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A prospective randomized case-controlled study

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INTRODUCTION: *Oxidative stress plays an important role in the pathogenesis of malign diseases. Prolidase is a member of the matrix metalloproteinase family, plays a major role in collagen metabolism, cell growth, and matrix remodeling. Elevated serum prolidase activity have beendemonstrated in several types of carcinoma. The aim of this study is to investigate the serum prolidase activity, total oxidant status (TOS), total antioxidant status (TAS) and to evaluate their relationship with tumor stage, lymph node metastasis, and tumor size in patients with breast carcinoma.*
METHODS: *Thirty-five patients with breast carcinoma and forty healthy controls were enrolled to this study.Serum TAS, TOS levels, and prolidase activities were measured and oxidative stress indices (OSI) were calculated.*
RESULTS: TOS, *OSI levels and prolidase activities were significantly higher in the patients with breast carcinoma compared to the control group (P < 0.001, P < 0.001, P = 0.002, respectively).TAS levels were significantly lower in the in the patients with breast carcinoma compared to the control group (P = 0.016).Positive correlations were found between prolidase activity, TOS, OSI levels and tumor stage, lymph node metastasis, and tumor size. A negative correlation was found between TAS levels and tumor size,hovewer there were no correlationsbetween tas levels and stage of the tumor,as well as lymph node infiltration.*
CONCLUSION: *We conclude that elevatedserum prolidase activity and oxidative stress may be associated with breast carcinoma. Increased serum prolidase activity may be related to stage and prognosis of breast carcinoma.*

KEY WORDS: Breast carcinoma, Oxidative stress, Proline dipeptidase

Introduction

Breast cancer takes the first place among cancers in women worldwide and ranks second after lung cancer in terms of death due to cancer [1-3]. Breast cancer occurs with uncontrolled proliferation and deterioration of cells and tissue structures. The most important factor determining the prognosis of the disease is early diagnosis[4,5]. Various risk factors are involved in the neoplastic transformation of breast cells. Some of these factors are being over 40 years of age, breast cancer history of first degree relatives, breast disease history, early menarche, late menopause, advanced age of childbirth, nulliparity [6]. In addition to these factors, oxidative stress also plays an important role in the pathogenesis of breast cancer [7,8]. Oxidant and antioxidant systems are normally on balance in living species. The shift of equilibrium in favor of the oxidant system is called oxidative stress condition [9,10].

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Cellular damage can be seen in case of oxidative stress. There are many different methods and markers for assessing oxidative stress and antioxidant status [11]. However, the measurement of these markers separately is time consuming and expensive. Therefore, total oxidant status (TOS) and total antioxidant status (TAS) are determined and oxidative stress index (OSI) is calculated in recent years [12,13].

Prolidase is a peptidase that cleaves proline and hydroxyproline from the carboxy terminal end of immunodipeptides and immunotripeptides and catalyzes the most important stage of extracellular matrix degradation [14,15]. Large amounts of proline and hydroxyproline is available in collagenous tissue. Prolidase has important roles in collagen cycle and matrix remodeling. It has been determined to be in leukocytes, plasma, dermal fibroblasts, kidney, brain, heart, thymus, and uterus [16]. Prolidase have various important roles in embryonic development, wound healing, inflammation, carcinogenesis, angiogenesis, physiological and pathological processes such as cell migration and differentiation [17].

The aim of this study is to investigate the serum prolidase activity, TOS, TAS levels and to evaluate their relationship with tumor stage, lymph node metastasis, and tumor size in patients with breast carcinoma.

Materials and Methods

1. STUDY DESIGN

This prospective randomized case-controlled study was carried out in General Surgery Department of the Dumlupinar University Faculty of Medicine between October 2015 and January 2016. This study was in accordance with the principles outlined in the Declaration of Helsinki. Ethical committe approval was received from the local Human Research Ethics Committee (Number: 2015-KAEK-86/13-1). Written informed consent was obtained from the all patients and volunteers. The study population consisted of thirty five patients (mean age ± standard deviation, SD;52.4 ± 13.4) diagnosed as breast carcinoma and forty healthy volunteers (mean age± SD; 49.6 ± 11.7). The following patients were excluded from the study: Patients with coexistent medical illness like chronic heart failure, uncontrolled hypertension, chronic renal failure, diabetes mellitus, autoimmun diseases, patients with continued use of nonsteroidal antiinflammatory drugs, supplemental vitamins or antioxidants capable of interfering with free radical production. The following control subjects were excluded from the study: The consumption of alcohol, supplemental vitamins, antioxidants, non-steroid antiinflammatory drugs intake and smoking. Subjects were also excluded if they had a history of breast carcinoma in their first degree relatives.

2. BLOOD SAMPLE COLLECTION AND LABORATORY ANALYSES

2.1. *Blood sample collection*

The blood samples of breast cancer patients were collected one day before the surgery. After overnight fasting, venous blood samples were collected from antecubital vein into an evacuated serum seperator clot activator tube (Vacuette® Z Serum Sep Clot Activator, GreinerBio-One, Kremsmunster, Austria) between 9 and 10 a.m. All samples were drawn by the same expert phlebotomists. All blood samples were centrifuged at 1500 × g for 10 min at 4 ºC within 1 h and serum aliquots were stored at -80ºC until biochemical analysis of total antioxidant status (TAS), total oxidant status (TOS), and prolidase measurements. The investigator executing the biochemical analyses was blinded to the randomization.

2.2. *Measurement of serum TAS levels*

Serum TAS levels were measured using a Beckman Coulter AU680 instrument (Beckman Coulter, Miami, FL, USA) with commercial reagents (Rel Assay Diagnostic, Gaziantep, Turkey). The method was based on novel automated measurement methods developed by Erel[18].In this method, free radical reactions were started with the production of hydroxyl radical by Fenton reaction, and the rate of the reaction was monitored by following the absorbance of colored dianisidyl radicals. Antioxidants in the sample suppressed the color formation to a degree that is proportional to their concentrations. Trolox, a vitamin E analog, was used as a calibrator. TAS levels were expressed as mmolTrolox equivalent/L.

2.3. *Measurement of serum TOS levels*

Serum TOS levels were measured using a Beckman Coulter AU680 instrument (Beckman Coulter, Miami, FL, USA) with commercial reagents (Rel Assay Diagnostic, Gaziantep, Turkey). The method was based on novel automated measurement methods developed by Erel[12]. The method is based on the principle of the oxidation of ferrous ion to ferric ion in the presence of various oxidants in the sample and the measurement of the ferric ion by xylenol orange. The color intensity is related to the number of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide (H_2O_2). TOS levels were expressed as μmol H_2O_2 equivalent/L.

2.4. *Calculation of oxidative stress index (OSI)*

The percent ratio of TOS to TAS was accepted as the OSI, an indicator of the degree of oxidative stress. To

perform the OSI calculation, the unit of TAS, mmol Trolox equivalent/L, was converted to μmol Trolox equivalent/L, and OSI was calculated as follows: OSI = [(TOS, μmol H_2O_2 equivalent/L) / (TAS, μmol Trolox equivalent/L) x 100][19]. The results are expressed as arbitrary units (AU).

2.5. *Measurement of serum prolidase activities*

Serum Xaa-prodipeptidase/prolidase(PEPD) activities were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Cusabio Biotech Co., Ltd., Wuhan, Hubei Province, China; catalog number: CSB-E16196h) on a microplate reader (BMG LabtechSpectrostar Nano, GmbH, Ortenberg, Germany) according to the manufacturer's protocol. Prolidase activities were expressed as mU/mL.

2.6. *Statistical analysis*

In this study, we firstly performed power analysis to detect the adequate number of participants. According to this we obtained a significance level (alpha) value of 0.05, a beta value of 0.20 and a power of 0.80. Based on these results, a sample size of 35 patients at least was necessary for each group.

Statistical analyses were performed using GraphPad Prism version 6.05 (GraphPad Software, Inc., CA, USA). All data sets were tested for normality using Shapiro Wilk test. Normally distributed data were expressed as mean ± standard deviation (SD). Not normally distributed data were expressed as median and interquartile range (IQRs). Parametric or non-parametric statistical tests were used according to the distribution of data. The comparisons of examined variables between study groups were tested using the two tailed student-t test for normally distributed data and using the Mann–Whitney U test for not normally distributed data. The correlation analyses were performed using the Spearman's correlation analysis,since data were not normally distributed. A P value < 0.05 was considered statistically significant.

Results

The patient group consisted of thirty five patients who were diagnosed as breast carcinoma and the control groupconsisted of forty healthy volunteers. The mean age of study population was 52.4 ± 13.4 in the patient group and 49.6 ± 11.7 in the control group. Demographic and clinical data of the patients are represented in Table I. Comparisons of serum TAS, TOS, OSI levels and prolidase activities between patients with breast carcinoma and control group are represented in Table II and comparison graphs of study groups are represented in Fig. 1. There was no difference between mean age of the study groups. Serum TOS, OSI levels and prolidase activities were significantly higher in the patients with breast carcinoma compared to the control group (P < 0.001, P < 0.001, P = 0.002, respectively, Table II, Fig. 1). Serum TAS were significantly lower in the in the patients with breast carcinoma compared to the control group (P = 0.016, Table II, Fig. 1).

The relationship between clinicopathological characteristics of the tumor and oxidative, anti-oxidative parameters of the patients with breast carcinoma are represented in Table III. Serum TOS, OSI levels and prolidase activities showed significant positive correlation with size of the tumor in the patients with breast carcinoma (r= 0.531, P=0.001; r= 0.483, P = 0.003; r = 0.714, P <0.001, respectively, Fig. 2). Although there was not a statistically significance, a negative correlation was observed between TAS levels and size of the tumor (r= -0.300, P= 0.08, Table III, Fig. 2). A positive correlation was found between serum TOS, OSI levels, prolida-

Table I - *Demographic and clinical data of the patients with breast carcinoma.*

Parameters	**n = 35**
Age (years) (mean ± SD)	52.4 ± 13.4
Type of the breast carcinoma (n)	
Invasive ductal carcinoma	26
Inflammatory carcinoma	1
Invasive lobular carcinoma	7
Invasive micropapillary carcinoma	1
Localization of the tumor (n)	
Unilateral right	13
Unilateral left	21
Bilateral	1
Stage (TNM) (n)	
1	9
2A	7
2B	6
3A	9
3B	4
Size of the tumor (cm)	2.5 (1.5 – 3.5)
Size of the tumor (T)(n)	
Stage T1	10
Stage T2	17
Stage T3	8
The number of the lymph node involvement	1.0 (0.0 – 3.0)
Spread to the lymph nodes (N)(n)	
Stage N0	15
Stage N1	13
Stage N2	5
Stage N3	2
Type of the surgical operation(n)	
Segmental resection	16
Modified radical mastectomy	19

Abbreviations: Data are presented as mean ± standard deviation (SD) or median and interquartile ranges (IQRs) or number.

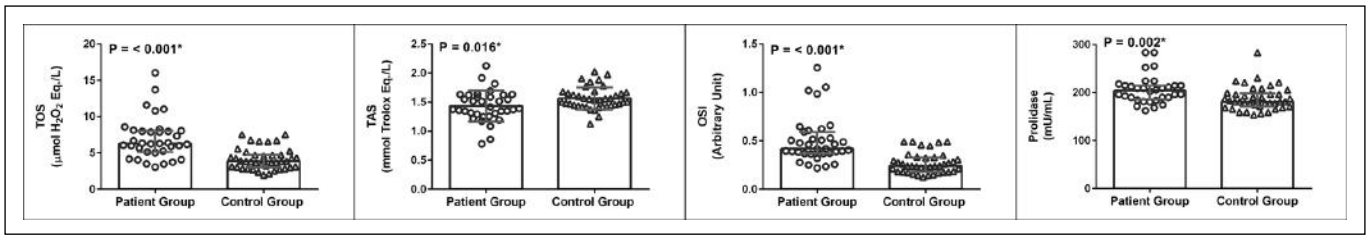

Fig. 1: Representative comparison graphs of study groups. Differences between the patient and the control groups for serum total oxidant status, total antioxidant status, oxidative stress indices levels, and prolidase activities. Normally distributed data are presented as mean ± SD (standard deviation), not normally distributed data are presented as median and interquartile ranges (IQRs). Normally distributed data were tested using the two-tailed Student-t test. Not normally distributed data were tested using the Mann–Whitney U-test. A P-value < 0.05 was considered statistically significant. TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index.

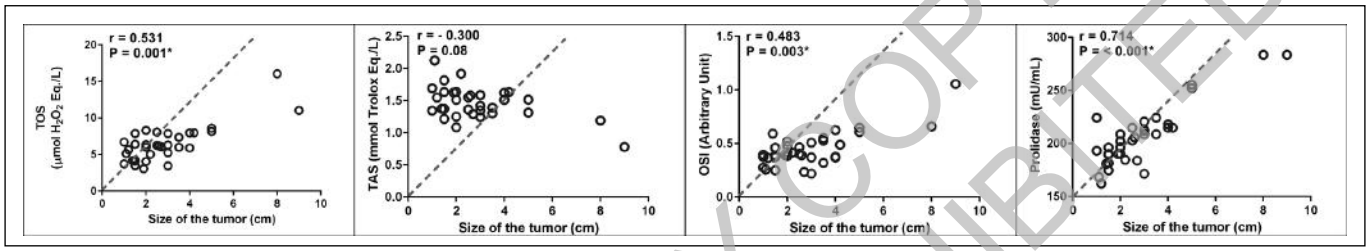

Fig. 2: Representative correlation graphs of variables. Correlations between size of the tumor and serum total oxidant status, total antioxidant status, oxidative stress indices levels, and prolidase activities in the patients with breast carcinoma.Data were tested using the Spearman's correlation analysis.A P-value < 0.05 was considered statistically significant.TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index.

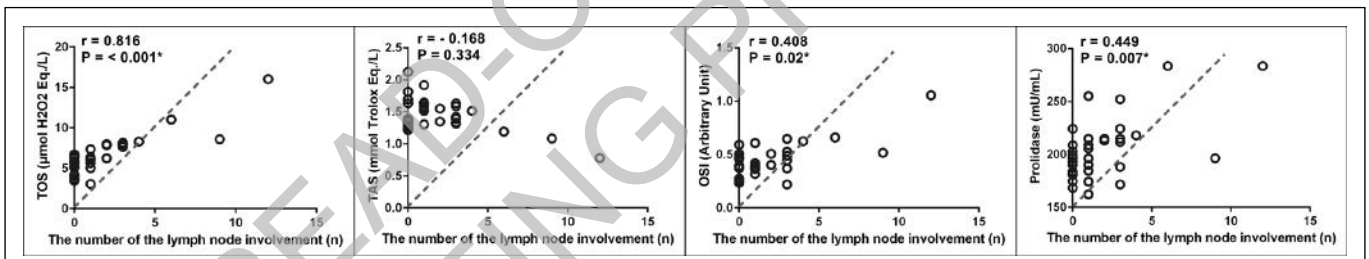

Fig. 3: Representative correlation graphs of variables. The relationships between the number of lymph node involvementand serum total oxidant status, total antioxidant status, oxidative stress indices levels, and prolidase activities in the patients with breast carcinoma. Data were tested using the Spearman's correlation analysis. A P-value < 0.05 was considered statistically significant. TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index.

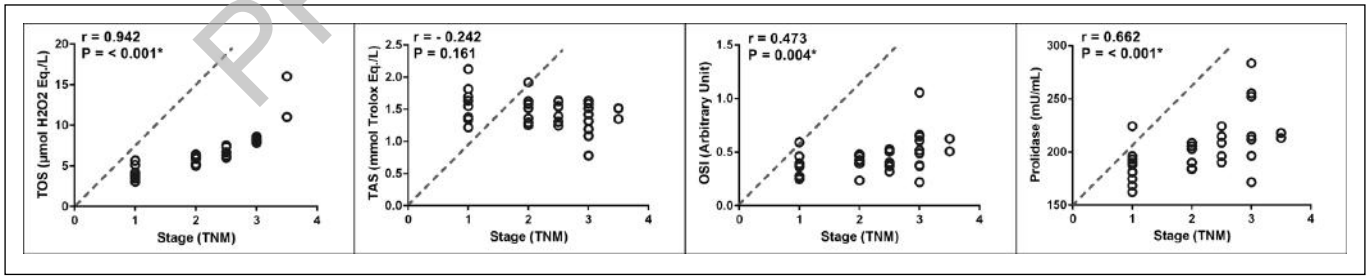

Fig. 4: Representative correlation graphs of variables. The relationships between the stage of tumor according to TNM staging and serum total oxidant status, total antioxidant status, oxidative stress indices levels, and prolidase activities in the patients with breast carcinoma. Data were tested using the Spearman's correlation analysis.A P-value < 0.05 was considered statistically significant.TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index.

Table II - *Comparisons of demographicdata and serum TAS, TOS, OSI levels and prolidase activities between patients with breast carcinoma and control group.*

Parameters	Patient Group (n = 35)	Control Group (n = 40)	95% CI of Difference	Statistical Analysis (*P*)
Age (years)	52.4 ± 13.4	49.6 ± 11.7	-2.98 to 8.58	0.337
TOS (μmol H_2O_2 Eq./L)	6.31 (5.14 – 8.07)	3.84 (3.01 – 4.73)	-3.45 to -1.55	< 0.001*
TAS (mmol Trolox Eq./L) (#)	1.44 ± 0.27	1.57 ± 0.19	0.026 to 0.24	0.016*
OSI (Arbitrary Unit)	0.42 (0.37 – 0.59)	0.24 (0.18 – 0.33)	-0.25 to -0.13	< 0.001*
Prolidase (mU/mL)	204.3 (186.0 – 215.0)	180.8 (171.2 – 198.7)	-27.97 to -6.20	0.002*

Abbreviations: TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, CI: Confidence intervale. Normally distributed data are presented as mean ± SD, not normally distributed data are presented as median and interquartile ranges (IQRs).Normally distributed data (#) were tested using the two-tailed Student-t test. Not normally distributed data were tested using the Mann–Whitney U-test. *: P-value < 0.05 was considered statistically significant.

Table III - *The relationship between clinicopathological characteristics of the tumor and oxidative, anti-oxidative parameters of the patients with breast carcinoma.*

	Size of the tumor (cm)		Lymph node involvement (n)		Stage (TNM Staging)	
	R	P	r	P	R	P
TOS (μmol H_2O_2 Eq./L)	0.531	0.001*	0.816	< 0.001*	0.942	< 0.001*
TAS (mmol Trolox Eq./L)	-0.300	0.08	-0.168	0.334	-0.242	0.161
OSI (Arbitrary Unit)	0.483	0.003*	0.408	0.02*	0.473	0.004*
Prolidase (mU/mL)	0.714	< 0.001*	0.449	0.007*	0.662	< 0.001*

Abbreviations: TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index. Data were tested using the Spearman's correlation analysis.
*: P value less than 0.05 was considered statistically significant. To analyse between oxidative parameters and stage of the tumor, stage 1 was numbered as 1, stage 2A was numbered as 2, stage 2B was numbered as 2.5, stage 3A was numbered as 3, stage 3B was numbered as 3.5.

se activities and the number of lymph node metastasis (r= 0.816, P<0.001; r = 0.408, P = 0.02; r = 0.449, P = 0.007, respectively, Fig. 3). Serum TAS levels were not correlated with the number of lymph node metastasis (r= -0.168, P= 0.334, Table III, Fig. 3).Serum TOS, OSI levels and prolidase activities showed significant positive correlation with stage of the tumor according to the TNM staging in the patients with breast carcinoma (r= 0.942, P<0.001; r= 0.473, P= 0.004; r= 0.662, P < 0.001, respectively, Fig. 4). Serum TAS levels were not correlated with stage of the tumor (r= -0.242, P= 0.161, Table III, Fig. 4).

Discussion

In this study, we investigated the serum TAS, TOS levels, and prolidase activity and also evaluated their relationship with tumor stage, lymph node metastasis, and tumor size in patients with breast carcinoma. The role of oxidative stress in breast carcinoma was evident as increased levels of TOS, OSI and activity of prolidase, as well as decreased levels of TAS in patients with breast carcinoma. The relationships between clinicopathological characteristics of the tumor and oxidative, anti-oxidative parameters of the patients with breast carcinoma were

evident as positive correlation between serumTOS, OSI levels and prolidase activities and size of the tumor, the number of lymph node metastasis, stage of the tumor.
Oxidative stress is defined as a decrease in antioxidant capacity or increase in oxidant status. However, there are additional effects of various oxidants and antioxidants in oxidative stress [20]. Free radicals can lead to cellular damage, chromosome breakage, and ultimately the development of cancer by initiating lipid peroxidation process and the DNA damage in cases where excessive production of reactive oxygen species (ROS) or inadequate antioxidant defense conditions[21]. What does ıt mean?
Although plasma levels of oxidants and antioxidants can be measured separately, the results can not fully reflect oxidative stress [22]. TAS, TOS and OSI are biomarkers which indicate oxidative stress and reflect the balance between oxidation and antioxidation [18]. Thus, we investigated the level of oxidative stress by measuring the serum TAS, TOS, and OSI levels in breast cancer patients and healthy controls. Serum TOS and OSI levels were increased in patients with breast carcinoma, whereas serum TAS levels were decreased in patients with breast carcinoma. In previous studies, increased levels of oxidative stress have been demonstrated in cancer patients [23,24]. Feng et al.[24] reported increased level of oxidative stress in breast cancer patients by measuring TAS, TOS and OSI values. In addition, they have investigated the relationship between breast cancer stage and levels of these parameters as similar to this study. In our study, in addition to the association between oxidative stress levels and tumor stage, the association between oxidative stress levels and tumor size,lymph node metastasis were also examined. Unlike from the study by Feng et al. [24] serum prolidase activities were studied in our study. Changes in prolidase activities were similar to changes in the level of oxidative stress. These results add a difference to this study.
Antioxidants are such compounds that retard, inhibit or block the oxidative damage by suppressing oxidative stress and eliminating free radicals [25]. Natural antioxidant defense consists of enzymatic and non-enzymatic endogenous antioxidants produced by our own body. The occurrence of the natural antioxidant defense deficiency contributes to carcinogenesis. Antioxidant therapy has been the subject of research in patients with inadequate antioxidant status [26]. Exogenous antioxidants have been shown to reduce the activity of ROS [27]. Thus, exogenous antioxidant treatment is one of the treatment options that can be applied in patients with elevated oxidative stress and cancer. In a previous study,it has been suggested that the clinical stage of breast cancer is associated with oxidative stress in agreement with this study [28].
Prolidase is an enzyme that plays an important role in collagen metabolism, cell growth and matrix remodeling [29]. It helps to re-synthesize proline and hydroxyproline by releasing the collagen. Extracellular collagenases initiate collagen destruction but the last step is catalyzed by intracellular prolidase [30]. It has also been suggested that prolidase is the speed-limiting step in the regulation of collagen biosynthesis [31]. Collagen is one of extracellular matrix components and is the main barrier against the invasion of neoplastic cells. Tumor cells secrete proteolytic enzymes and thus exceed beyond the basal membrane and extracellular matrix [32]. Due to catalyzation by prolidase is the last step of intracellular collagen destruction, it is thought that prolidase is related to cancer development. Prolidase enzyme activity was investigated in several chronic diseases such as non-ulcer dyspepsia and chronic liver disease [33]. In addition, an increase in serum prolidase activity was demonstrated in several types of cancer such as lung, bladder, gastric, ovarian, and endometrial cancer [34-37]. In a previous study by Cechowska et al. [38],increased serum prolidase activity has been demonstrated in breast cancer tissue consistent with our study[38]. Unlike to the study by Cechowska et al.[38], the relationships between serum prolidase activity and tumor stage, tumor size, and lymph node infiltration were investigated in our study.
In this study, while serum prolidase activities and TOS, OSI levels were significantly increased,serum TAS levels were significantly decreased in breast cancer patients. Serum prolidase activities and TOS, OSI levels positively correlated with tumor stage, lymph node metastasis, and tumor size. To our knowledge our work is one of the few studies in the literature investigating the relationship of serum prolidase activity and oxidative stress levels with tumor stage, size, and lymph node metastasis.

Conclusion

In summary, an increase in collagen turnover in breast cancer patients is an expected condition. An increase in serum prolidase activity was observed in breast cancer patients due to increased collagen turnover. It has been observed that increased serum prolidase activity is associated with increased oxidative stress. Additionally a significant decrease was observed in the antioxidant status. We conclude that elevated serum prolidase activity and oxidative stress may be associated with breast carcinoma and increased serum prolidase activity may be related to stage and prognosis of breast carcinoma. We suggest that treatments which can reduce or suppress prolidase activity, may contribute to the breast cancer therapy. In addition, antioxidant treatment may be effective in protection from cancer and also in the treatment of cancer. Treatments that reduce or supress prolidase activity would be the subject of research in cancer patients in the future. Major limitation of this study is relatively small sample size. Further prospective studies with large cohort are needed to clarify our hypothesis.

Riassunto

Lo stress ossidativo gioca un ruolo importante nella patogenesi delle malattie neoplastiche. La prolidasi è un costituente della matrice metalloproteinasica, gioca un ruolo maggiore nel metabolismo del collagene, nell'accrescimento cellulare e nel rimodellamento strutturale. Una elevata attività prolidasica è stata dimostrata in molti casi di carcinomi. Lo scopo del presente studio è quello di indagare sull'attività sierica della prolidasi, dello stato ossidativo (TOS) ed antiossidativo (TAS) totale, e di valutare il loro rapporto con lo stadio del tumore, delle metastasi linfonodali e della massa neoplastica in pazienti con carcinoma mammario.

Per lo studio sono state arruolate 35 pazienti con carcinoma della mammella e 40 soggetti di controllo. Sono stati rilevati i livelli di TAS, TOS e dell'attività prolidasica, calcolando gli indici di stress ossidativo (OSI).

Come risultato i livelli di TOS, di OSI e dell'attività prolidasica sono risultati significativamente più elevati nelle pazienti con carcinoma mammario rispetto al gruppo di controllo (rispettivamente ($P < 0.001$, $P < 0.001$, $P = 0.002$). I livelli di TAS sono risultati significativamente inferiori nelle pazienti con carcinoma mammario rispetto al gruppo di controllo ($P = 0{,}016$). Sono stati rilevate correlazioni positive tra attività prolidasica, ed i livelli di TOS e di OSI con lo stadio tumorale, le metastasi linfoniodali e le dimensioni del tumore. Negativa è risultata la correlazione tra i livelli di TAS e le dimensioni del tunore, ma nessuna correlazione tra i livelli di TAS e lo stadio del tumore, come pure con l'infiltrazione linfonodale.

Si conclude che l'elevata attività prolidasica del siero e lo stress ossidativo possono associarsi col carcinoma mammario. L'accresciuta attività prolidasica può essere messa in relazione con lo stadio e la prognosi del carcinoma mammario.

References

1. Moore MA, Eser S, Igisinov N, et al.: *Cancer epidemiology and control in North-Western and Central Asia - past, present and future.* Asian Pac J Cancer Prev, 2010; 11:17-32.

2. Parkin DM, Bray F, Ferlay J, et al.: *Global cancer statistics, 2002.* CA Cancer J Clin, 2005; 55:74-108.

3. Franceschini G, Leone AD, Masetti R: *The Breast Unit. Update on advantages and the open issues.* Ann Ital Chir, 2014; 85:407-12.

4. Benson JR, Jatoi L, Keisch M, et al.: *Early breast cancer*. Lancet, 2009; 373:1463-479.

5. Franceschini G, Sanchez AM, Leone AD, et al.: *Update on the surgical management of breast cancer.* Ann Ital Chir, 2015; 86:89-99.

6. Kaminska M, Ciszewski T, Lopacka-Szatan K, et al.: *Breast cancer risk factors*. Prz Menopauzalny, 2015; 14:196-202.

7. Thanan R, Oikawa S, Hiraku Y, et al.: *Oxidative stress and its significant roles in neurodegenerative diseases and cancer*. Int J Mol Sci, 2014; 16:193-217.

8. Roque AT, Gambeloni RZ, Felitti S, et al.: *Inflammation-induced oxidative stress in breast cancer patients*. Med Oncol, 2015; 32:263.

9. Valko M, Leibfritz D, Moncol J, et al.: *Free radicals antioxidants in normal physiological functions and human disease*. Int J Biochemcell Biol, 2007; 39;44-84.

10. Jomova K, Valko M: *Advances in metal-induced oxidative stress and human disease.* Toxicology, 2011; 283:65-87.

11. Kohen R, Nyska A: *Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification.* Toxicol Pathol, 2002; 30:620-50.

12. Erel O.: *A new automated colorimetric method for measuring total oxidant status.* Clin Biochem, 2005; 38:1103-11.

13. Erel O: *A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation.* Clin Biochem, 2004; 37:277-85.

14. Karna E, Surazynski A, Palka J: *Collagen metabolism disturbances are accompanied by an increase in prolidase activity in lung carcinoma planoepitheliale.* Int J Exp Pathol, 2000; 8:341-47.

15. Myara I, Charpentier C, Lemonnier A: *Optimal conditions for prolidase assay by proline colorimetric determination: Application to iminodipeptiduria*. Clin Chim Acta, 1982; 125:193-205.

16. Zanaboni G, Dyne KM, Rossi A, et al.: *Prolidase deficiency: Biochemical study of erythrocyte and skin fibroblast prolidase activity in Italian patients*. Haematologica, 1994; 79:13-8.

17. Palka JA, Phang JM: *Prolidase activity in fibroblasts is regulated by interaction of extracellular matrix with cell surface integrin receptors*. J Cell Biochem, 1997; 67:166-75.

18. Erel O: *A novel automated method to measure total antioxidant response against potent free radical reactions.* Clin Biochem, 2004; 37:112-19.

19. Akcilar R, Akcilar A, Savran B, et al.: *Effects of ukrain in rats with intestinal ischemia and reperfusion*. J Surg Res, 2015; 195:67-73.

20. Wayner DD, Burton GW, Ingold KU, et al.: *The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma*. Biochim Biophys Acta, 1987; 924:408-19.

21. Goldstein BD, Witz G: *Free radicals and carcinogenesis.* Free Radic Res Commun, 1990; 11:3-10.

22. Sharma SB, Dwivedi S, Kumar N, et al.: *Studies on oxidative stress, serum iron and iron binding capacity in subjects prone to the risk of coronary artery disease.* Indian Heart J, 2000; 52:583-86.

23. Sener DE, Gonenc A, Akinci M, et al.: *Lipid peroxidation and total antioxidant status in patients with breast cancer.* Cell Biochem Funct, 2007; 25:377-82.

24. Feng JF, Lu L, Zeng P, et al.: *Serum total oxidant/antioxidant status and trace element levels in breast cancer patients.* Int J Clin Oncol, 2012; 17:575-83.

25. Sirerol JA, Rodriguez ML, Mena S, et al.: *Role of natural stilbenes in the prevention of cancer.* Oxid Med Cell Longev, 2016; 2016:3128951.

26. Mut-Salud N, Alvarez PJ, Garrido JM, et al.: *Antioxidant intake and antitumor therapy: Toward nutritional recommendations for optimal results.* Oxid Med Cell Longev, 2016; 2016:6719534.

27. Yasueda A, Urushima H, Ito T: *Efficacy and interaction of antioxidant supplements as adjuvant therapy in cancer treatment: asystematic review.* Integr Cancer Ther, 2015; 15:17-39.

28. Carioca AA, Verde SM, Luzia LA, et al.: *Association of oxidative stress biomarkers with adiposity and clinical staging in women with breast cancer.* Eur J Clin Nutr, 2015; 69:1256-261.

29. Pirincci N, Kaba M, Gecit I, et al.: *Serum prolidase activity, oxidative stress, and antioxidant enzyme levels in patients with renal cell carcinoma*. Toxicol Ind Health, 2016; 32:193-99.

30. Ilikhan SU, Bilici M, Sahin H, et al.: *Assessment of the correlation between serum prolidase and alpha-fetoprotein levels in patients with hepatocellular carcinoma*. World J Gastroenterol, 2015; 21:6999-7007.

31. Surazynski A, Miltyk W, Palka J, et al: *Prolidase-dependent regulation of collagen biosyn*thesis. Amino Acids, 2008; 35:731-38.

32. Chen WT: *Membrane proteases: Roles in tissue remodeling and tumour invasion*. Curr Opin Cell Biol, 1992; 4:802-09.

33. Kumari S, Verma AK, Rungta S, et al.: *Serum prolidase activity, oxidant and antioxidant status in nonulcer dyspepsia and healthy volunteers.* ISRN Biochem, 2013; 2013:182601.

34. Arioz DT, Camuzcuoglu H, Toy H, et al.: *Serum prolidase activity and oxidative status in patients with stage I endometrial cancer.* Int J Gynecol Cancer, 2009; 19:1244-247.

35. Guszczyn T, Sobolewski K: *Deregulation of collagen metabolism in human stomach cancer*. Pathobiolog, 2004; 71:308-13.

36. Camuzcuoglu H, Arioz DT, Toy H, et al.: *Assessment of preoperative serum prolidase activity in epithelial ovarian cancer.* Eur J Obstet Gynecol Reprod Biol, 2009; 147:97-100.

37. Gecit I, Aslan M, Gunes M, et al.: *Serum prolidase activity, oxidative stress, and nitric oxide levels in patients with bladder cancer*. J Cancer Res Clin Oncol, 2012; 138:739-43.

38. Cechowska-Pasko M, Pałka J, Wojtukiewicz MZ: *Enhanced prolidase activity and decreased collagen content in breast cancer tissue.* Int J Exp Pathol, 2006; 87:289-96.