Research Article

Analysis of Oxidative Stress in Patients with Breast Cancer and Obesity

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Abstract

Background: Breast cancer is a major source of public health concern. Our work aims to study the role of oxidative stress in breast pathophysiology. **Materials & Methods:** A group of 39 patients diagnosed and treated for breast cancer was followed in a prospective, observational, non-randomized study to assess the serum oxidative stress levels. Thus, the following parameters were determined: Malondialdehyde (MDA) concentration as a measure of lipid peroxidation reaction, serum thiol concentration as end products of oxidative degradation of proteins, and total level of antioxidants. **Results:** 81.9% of these patients have an excess of adipose tissue (overweight or obesity). A positive correlation was obtained between the serum peroxides values and obesity as measured by Body Mass Index (BMI) ($p<0.01$) and waist circumference ($p<0.05$). The mean values of serum thiols were higher in obese patients versus non-obese ones (341 µmol/l *vs.* 336 µmol/l), but without statistical significance. Regarding the influence of obesity on antioxidants levels, no statistically significant results were obtained. **Conclusion:** Breast cancer patients had significantly high levels of oxidation markers such as MDA and low levels of antioxidant markers, respectively thiols and total antioxidants. The obtained oxidative stress parameters are independent of obesity and are mainly related to the presence of breast cancer.

Keywords: Breast cancer; Oxidative stress; Obesity; Serum peroxide; Antioxidant markers

Introduction

Breast cancer is a major source of public health concern. The latest data published by the International Agency on Cancer Research (IARC) report that 110.000 (23.6%) cases of the obesity related cancers are attributed to breast cancer in postmenopausal patients, also suggesting that at least 28.000 of those could have been prevented. [1] The results of a meta-analysis from 2014 on 200.000 patients diagnosed and treated for breast cancer included in 82 clinical trials concluded that patients with a high Body Mass Index (BMI) had lower survival rates, compared to those with normal BMI. The same meta-analysis also reported that other individual prognostic factors such as menopausal status or hormonal receptors status were not found to be related to the survival rates. [2]

After molecular testing, three mechanisms were identified to justify the relationship between the excess of adipose tissue and the development of a tumor favorable microenvironment. The first would be an increase in the synthesis of cytokines triggering the cascade of inflammation, with the onset of a chronic inflammatory status. Secondly, hyperinsulinemia, as a consequence of increased insulin resistance, was also incriminated in cancer development due to increasing levels of circulating Insulin-like Growth Factor (IGF), an important proangiogenic and antiapoptotic factor. The third mechanism would be hyperplasia and hypertrophy of fat cells, with the installation of a hypoxic state, causing increased levels of oxidative stress, stimulation of angiogenesis, increased hypoxia-inducible factor-1 expression thus promoting angiogenesis and tumor development. [3]

Due to their high prevalence rate worldwide, breast, cervical and ovarian cancers were intensively investigated for identifying treatment response and prevalence prognostic factors. Environmental, hormonal, viral, but also stage and treatment-related factors were linked to the development or treatment response of these tumors. [4-9]

The development and progression of breast cancer have been linked to the interaction between tumor cells and the tumor microenvironment. How the tumor microenvironment is altered by the level of oxidative stress is considered an essential molecular mechanism that explains why the adipose tissue influences the prognosis of breast cancer. [10] Oncobiology studies data lead to the conclusion that breast carcinoma should not be seen as an isolated group of cells that have undergone mutations, but as a microenvironment consisting of breast cancer cells, fibroblasts, adipocytes, immune and endothelial cells. [11] The interactions between cancer cells and stroma are involved in regulating signaling pathways in the evolution of this tumor. One of the most important factors influencing the

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development, progression and metastasis of cancer is oxidative stress. Reactive Oxygen Species (ROS) interact with various cellular components such as proteins, lipids, and nucleic acids resulting in structural and functional alterations of these components, thus further leading to an irreversible disruption of the cellular functions. Damage to DNA, proteins, cell membrane and mitochondria are involved in carcinogenesis, although no specific biochemical markers have yet been identified. In addition, information on biochemical changes in tissues and blood, especially antioxidant status, and its correlation with the clinical stage of the disease, is lacking.

The oxidant-antioxidant balance in the tissues is believed to aid the development and progression of cancer. Previous research has shown changes in this balance during the colorectal oncogenic process. [9]

In Romania, breast cancer is the leading cause of death. To this end, we are interested in this work a relationship between breast cancer, redox status and obsession.

Materials and Methods

The study is prospective, observational, non-randomized, performed on a sample of 39 patients, evaluated between January 2019 and October 2019 at Elias University Emergency Hospital, a representative sample for a population of patients diagnosed with breast cancer in a center with experience in the diagnosis and treatment of oncological diseases.

The database was completed using information from the patient's medical file, as well as laboratory results obtained by dosing the parameters of oxidative stress in the venous blood. Thus, recorded data were represented by BMI, waist circumference and serum levels of Malondialdehyde (MDA), thiols, and total antioxidants.

All patients signed an informed consent for data processing and for biological sampling (5 ml venous blood). Also, the approval of the Institutional Ethics Commission (no. 5748/13.08.2018) was obtained for conducting the study.

The inclusion criteria in the study were represented by

Patients diagnosed with stage I, II, or III invasive ductal or lobular carcinoma undergoing chemotherapy in the adjuvant or neoadjuvant setting or monitoring for the first two years after adjuvant or neoadjuvant chemotherapy

The absence of another associated type of neoplasia

Patients for whom all the information necessary to complete the database were available

Patients were included in the study in the order of hospitalization for chemotherapy. They were in different stages of treatment: Adjuvant chemotherapy, neoadjuvant chemotherapy, adjuvant endocrine therapy or radiation therapy, but all of these patients underwent adjuvant or neoadjuvant chemotherapy in the first two years after diagnosis.

The evaluation of patients consisted of collecting 5 ml of venous blood, as well as filling in a patient evaluation form.

We aimed to identify a correlation between excess adipose tissue assessed by calculating BMI and measuring waist circumference with serum levels of oxidative stress parameters.

Determination of oxidative stress parameters

To assess the level of oxidative stress in the previously presented group, serum parameters related to the oxidative attack on lipids, proteins and the determination of the level of activation of endogenous defense systems were evaluated.

All determinations were performed according to standardized techniques, processed from the serum collected from the patients included in the study.

Lipid peroxidation was assessed by measuring the serum concentration of MDA by the Carbonneau spectrophotometric method. This method is based on the production of a colored adduct (MDA-TBA2) with a maximum absorption at 532 nm depending on the concentration. [12,13] Normal values are between 0 µmol-4 µmol/100 ml serum.

SH-albumin thiol groups were measured by reaction with Ellman's reagent (5,5 dithiobis-2-nitrobenzoic acid) which reached a maximum intensity at 412 nm according to SH-groups. [14] Normal levels range are from 370 µmol/ l-450 µmol/l.

Total antioxidants were measured based on the ability of the serum to reduce iron (at low pH, the FeIII-tripyridyl-s-triazine complex is reduced to the ferrous state and forms an intense blue complex, and the maximum color intensity is at the length of 593 nm wave). [15] Normal values are between 0.9 µmol/l-1.4 µmol/l.

Statistical analysis

The R program version 3.6.2 (2019) was used for statistical analysis. In addition to the standard packages, the following packages have also been used:

Survival Therneau T (2015). A package for survival analysis in S. Version 2.38, <URL:https://CRAN.R-project.org, Alboukadel Kassambara, and Marcin Kosinski (2018)

Survminer: Drawing Survival Curves using ggplot2. R package version 0.4.3. https://CRAN.R-project.org

In this study, BMI and abdominal circumference were used as indicators of obesity. The analysis algorithm consisted in estimating the possible correlations between BMI/abdominal circumference and oxidative stress evaluation parameters, using the correlation index r Pearson/ρ Spearman, then making a comparative analysis by batches of these indices (obese *vs.* non-obese), using depending on the distributions of the variables either a bidirectional Welch t test for two independent samples, either a Wilcoxon Rank Sum test.

Results

The characteristics of the patients included in the studied group are presented in Table 1.

It was a higher percentage for patients for patients diagnosed with breast cancer in the 50-70 age range most of the patients presented in T2 and T4 stages, and the most common subtype encountered was luminal B.

Although in this group the patients were not selected according to the BMI, there are a significant percentage of overweight and obese patients. We note that the percentage of obese patients is 46.1%, respectively 35.8% for the overweight ones. Thus, 81.9% of the investigated patients have excess of adipose tissue. We mention that the height and weight taken into account were those from the diagnosis. The waist circumference at the time of signing the informed consent was greater than 88 cm in most patients (74.4%) [Table 1].

Table 1: Patient related characteristics.

Characteristic	Number and percentage of patients
Age at diagnosis	
<50 years	13 (33.3%)
50-70 years	22 (56.4%)
>70 years	4 (10.2%)
BMI	
18-25 kg/sqm	7 (17.9%)
25-30 kg/sqm	14 (35.8%)
>30 kg/sqm	18 (46.1%)
Waist circumference	
<80 cm	4 (10.2%)
81-88 cm	6 (15.4%)
>88cm	29 (74.4%)
Intrinsic subtype	
Luminal A	8 (20.5%)
Luminal B	19 (48.7%)
Luminal B Her2 positive	5 (12.8%)
Her2 positive	2 (5.2%)
Triple negative	5 (12.8%)
Stage T	
T1 (<2 cm)	7 (18%)
T2 (2-5 cm)	18 (46%)
T3 (>5 cm)	1 (2.6%)
T4 (invasion of the chest wall or skin)	13 (33.4%)
Invasion of regional lymph nodes	
Present	30 (77%)
Absent	9 (23%)

Analysis of the influence of obesity on lipid peroxides levels

As is well known, because lipids have a large distribution in the body and in the cell membrane, the first attack of oxygen free radicals is at this level.

To perform this analysis, lipid peroxides levels were determined in the serum of the patients included in the study according to the method presented in the materials and methods section. The Pearson correlation index was determined between the calculated obesity parameters (BMI/waist circumference) and serum levels of lipid peroxides. The results obtained [Table 2] showed a statistically significant positive average correlation ($p<0.01$) between BMI and peroxides value; there was also a statistically weak positive mean correlation ($p<0.05$) between the waist circumference and biochemically determined value of lipid peroxides.

The serum peroxide values measured in the studied patients and presented in Table 3. Areincreased compared to those considered within normal limits.

The data presented in the histograms in Figure 1 represent the values of the correlation between BMI and the intensity of the lipid peroxidation reaction. After analyzing these histograms, a distribution model close to the normal distribution is highlighted,

Table 2: Calculation of the Pearson correlation index between obesity (BMI/waist circumference) and serum peroxides.

Obesity parameter	Peroxide sr Pearson (Value p)
BMI	0.436 (0.0061)
Waist circumference	0.338 (0.0379)

Table 3: Descriptive analysis of the level of peroxides on the two lots.

Peroxides (µmol/100ml)	Obesity	Non-Obesity
Mean ± S.D	6.25 ± 0.86	5.81 ± 0.99
Median (IQR)	6.28 (1.00)	5.92 (1.51)
Min to Max	4.48 la 8.13	4.39 la 7.70
Skewness	-0.08	0.14

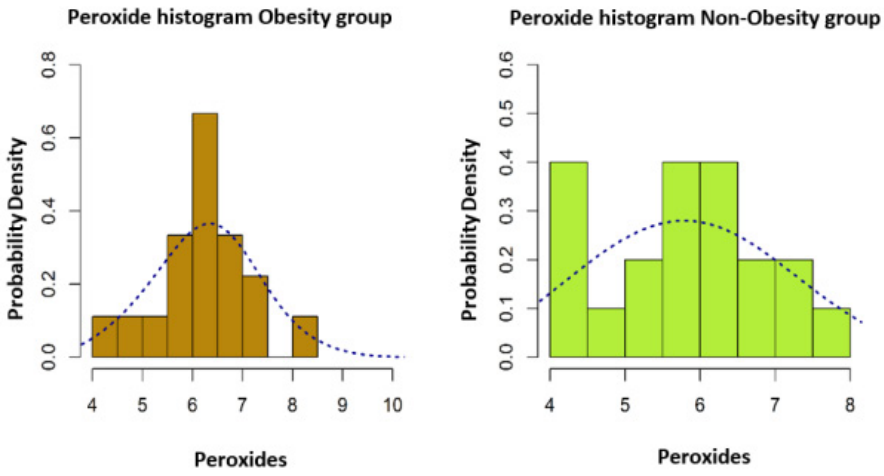

Figure 1: Histogram of serum peroxide distribution in the two groups (obese *vs.* non-obese patients).

which is why a bidirectional Welch test was applied to two independent samples.

The differences between the values obtained are without statistical significance (p>0.05), but as shown in the graph in Figure 2, there is a change in the mean values of lipid peroxides in the group of obese patients, compared to lower values in those without metabolic changes.

Thus, the values of the final product of the lipid peroxidation reaction are not statistically significantly modified in obese versus non-obese patients, but there are small numerical differences that may represent an increase in lipid metabolism and may direct their peroxidation [Figure 3].

Analysis of the influence of obesity on thiol levels

After initiating chain reactions, excess ROS attack protein structures, inducing a number of molecular, structural and functional changes at this level.

In the next stage of the study, we correlated the values of obesity parameters with the level of thiols obtained from oxidative degradation reactions of proteins.

According to Table 4 which illustrates the mean and median serum thiol values in the two groups of obese/non-obese patients respectively, the results obtained do not correlate with statistical significance (p>0.05).

The two distributions were tightened and a bidirectional Welch test is used for comparison for two independent samples. The difference is without statistical significance (p>0.05).

The graph in Figure 4 does not show significant differences between serum thiol levels in obese patients compared to non-obese patients.

Analysis of the influence of obesity on antioxidants levels

Because there are data in the literature that support the existence of an antioxidant defense deficiency in oncological patients, we tried to identify whether excess adipose tissue influences the serum level of antioxidants. Table 5 describes the characteristics of the two groups of obese/non-obese patients, depending on the serum levels of total antioxidants.

Thus, the Pearson correlation index was evaluated in obese patients compared to the normal-weight ones and the histograms of the two distributions are shown in Figure 5.

Histogram of serum antioxidant distribution within the two groups (obese *vs.* non-obese patients)

Both distributions were characterized by a significant negative

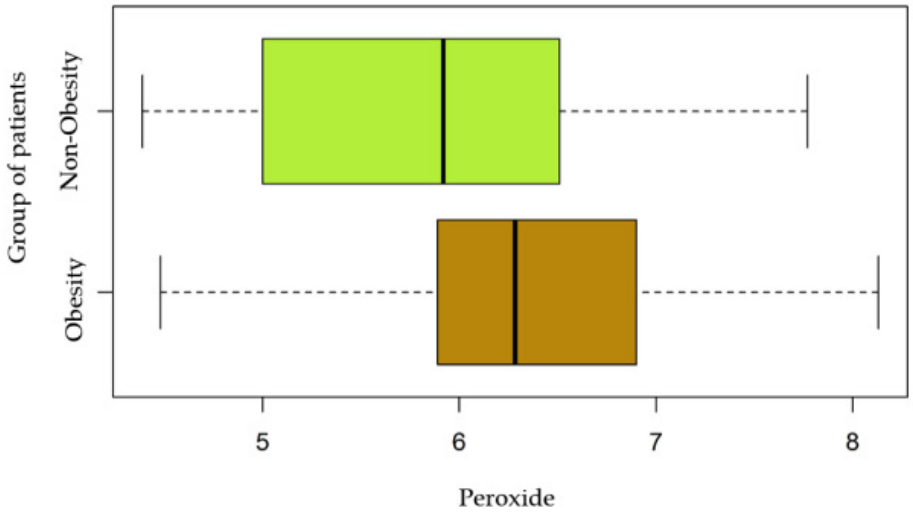

Figure 2: Comparative graph of serum peroxide concentration in obese *vs.* non-obese patients.

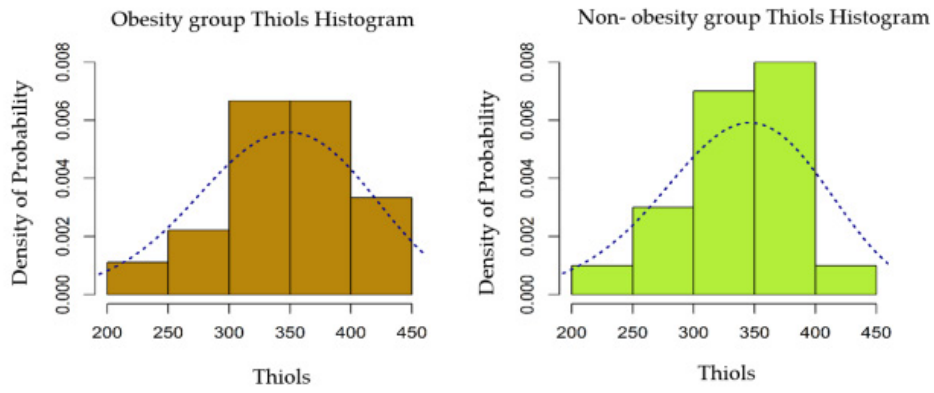

Figure 3: Histogram of serum thiol level distribution in obese *vs.* non-obese patients.

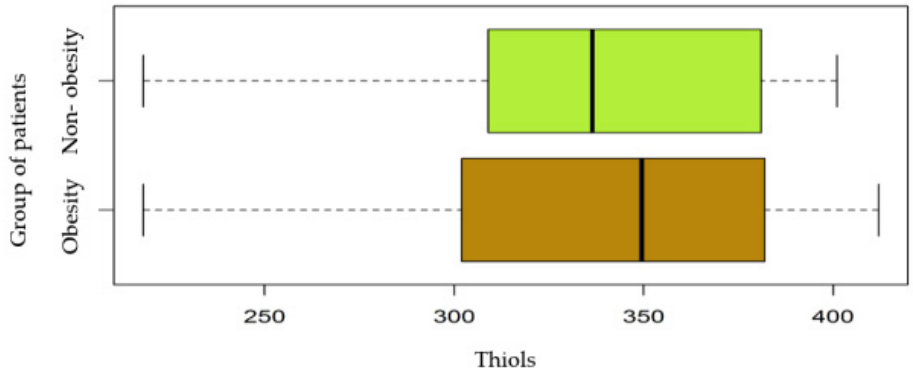

Figure 4: The comparative graph on serum thiol concentration in obese *vs.* non-obese patients.

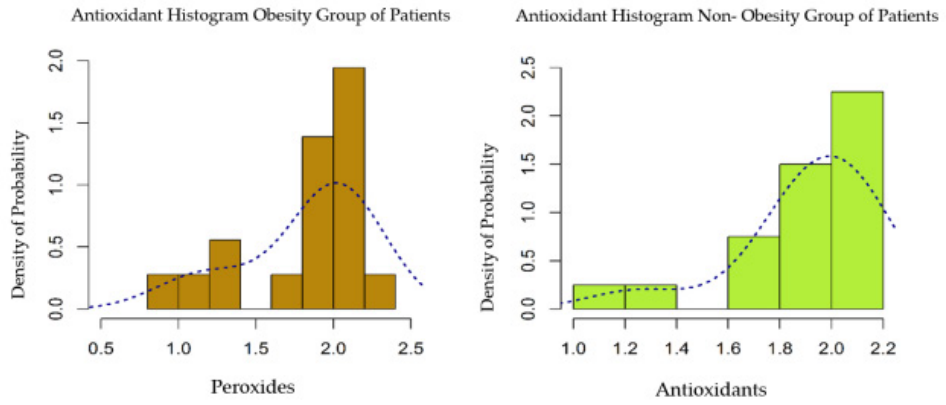

Figure 5: Histogram of serum antioxidant distribution within the two groups (obese *vs.* non-obese patients).

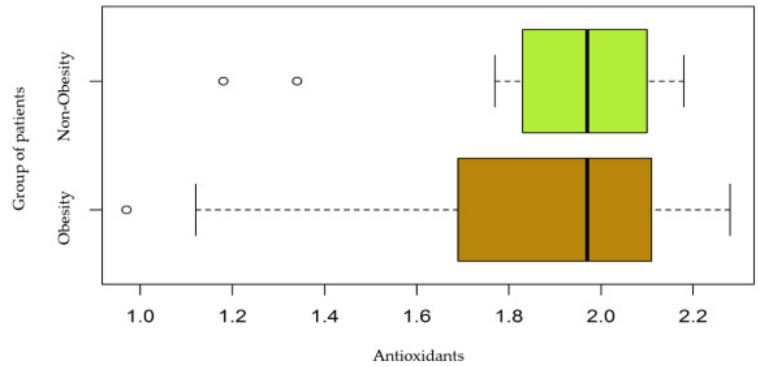

Figure 6: Comparative graph of the serum concentration of antioxidants in the two groups.

Table 4: Descriptive analysis of thiols on the two lots.

Thiols (µmol/l)	Obesity	Non-Obesity
Mean ± D. S	341.27 ± 50.61	336.65 ± 48.74
Median (IQR)	349.50 (80.00)	336.50 (72.00)

asymmetry. A Wilcoxon Rank Sum test was used for comparison. The differences were without statistical significance ($p>0.05$).

The graph shown in Figure 6 does not support a difference in serum antioxidants levels between obese and non-obese patients.

Discussion

This research paper was designed for clarifying the role of oxidative stress in influencing the prognosis of obese patients diagnosed with breast cancer. Both breast cancer and obesity are associated with disorders of the homeostasis of ROS, but how oxidative stress parameters influence the tumor microenvironment and subsequently the prognosis of these patients is still investigated. [16]

Table 5: Descriptive analysis of the level of antioxidants for the 2 batches.

Antioxidants (µmol/l)	Obesity	Non-Obesity
Mean ± S.D	1.82 ± 0.39	1.91 ± 0.25
Median (IQR)	1.97 (0.42)	1.97 (0.27)
Min la Max	0.97 la 2.28	1.18 la 2.18
Skewness	-1.09	-1.64

Obesity was quantified by measuring both BMI and waist circumference in the context of studies supporting the role of abdominal obesity in increasing the risk of breast cancer. [17]

The evaluation of the oxidative stress was made by serum investigation of some parameters involved in the oxidative attack on lipids, proteins and the determination of the activation level of the endogenous antioxidant defense systems. In this regard, serum concentration of MDA was determined as a measure of the lipid peroxidation reaction, serum thiol concentration as a final product of oxidative degradation of proteins and, in particular, of those containing sulfur and serum level of total antioxidants was measured too.

Given the role of oxidative stress in the pathogenesis of breast cancer, Pala et al. suggested that the disease is characterized by "pro-oxidants" that alter the redox state of thiol/disulphide and affect glucose tolerance, generating the so-called " mitochondrial oxidative stress." This means that the intracellular thiol/disulphide state is moved to an oxidative state, due to the persistent creation of a large number of ROS. [18]

Regarding MDA, a statistically significant positive mean correlation was identified between BMI and serum value of lipid peroxidation compounds ($p<0.01$). This correlation is supported by the hypothesis that adipose tissue, by maintaining a high degree of systemic inflammation and increased activity of macrophages, leukocytes and polymorphonuclear cells, causes excess production of ROS with a proportional increase in oxidative stress.

Another significant aspect is the identification of an increased serum level of peroxides in the studied patients, compared to the serum values considered normal. Thus, compared with the value considered normal 0 µmol-4 µmol/100 ml serum, in obese patients the average value was 6.25 µmol/100 ml and in non-obese patients it was 5.81 µmol/100 ml. These values are considered in the context of a lipid peroxidation reaction accentuated by the presence of neoplastic disease.

Data from the literature mention that lipid peroxidation, an intensely process once initiated, occurs as a chain reaction. Obesity can decrease the incidence of breast cancer in women through a specific mechanism that involves generation of lipid peroxidation products. [19] So, lipid peroxidation an episodic phenomenon involved in apoptosis, the cell cycle for the development and differentiation of tumor cells provides some protection in breast cancer. [20,21] This consideration of lipid peroxidation as a protective factor in breast cancer does not contradict the conventional view that oxidative degradation of lipids is an undesirable cytotoxic process, but emphasizes the protective phenomenon only in one stage. [20] This may explain the values obtained in this study, widely distributed, significantly not correlated with the parameters of obesity. Data from the literature indicate that the associations between dietary factors and breast cancer remain controversial, but several results suggest that lipid peroxidation may have a protective effect in patients with breast cancer and metabolic disorders, such as obesity. [22]

In the context that visceral obesity quantified by measuring waist circumference has an influence on increased mortality and morbidity, [23] this study sought to demonstrate a correlation between increased serum lipid peroxidation levels and waist circumference. By calculating the Pearson correlation index, a statistically significant weak positive correlation is found ($p<0.05$). Data from the literature, such as this research, indicate that the determination of lipid peroxidation compounds could be a valuable tool in assessing the prognosis of breast cancer, requiring additional studies to standardize the methodology. [24]

The determination of serum thiols evaluated the antioxidant barrier of proteins in normal weight patients compared to obese ones. Although mean and median values were slightly increased in the group of obese patients compared to those of normal weight, this difference was statistically insignificant (341 µmol/l *vs.* 336 µmol/l). However, lower values are obtained compared to normal ranges (370 µmol/l-450 µmol/l).

The Sulfhydryl (SH) groups in the composition of thiols act as a substrate for antioxidant enzymes, but they also have the role of blocking free radicals. In the human body, these-SH groups are a component of albumin mainly, but there also components of low molecular weight proteins such as cysteine, cysteinyl glycine, glutathione, homocysteine and γ-glutamyl cysteine. Under oxidative stress, thiol groups form disulfide bonds which can be reduced back to thiol groups that reenter the circuit and thus thiol-disulfide homeostasis is maintained. A study from 2019 conducted on a number of 37 patients with breast cancer compared to 31 healthy patients in which the values of serum thiols were analyzed, but also of compounds containing thiol disulfide groups, showed a statistically significant difference between the two groups in terms of serum concentration of groups containing thiol-sulfide groups. [25]

Endocrine functions, altered in obese patients due to the accumulation of visceral adipose tissue become important sources of oxidative stress manifested by decreased antioxidant capacity. The oxidant-antioxidant balance is necessary to maintain optimal physiological conditions in the body. Thiol-mediated redox mechanisms involve antioxidant reactions. The disulfide bridges between two cysteine amino acids regulate protein oxidation. An increase in thiol levels or thiol/disulfide ratios represents the intensity of antioxidant protection. Dynamic homeostasis of the thiol-disulfide ratio is necessary for antioxidant protection, regulation of enzymatic activity and detoxification, being also involved in the pathogenesis of various chronic diseases, such as diabetes, cardiovascular disease and cancer. [26]

Lipid peroxidation products, including MDA, are commonly used as biomarkers of oxidative stress because they may contribute to or reflect the amplification of cellular damage resulting from the generation of oxidized compounds. In addition, thiols, resulting from glutathione, cysteine and cysteine glycine, are natural reservoirs with reducing power and act as intracellular and extracellular redox buffers. [27]

Of all antioxidants, thiols are a major defense against ROS. The redox states of thiols play a critical role in determining the structure and function of proteins, regulating the enzymatic activity of transcription factors and antioxidant protection. Oxidative stress results from the imbalance created between the excess of ROS and the decrease of the antioxidant barrier.

For the complete evaluation of the oxidative stress parameters in this study, the serum level of total antioxidants in the study group was evaluated to assess a possible difference between obese patients compared to normal weight ones. The results do not support a statistically significant difference between the two groups of patients, thus do not demonstrate a benefit of antioxidant therapy in the prevention of breast cancer [28] or as an additive treatment in the therapeutic approach of this type of cancer. [29]

Cancer is a dynamic, multi-factorial and intrinsically complex disease. Despite these aspects, the tumor growth environment in each patient is much more stable and uniform, because most of the factors in this environment come from the predictable determinants of the patient's physiology. Thus, targeting this tumor growth microenvironment, more predictive treatment results can be obtained not only in breast cancer patients [30,31] but in a wider range of tumor types. Because the vast majority of tumors are surrounded by adipocytes and serve as active endocrine tissue, there may be direct effects of adipocytes on tumor growth that make adipocytes as a whole, viable targets for new therapeutic strategies in cancer.

As obesity and diabetes affect an increasing number of people, it is essential to understand the mechanisms by which they contribute to the development and progression of specific cancers. Targeting cancer with specific therapies, but ignoring systemic metabolic dysfunction, can contribute to resistance to cancer therapy and treatment failure. [32-34]

Conclusion

In the studied group, the observed oxidative stress is independent of obesity and is mainly related to the presence of breast cancer.

Breast cancer patients had significantly high levels of oxidation markers such as MDA and low levels of antioxidant markers, respectively thiols and total antioxidants. The results suggest an imbalance in redox homeostasis in these patients which can lead to an imbalance in pro and antiapoptotic processes, altered gene expression and mutations, all involved in the process of carcinogenesis. The study reiterates the importance of reducing oxidative stress to prevent the development and progression of many chronic diseases in which it plays an important role.

It is also known that oxygen radicals can involve in the process initiation of breast cancer and progression, depending on several genetic factors, hormonal, environmental, but also behavioral, namely nutrition, therefore. It is important to take into consideration a healthy lifestyle with an activity regular physical exercise in order to avoid any complications leading to serious damage and damaging.

Conflict of Interest

The Authors declare that they have no competing interests in relation to this study.

Authors' Contributions

IP and LNG was responsible writing of the manuscript. AZ, GLS were responsible for reviewing and editing of the manuscript. DEG, IN, LSCM, AMS and MIG made substantial contributions to the conception or design of the work. RA were responsible for the critically reviewed the manuscript. All Authors read and approved the final manuscript.

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