress present and can be calculated in all body fluids as the ratio of total oxidative stress to total antioxidant [TOS]/ [TAS] [9].

Saliva is a colorless fluid with a thickness appearance that exists in the human mouth and is secreted by salivary glands. The human salivary gland produces (500-1500 ml) of this fluid per day. It consists of hundreds of molecules, where water formed about 99 % of its content, while the remaining 1% is a mixture of organic and inorganic species [10]. The natural pH of saliva is around (6.7-7.3) and the change in this pH value and in the volume of salvia is based on the physiological and pathological condition of the body. The idea of using saliva as a diagnostic fluid for blood was suggested during the last decades when many researches started to look at the variations in biochemical parameters in blood serum or plasma with that occurring in saliva and the correlation between these variations [11,12].

This study discussed the evaluation of the total redox balance in Iraqi patients with breast tumors before and after transformation to the malignant stage. As well as to evaluate the correlations between (saliva& serum) fluid of the total redox balance to seek out the possibility of using saliva as alternative fluid.

Materials and Methods

Chemicals: All chemicals used in this study were highly analytical grade.

Measurement of total oxidant status [TOS]

On the basis of Erel's method, the total oxidant status value in the saliva and serum samples of all present studied groups was measured. In this experiment, the calibration was done with a standard solution (hydrogen peroxide). The absorbances of the standard hydrogen peroxide solutions measured at a wave length λ=560 nm were plotted against their concentrations to construct the standard plot. The straight line equation derived from their standard curve was used to quantify the level of total oxidant status in the samples studied [13].

Determination of total antioxidant capacity [TAC]

The total antioxidant status was determined using an easy method by Erel, 2004. The assay was calibrated with standard glutathione solutions and the measured absorbance at a wave length of λ=444 nm was plotted against its concentrations to construct the standard plot. The straight line equation derived from this standard curve was used to quantify total antioxidant capacity (TAS) in the studied samples [14].

Oxidative stress index (OSI)

The value of the oxidative stress index (OSI) was calculated from the following equation [15].

OSI = [TOS] (µmol H2O2/L)/[TAC] (µmol glutathione/L)

Data Analysis

The program SPSS (version 26) (One -Way ANOVA and Pearson correlation) were used to analyze the obtained results and to perform the correlation relationships, respectively. Throughout this work, the results obtained were reported as mean value ±standard deviation. The difference are considered as a highly significant if ($P \leq 0.01$), significant when the ($P \leq 0.05$) and non-significant if ($P < 0.05$) [16].

Exclusion criteria

Patients and controls who had an unbalanced diet (such as vegetarians) liver disease, active inflammatory conditions, chronic pancreatitis, chronic renal failure, chronic or acute inflammatory diseases, as well as those patients who started taking drugs, and those who had a history of smoking, lipid lowering therapy, or alcohol consumption, or taking antioxidant supplements were excluded. The study protocol conforms to the ethical guidelines, endorsed by the College of Science, University of Baghdad Ethics Committee.

Subjects and Sampling

All the patient participants enrolled in the present study (59) attended the Al-Ilwia Hospital Center for Early Cancer Detection/ Baghdad/ Iraq and were diagnosed by specialists at the same center. These patients were assigned to two groups according to tumor type: premalignant group (32) and malignant group (27) ,control group age matched apparently healthy Iraqi women, (27) were also included in the study. The analyzed samples were blood serum and saliva samples, which were collected from the same individual and as described below.

Fasting sera and unstimulated saliva samples were collected in plain tubes. Samples of patients with breast tumors were collected after diagnosis just before receiving any type of treatment. Before collecting saliva samples, participants were asked to rinse their mouth with saline. Saliva samples were centrifuged at (2400×g) for 15 minutes at 4 C°, then the supernatant was kept frozen to be used for the desired measurements. At the same time, blood samples were withdrawn from the same individual in a serum tube, then centrifuged at (3000 x g) for 5 minutes. Serum samples were collected and frozen at (-20C°) to be used for measurements of the different parameters.

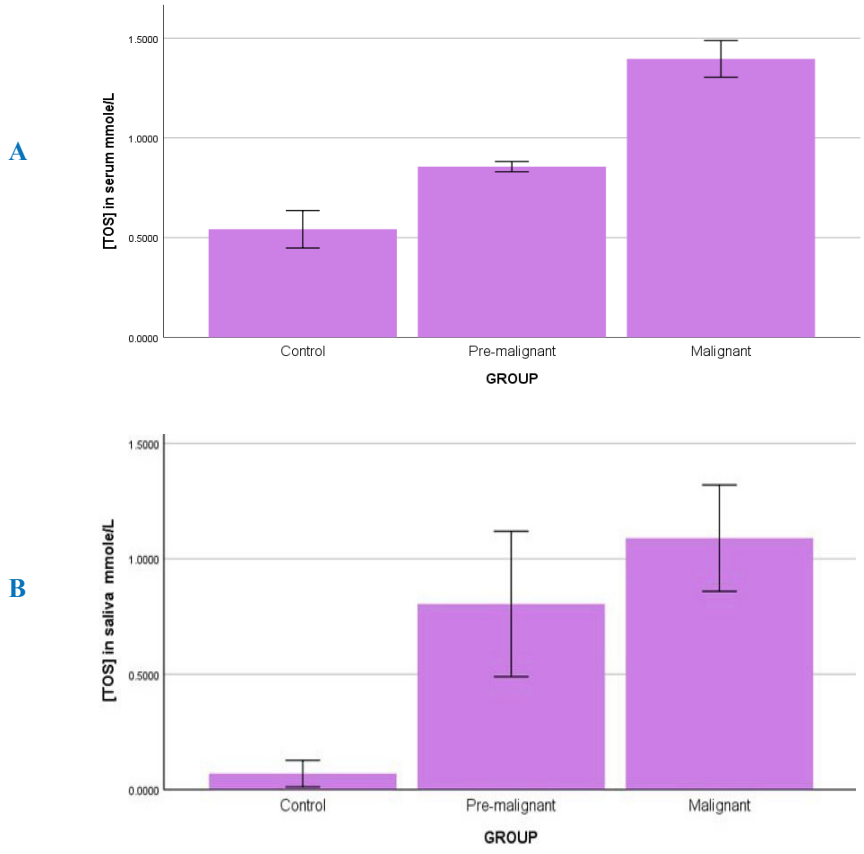
Table (1): The number of cases and mean age value for all studied groups ± SD

Group	N	Age (year)	Note
Control	27	45.07±15.36	
Benign tumor (Premalignant)	32	47.18±13.95	Before receiving any type of treatment.
Breast cancer (Malignant)	27	49.96±12.78	Before receiving any treatment(early diagnosed) at zero stage

Result

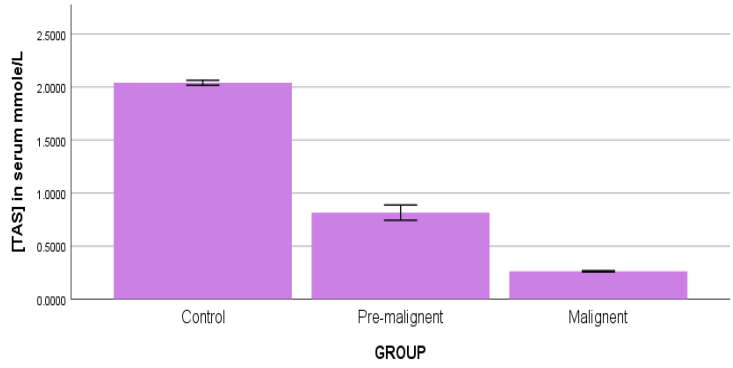
Total oxidant status [TOS], which represents the amount of oxidant substances in body fluid, was measured in the present study in non- stimulated saliva and serum samples of the three studied groups using Erle's colorimetric method (Erle., 2005) [13].

Fig.1: The mean value of [TOS] ±standard deviation in (A) serum and in (B) samples of the healthy and patient groups.



**The difference is highly significant at the P ≤ 0.01 level and significant at P ≤ 0.05.
a: The P value ≤ 0.0 1 refers to difference in comparison between the premalignant and control group. b: The P value ≤ 0.0 1 refers to difference in comparison between the malignant and control group. c: The P value ≤ 0.0 1 refers to difference in comparison between the premalignant and malignant groups.

A



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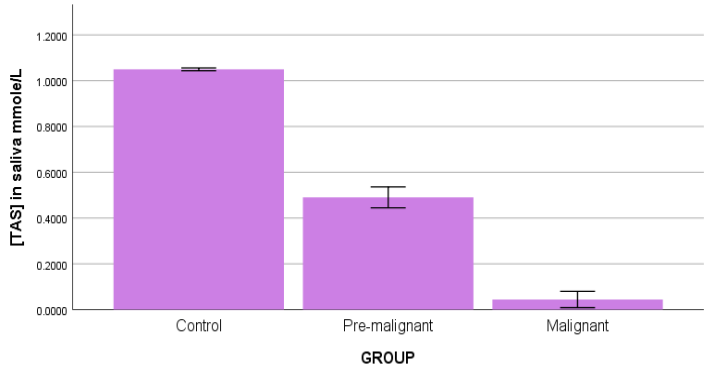
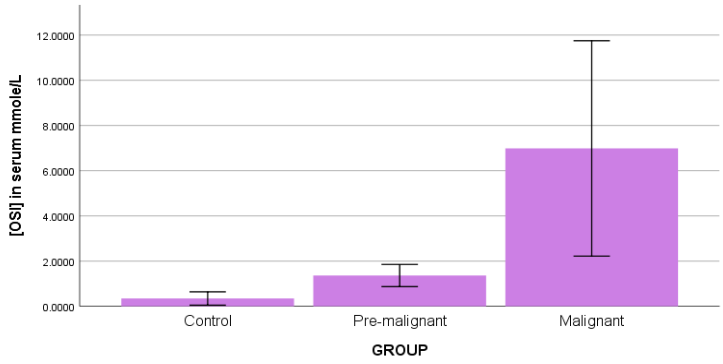


Fig. (2): The mean value of [TAS] ±standard deviation in serum and saliva samples of the healthy and patient groups.

The P value ≤ 0.0 1 refers to difference in comparison between the premalignant and control group. B: The P value ≤ 0.0 1 refers to difference in comparison between the malignant and control group. C: The P value ≤ 0.0 1 refers to difference in comparison between the premalignant and malignant groups.

A



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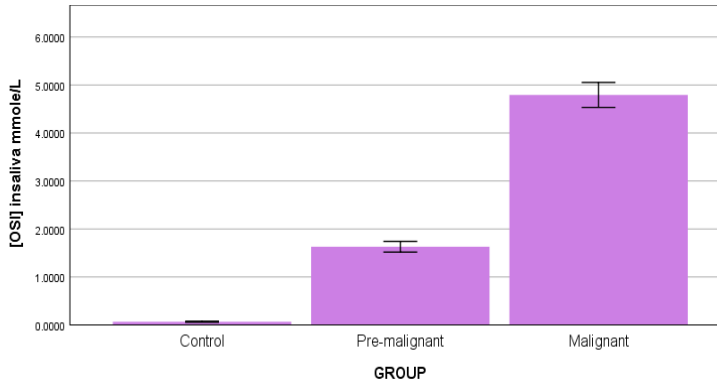

Fig. (3): The mean value of [OSI] ±standard deviation in serum and saliva samples of the healthy and patient groups.

a: The P value 0.0 1 refers to the difference in comparison between the premalignant and control group. b: The P value ≤ 0.0 1 refers to difference in comparison between the malignant and control group. c: The P value ≤ 0.01 refers to difference in comparison between the premalignant and malignant group.

Table (2) Correlation between serum and saliva for all studied groups.

Saliva / Serum	Control R = Pearson correlation p-value	Benign R = Pearson correlation p-value	Breast cancer R = Pearson correlation p-value
TOS	R=0.017,p=0.871	R=0.403* ,p=0.037	R=0.342 *,p=0.046
TAS	R=0.158, p=0.288	R=0.224, p=0.261	R=0.211*,p=0.042
OSI	R=0.016 ,p=0.784	R=0.129, p=0.543	R=-0.256 ,p=0.431

Discussion

It is clear from the results in the above Fig. (1) a highly significant increase is present in [TOS] ($P \leq 0.01$) in serum as well as in saliva samples of pre-malignant and malignant groups while compared with that of the corresponding control groups. And significantly increased ($P \leq 0.01$) as it is clear when [TOS] was compared between the pre-malignant group with that of malignant group ($P \leq 0.01$). Such results indicated the presence of a large amount of oxidants in the patient's groups in comparison to the healthy control group, as well as upon the transformation from the premalignant stage to the malignant stage of the breast tumors. Such high concentration of the oxidants was previously reported in many researches, resulting in pathogenesis of many diseases, one of them is cancer [17]. The high [TOS] measured in each of the patient group is similar to the results of a study in China [18] as well as the results of a study of Mahdi et al., 2018 in Ethiopia that carried out using serum and tissues of women with breast cancer [19].

The results of the TAS measurement, which determine the combined capacity of all antioxidant defense systems to combat and prevent oxidative damage, are presented in Fig. (2). The above results obviously, showed a highly significant decreased [TAS] in serum and saliva samples of the two patient groups ($P \leq 0.01$) compared with that of the control group. Likewise, a highly significant decrease in this level was clear ($P \leq 0.01$) when compared to [TAS] in the benign patient group with the breast cancer patient group in both fluids. The results of the present study were consistent with the results of Feng et al., 2012 study, which measured a low level of [TAS] in the serum of Chinese women with benign tumor and breast cancer compared to the healthy group. It was also consistent with the results of Mehdi et al., 2018 research in Ethiopia who reported a lower serum [TAS] level in the serum of the group with serious breast tumor [18,19].

The results illustrated in Fig. (3) show a highly significant increase ($p \leq 0.01$) in this index in serum and saliva of the pre- malignant and malignant breast tumor patient groups compared to the corresponding value in the control group. These results agree with the results of Feng et al., 2012 in China [18] and Eraldemir, 2019 in Turkey [20] reporting the presence of a high value of OSI in serum of studied breast cancer women with type 2 diabetes mellitus who underwent surgery compared to the control group This index was also observed to increase significantly ($p \leq 0.01$) in saliva and serum samples from women with breast cancer compared to the corresponding fluid from the premalignant (benign) group, a result that was in agreement with the result of the Sawczuk et al. 2019 study that reported an elevated [OSI] in saliva from women with malignant tumor with and without mutation in BRCA1, (BRCA1 encodes a suppressor protein) [21]. As the disease progressed from pre-malignant to malignant stages, significant differences were observed among the three study groups as illustrated in Fig. (1) and Fig. (3) with TOS and OSI gradually increased. The calculated increased level of OSI in the malignant tumor patients pointed out to the presence of an elevation in the different reactive species, which means a shift in the redox status toward the oxidant state.

The total oxidant' level was accompanied with a decreased concentration of antioxidant defense system in the serum and saliva of the two types of the patient groups compared to healthy women on one hand and between the two patient groups on the other hand. Now-days these free radicals are known to play an important role, as a major player in cellular physiology and pathophysiology [22]. Cancer cells are exposed to high levels of ROS, but the question is is this phenomenon the cause or outcome of carcinogenesis. Carcinogenesis is defined as a complex process which includes a series of cellular and molecular events that encourage the normal cell to transform into a cancer cell, all of these events are accompanied by specific features, like an enduring stimulus to genetic instability, angiogenesis and metabolic deregulation, absence of immune surveillance, proliferation, and resistance to cell death. Accumulation of all these changes stimulates tumor initiation and progression, resulting in an increase in the level of oxidative DNA that is reported to be responsible for various types of cancer [22, 23].

Typically, antioxidants, when present in high concentrations, are considered a safe and defense system that scavenges free radicals and thus prevents the occurrence of many diseases [24]. But if this system cannot cope properly and the free radicals can trigger a negative chain reaction that can destroy different components of the cell such as the cell mem-

brane, block the activity of the major enzymes, and prevent proper functioning of the body [25]. This can be achieved through the structural modification of proteins present in cells that leads to cell dysfunction and disruption of vital cell function [26]. The decreased level of antioxidants reported in the present study could be assumed to be the result of the modifications of antioxidant enzymes that occur as a direct or indirect result of the presence and activity of the different free radicals. In our previous study, the activity of xanthine oxidase and sulfhydryl enzyme was found to increase in serum and saliva samples from both groups of patients. The increased activity of sulfhydryl oxidase results in the conversion of xanthine dehydrogenase to xanthine oxidase, and the increased activity of the subsequent enzyme produced hydrogen peroxides and superoxide anion, both reported to be present at high concentrations in different types of malignancy, including breast cancer [27]. Thus, the increased oxidative stress seems to be responsible for the initiation and progression of breast cancer. The reduction of antioxidant activity in saliva reflects the susceptibility of the salivary gland to oxidative damage that increases the risk of OS related diseases. The combined evaluation of TOS, TAS and OSI could be more beneficial for clinical assessment of dental care, oral inflammatory and mucosal ulceration) that accompany the occurrence of cancer [28]. Thus, the observed increase in OSI between the premalignant tumor group and the serious malignant group can be explained by the presence of a high level of oxidants with a deficiency of the antioxidant system, which leads to escape of cancerous cells and activation of invasion of metastasis [23].

Conclusions

Oxidative stress plays a critical role in breast cancer progression, where combined determination of [TOS], [TAS], and OSI could be more beneficial for clinical evaluation. Furthermore, these parameters may serve as important indexes for monitoring the occurrence and progression of breast cancer, as well as may be an ideal therapeutic target for the regulation of this type of disease.

Furthermore, the results of this study indicated that in the patient with malignant tumor, saliva may be used as an alternative medium rather than blood to detect this disease.

Authors' contribution

Hathama Razooki Hasan supervised the study, checked and revised the manuscript draft and the final version. Samar Ahmed Jabbar supervised patient sampling, conducted lab work, and wrote the first draft. The authors approved the final version.

Ethical approval

Ethical permission was obtained from this hospital and from all patients included in this study to carry out the investigation. On January 8 , 2023, the Iraqi Ministry of Health's Ethics Committee approved the study under reference number 80220.

Conflict of interest: Non

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