Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Oxidative stress and galectin-3 levels during skin grafting after hand injury: gender differences

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Article Info

Article history:

Received: November 21, 2023
Accepted: March 18, 2024
Published: July 31, 2024

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Abstract

The study included 40 patients of both genders who underwent skin transplantation after a hand injury. The study aims to evaluate the oxidative stress parameters in patients' blood and serum levels of galectin-3 in order to investigate gender differences pre- and post- skin transplantation. The results of the study suggest a significant increase in superoxide anion radical levels, catalase activity, and reduced glutathione levels in females before skin transplantation. The surgical treatment caused significant increase in superoxide anion radical and hydrogen peroxide levels as prooxidants in males, while superoxide dismutase and catalase activity were also increased 7 days after the procedure. In females, superoxide anion radical and TBARS levels increased after surgical procedure as well as the activity of catalase. Regarding galectin-3 levels, a significant interaction between gender and time was observed (gender×time; p=0.000). Correlation analysis of different oxidative stress markers with gal-3 revealed the existence of a significant negative correlation of superoxide anion radical, catalase, and reduced glutathione with gal-3, but only in female patients. It can be concluded that OS as well as galectin-3 play important roles at least in the first 7 days of the postoperative period.

Keywords: Oxidative stress, Skin transplantation, Galectin 3, Hand injuries, Gender differences.

1. Introduction

The most commonly injured part of the human body is the hand, with enormous socio-economic burden and morbidity [1]. Treatment of traumatic hand injuries ranges from simple suture reparation, symptomatic treatment, and immobilization, to complex microvascular reparation and repair procedures performed in several acts with uncertain outcomes. In many cases, these injuries (work-related injuries, cuts, avulsions, abrasions, motorcycle accidents, etc.) leave skin defects requiring specific treatment to

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Doi: http://dx.doi.org/10.14715/cmb/2024.70.7.6

cover the existing defects. There are different methods and techniques used in the management of these wounds such as STSG (split-thickness skin graft), FTSG (full-thickness skin graft), flaps, etc [2]. STSG consists of the epidermis and a part of the dermis, which relies on a vascularized wound surface for ingrowth [3]. Advantages of STSG are as follows: low metabolic activity as compared to FTSG (better for shallow skin defects and unhealthy wound beds like chronic ulcers); lesser amount of primary graft contracture (lower concentration of elastic fibres in the graft); the least amount of donor site morbidity; faster regrowth of new skin. Disadvantages include poor aesthetic match (STSGs are often meshed or have different colorizations), susceptibility to trauma, hyposensitisation of the recipient areas, and necessity for wound care in both recipient and donor sites [3].

Traumatic injuries are one of the leading causes of local and systemic oxidative stress (OS) [4]. The frequency of male gender is disproportionately high in practically all types and mechanisms of injury, which hides the impact of trauma on the female population [5]. Pioneering studies have provided proof-of-concept about central role of OS as a major modulator of the regeneration of skeletal muscle tissue after traumatic injury [6]. Almost all forms of surgery can be negatively influenced by OS, which served as a gateway to many therapeutic trials aiming to modify the oxidative response, with variable results. This opens a wide field for further research in order to closely determine the role of OS, its effects, and best available treatment modalities significant for future clinical practice in surgery and internal medicine [4]. Despite the fact that working-age population (18-64 years of age) is the most likely to be injured, only limited publications have addressed this complicated issue [7].

Galectin-3 (gal-3) is a multifaceted molecule and emerging studies have highlighted its central role in various cellular processes. Gal-3 isin the inflammatory response originating from traumatic events and various diseases. This protein, belongs to the group of lectins, and plays pivotal role in different physiological and pathophysiological processes, immune and inflammatory reactions. Gal-3is a marker of fibrosis, cell adhesion, apoptosis, and neoplastic development. Different studies have reported negative and positive effects of gal-3, depending on its expression and function in various tissues [8]. It is significantly expressed within skin cells, such as keratinocytes, melanocytes, fibroblasts, dendritic cells and monocytes, hair follicles, sweat and sebaceous glands, where it regulates different mechanisms [9]. Gal-3 is also involved in cell maturation and differentiation. Moreover, Gal-3-mediated anti-apoptotic effects have been reported in the scientific literature [10]. There is evidence of gender-specific variability in the levels of gal-3 and females probabilistically have greater levels of gal-3 in serum [11].

Taking into account the mentioned facts supporting the gender differences in OS parameters and gal-3 levels in blood, we aimed to investigate their levels pre- and post-STSG surgery in patients with hand injury.

2. Materials and methods

2.1. Ethical concerns and study design

This is a prospective, cross-sectional study conducted in the Center for Plastic and Reconstructive Surgery of University Clinical Centre Kragujevac (Kragujevac, Serbia) in the period from January 2018 until January 2020. The study was conducted in accordance with the Declaration of Helsinki (revision 2013), the principles of Good clinical practice and regulations, and it was approved by the Ethical Committee of University Clinical Center Kragujevac (No.01/18/1992). Before all study procedures, all patients voluntarily gave their written informed consent for participation in the study.

The study included 40 patients (20 males and 20 females), aged between 18 and 65 years (working-age population) who suffered from hand injury and required secondary wound coverage with STSG. Apart from age, pre-requisites for the inclusion criteria involved the presence of a split-thickness skin graft donor site wound with a minimum size of 15 cm^2 and with a minimum width of 3 cm. The exclusion criteria for participating in the study were the following: the presence of any type of chronic or currently active skin disease which the investigator considered could adversely affect the skin-grafting procedure and healing. Moreover, any form of vasculopathy, diabetes mellitus, cardiovascular disease and systemic disease with OS etiology as well as long-term corticosteroid therapy were considered carefully in the exclusion criteria for participation of the subjects in this study.

2.2. The procedure of skin harvesting for an STSG and transplantation procedure

The skin harvested for an STSG of 300 μm thickness was performed with a skin dermatome. The donor site was the inner side of the upper thigh. Before taking the transplant, the skin was surgically prepared and washed with saline. After the skin harvesting, a uniform piece of STSG was obtained and the donor region of the graft was processed using the standard technique - an occlusive bandage according to Allen-Kocher. The graft edges were aligned with scissors and then perforated with a scalpel. The graft was placed on vaseline gauze with its outer side and folded several times depending on its size. The prepared graft was placed in a sterile container with a lid and with two or three small gauzes soaked in sterile 0.9% NaCl solution. The sterile container was placed in the refrigerator at the temperature of 4°C.

The hand wound was cleaned, debridement procedure was done properly, and the wound was prepared for coverage with STSG. The recipient site was thoroughly cleaned and debrided back to all viable tissue. Prevention of any fluid or air pockets present beneath the transplant was achieved by previously meshing the graft. To prevent the graft from migrating into the wound bed, it was securely maintained in the place. If there was any doubt about the cleanliness of the wound, the dressing was removed after at least three days; if the wound was extremely clean, the dressing was removed after seven days.

After skin grafting the patients were kept under observation daily for 7 days, and blood samples were collected for analysis at 7^{th} postoperative day.

2.3. Blood sampling and biochemistry

Blood samples of all patients were obtained from the peripheral venous circulation (cubital vein) just before the skin grafting procedure (day 1) and one week after skin grafting (day 7). Blood samples were then processed to obtain serum and plasma for additional biochemical examination: the blood was left to coagulate for two hours

at room temperature and centrifuged to collect serum samples; while after centrifuging the blood collected in anticoagulant tubes, plasma samples were obtained. Erythrocyte lysates were made by washing the erythrocyte suspension three times in ice-cold saline first and then lysing it in three volumes of ice-cold distilled water. Serum, plasma, and erythrocyte lysate samples were kept at -20°C until the time of analysis.

2.3.1. Biochemical analysis

All biochemical analyses were conducted in a specialized biochemical laboratory of the University Clinical Center Kragujevac. Serum levels of urea (reference range urea 3–8 mmol/L), creatinine (reference range 49–106 μmol/L), and glucose (3.80-6.10 mmol/L) were measured by using standard kits in an automatic clinical chemistry analyzer (AU680 Clinical Chemistry Analyzer by Beckman Coulter).

The complete blood cell count (CBC) was determined by using a hematology analyzer (DxH 800 Hematology Analyzer by Beckman Coulter). The following haematological parameters were analysed: hemoglobin (Hb; reference range 138–175 g/L for males and 110–157 g/L for females), hematocrit (HCT; 0.415-0.530 L/L for males and 0.356–0.470 L/L for females), erythrocytes (Er; range 4.34–5.72 × 10^{12}/L for males and 3.86–5.08 × 10^{12}/L for females), leucocytes (Le; 3.70–10.0 ×10^{9}/L), and platelets (PLT;135–450 × 10^{9}/L).

The parameters of blood coagulation (prothrombin time (PT) – reference range 11.8-15.3s and activated partial thromboplastin time (aPTT) – 25-35s) were measured by ACL TOP 350CTS (Beckman Coulter Inc. Brea, USA).

2.3.2. Determination of OS parameters

The following prooxidative markers were measured from plasma samples of all patients included in the study: index of lipid peroxidation measured as TBARS (thiobarbituric acid reactive substances), hydrogen peroxide (H_2O_2), nitrites (NO_2^-), superoxide anion radical (O_2^-), while the activity of antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), and the level of reduced glutathione (GSH) were determined from erythrocyte lysates. All OS parameters were measured spectrophotometrically (Shimadzu UV-1800 spectrophotometer, Japan, manufacturer number 00182), as described previously [12].

2.3.2.1. Determination of TBARS

The index of lipid peroxidation in the plasma samples was determined by measuring TBARS using 1% thiobarbituric acid (TBA) in 0.05M sodium hydroxide (NaOH) incubated with the sample at 100˚C for 15 minutes. Measurement was performed spectrophotometrically at 530 nm, while distilled water served as a blank probe [13].

2.3.2.2. Determination of H_2O_2

The determination of hydrogen peroxide (H_2O_2) was based on the phenol red oxidation by H_2O_2, a reaction catalysed by horseradish peroxidase. The level of H_2O_2 was then measured spectrophotometrically at 610 nm, while the distilled water served as a blank probe [14].

2.3.2.3. Determination of NO_2^-

Levels of nitrites were determined according to Green's method using a Griess reaction. NO_2^- was determined as an index of NO production with the Griess reagent [15]. 0.1 ml 3 N perchloride acid, 0.4 ml 20 mM ethylenediaminetetraacetic acid, and 0.2 ml of sample were on ice for 15 minutes and then centrifuged for 15 minutes at 6000 rpm. 220 μl K_2CO_3 was added after pouring off the supernatant. The measurement of NO_2^- was performed spectrophotometrically at 550 nm, while distilled water served as a blank probe.

2.3.2.4. Determination of O_2^-

The level of the superoxide anion radical (O_2^-) was determined after the reaction of nitroblue tetrazolium in TRIS buffer with the plasma samples. The O_2^- measurement was performed spectrophotometrically at 530 nm, while distilled water was used as a blank probe [16].

2.3.2.5. Determination of CAT

For measuring the level of CAT, 50 μl CAT buffer, 100 μl sample, and 1 ml 10 mM H_2O_2 were used. The CAT levels were determined spectrophotometrically at 360 nm and expressed in U/g Hb × 10^3 plasma. Distilled water served as a blank probe [17].

2.3.2.6. Determination of SOD

As described previously [18], the levels of SOD activity were determined by the epinephrine method. 100 μl sample and 1 ml carbonate buffer were mixed, then 100 μl epinephrine was added. The SOD level measurement was performed spectrophotometrically at 470 nm, and the SOD level was expressed in U/g Hb × 10^3 RBC.

2.3.2.7. Determination of GSH

The determination of reduced glutathione (GSH) level was based on GSH oxidation via 5,5-dithiobis-6,2-nitrobenzoic acid. To obtain the GSH extract, we combined 0.1 ml of 0.1% EDTA, 400 μl plasma, and 750 μl precipitation solution (containing 1.67 g metaphosphoric acid, 0.2 g EDTA, and 30 g NaCl) and the mixture was filled with distilled water until 100 ml. Afterwards, the vortex machine was used for mixing, extraction on cold ice was performed for 15 minutes, and the samples was centrifuged at 4000 rpm for 10 minutes. For the blank probe, we used distilled water, while measuring was performed at 420 nm [19].

2.3.3. Determination of gal-3 levels by Enzyme-linked immunosorbent assay (ELISA)

The serum gal-3 levels were measured by Enzyme-linked immunosorbent assay (ELISA). Microplates were precoated with capture polyclonal antibody (goat) to human gal-3 (Human galectin-3 ELISA solid Phase Sandwich Elisa, R&D system, Inc) and washed three times with wash buffer 1% Tween 20 (Sigma–Aldrich, St. Louis, MO) in PBS. 50 μl of samples was added to each well in duplicate, with 50 μl of sample diluent. Detection antibody diluted in Reagent Diluent was then added to each well and incubated at room temperature (RT) for 2 h on a microplate shaker set at 200 rpm. After washing and incubation period, the reaction was stopped by adding the stop solution. The absorbance of each sample was determined at 450 nm using a microtiter plate reader (UT-2100C, MRC, UK, manufacturer number 452104038IEX). A standard curve ranging from 0.156 to 10 ng/ml of gal-3 was generated for each ELISA.

2.4 Statistical analysis

The calculation of the sample size was made based on the results of a previously published study [20] in which the influence of STSG preservation on tissue integrity, cell proliferation and apoptosis as well as vascularization was monitored. The T-test for a paired sample, two-tailed, was used for the calculation, assuming an alpha error of 0.05 and a study power of 0.8 (beta error 0.2) and using the appropriate computer program.

Prior to statistical analysis, all data were checked for normality using the Kolmogorov-Smirnov test. Depending on the distribution, the data were evaluated using two-way ANOVA if data were normally distributed, and non-parametric data were analyzed using the Kruskal–Wallis followed by post hoc Mann–Whitney test. These analyses were carried out using SPSS statistical program version 22.0. The p-value below 0.05 was considered as statistically significant, while statistical p-value below 0.01 was considered as highly significant.

3. Results

3.1. General data on patients

Twenty male and twenty female patients, aged 47.80±9.89 and 49.4±8.13, respectively, were included in the study. The standard laboratory analysis parameters are presented in Table 1. We did not find statistically significant variability between male and female patients pre- and post-STSG transplantation in any analysed parameter.

3.2. OS parameters

The OS markers from patients' plasma and erythrocyte lysates are presented in Figures 1 and 2. As shown in Figure 1A female patients had significantly higher ($p<0.05$) values of O_2^- before skin grafting. In both genders, there was a significant increment in the value of this parameter ($p<0.01$) 7 days after skin transplantation compared to the values before skin grafting, while gender differences were not registered at that time point.

Figure 1B shows significant alterations in H_2O_2 levels. Levels of H_2O_2 were significantly lower ($p<0.05$) in female patients before skin grafting, and this alteration persisted 7 days after the skin transplantation procedure ($p<0.01$). A significant increase of H_2O_2 levels ($p<0.05$) after 7 days of the procedure was observed in male patients, while in female patients the values of this parameter remained unchanged.

TBARS values did not differ between genders prior and after skin grafting, as shown in Fig. 1C. After 7 days of skin transplantation, TBARS levels significantly increased ($p<0.01$) in female patients, while in males there was no registered alteration of TBARS levels at that time point.

NO_2^- levels were not altered between groups regarding gender and surgery (Figure 1D). Levels of SOD did not differ between genders prior and after skin grafting, while after 7 days of surgical procedure, the levels of SOD increased only in male gender ($p<0.01$), as shown in Figure 2 A.

There was a significant rise in the values of CAT values in female patients before skin grafting ($p<0.05$), while skin transplantation led to a significant increase of CAT levels in both genders ($p<0.01$). Moreover, the gender differences in CAT levels persisted 7 days after the surgical procedure, as shown in Figure 2B.

Figure 2C shows the alteration of GSH levels. Prior and after surgery, GSH levels were significantly higher in female patients ($p<0.05$). Skin transplantation procedure did not alter GSH values compared with values before skin grafting in both genders.

3.3. Serum gal-3 levels

Two-way ANOVA revealed that serum gal-3 levels were not affected by factors of gender and time individual-

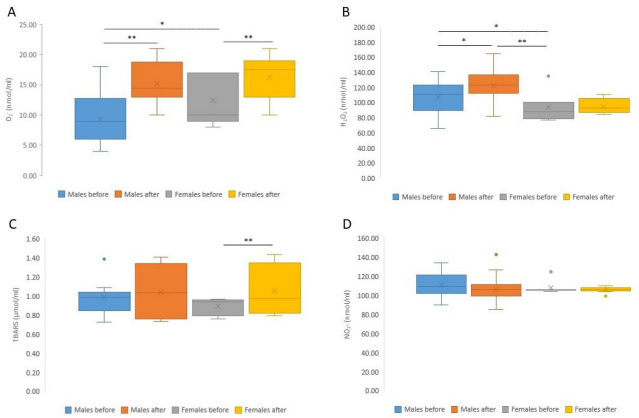

Fig. 1. Levels of prooxidative parameters: A - superoxide anion radical (O_2^-), B - hydrogen peroxide (H_2O_2), C - index of lipid peroxidation (TBARS) and D - nitrites (NO_2^-) presented by boxplots.** statistical significance at the level of $p<0.01$, * statistical significance at the level of $p<0.05$ between groups. Kruskal-Wallis test and post hoc Mann-Whitney analysis were performed.

Table 1. Comparison of patients' general data. Values are expressed as median (interquartile range) or mean±SD depending on their distribution. Two-way ANOVA or Kruskal Wallis test was performed for statistical analysis.

	Male before	Male after	Female before	Female after	Statistical significance
WBC ($\times 10^9$/L)	8.85 (3.85)	6.90 (4.83)	7.6 (4.45)	7.25 (3.60)	n.s.
RBC ($\times 10^{12}$/L)	4.33 ± 0.6	4.29 ± 0.58	4.40 ± 0.43	4.33 ± 0.46	n.s.
Hgb (g/L)	134.5 ± 17.12	131.4 ± 18.98	130.35 ± 10.29	126.6 ± 10.82	n.s.
Hct (L/L)	0.39 ± 0.06	0.39 ± 0.06	0.38 ± 0.04	0.37 ± 0.04	n.s.
Plt ($\times 10^9$/L)	254.6 ± 45.26	253.55 ± 43.47	245.3 ± 44.38	231.3 ± 43.16	n.s.
Glucose (mmol/L)	5.75 (1.83)	5.15 (1.17)	5.60 (1.63)	4.95 (0.97)	n.s.
Pt (s)	12.74 ± 1.98	12.30 ± 1.83	12.25 ± 1.80	12.03 ± 1.80	n.s.
INR (s)	1.11 (0.49)	1.10 (0.54)	1.00 (0.86)	1.02 (0.58)	n.s.
Urea (mmol/L)	4.90 (2.73)	4.60 (1.38)	4.75 (1.88)	4.25 (2.10)	n.s.
Creatinine (μmol/L)	88.65 ± 15.21	77.9 ± 19.11	80.8 ± 10.55	74.55 ± 9.24	n.s.

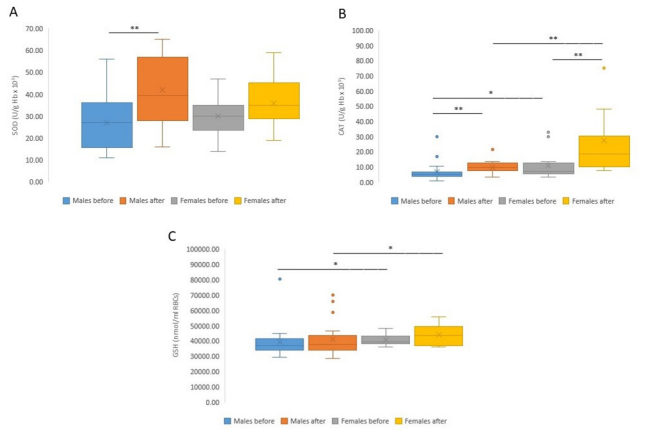

Fig. 2. Markers of antioxidative defence system. A - Activity of superoxide dismutase (SOD), B - Activity of catalase (CAT), C – concentration of reduced glutathione (GSH) presented by boxplots** Statistical significance at the level of p<0.01 between groups. * Statistical significance at the level of p<0.05 between groups. Kruskal-Wallis test and post hoc Mann-Whitney analysis were performed.

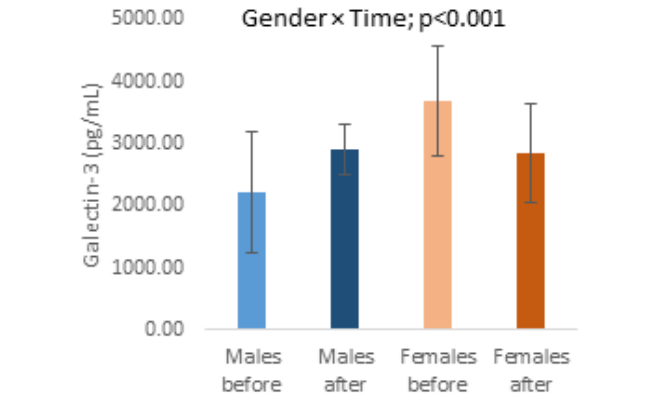

Fig. 3. Levels of gal-3. Bars represent means ± SD of gal-3 serum concentration. Two-way ANOVA was used for analysis; factor gender and factor time were evaluated.

ly, while interaction of two factors was significant regarding this parameter (p=0.000), as shown in Figure 3. A simple main effect was determined to obtain further insights. Males differ significantly by the gal-3 variable at different sampling times – before and after surgery ($F(1,19) = 6.117$; $p < .05$, $\eta 2 = .516$), 51.6% of the variance. Women also differ significantly in terms of gal-3 variables in the first and second sampling moments ($F(1,19) = 20.270$, $p < .001$; $\eta 2 = .953$), 95.3% of the variance. Men and women differ significantly in basic conditions, before the surgery ($F(1,38) = 23.465$; $p < .001$; $\eta 2 = .382$), 38.2% of the variance. Men and women do not differ significantly at the second sampling point, after the surgery ($F(1,39) = .135$; $p = .715$; $\eta 2 = .004$).

As shown in Fig. 4 A, ROC in male patients after skin-grafting surgery showed the following parameters: sensitivity was 0.695 (CI 0.531-0.859, p<0.05), specificity was 0.305 and cut-off value was 2358,75 pg/ml, while after surgery an area under the curve in male patients was lower than 0.5 (not shown). In the female patients before skin-grafting surgery (Fig. 4B) sensitivity was 0.780 (CI 0.635-0.925, p<0.01), specificity was 0.220 and cut-off value was 3038,26 pg/ml, while prior STSG surgery an area under the curve in male patients was lower than 0.5 (not shown).

3.4. Correlation between parameters of OS and gal-3

As shown in Table 2, there was no significant correlation between any parameter of OS and gal-3 in all patients. Moreover, when we observed the male group of patients, there were no significant correlations found between these parameters. On the other hand, in the female group of patients, significant negative correlation between gal-3 and O_2^- (R=-0.349; p=0.027) was registered. Importantly, we noticed a significant negative correlation between gal-3 and CAT (R=-0.420; p=0.007), and significant nega-

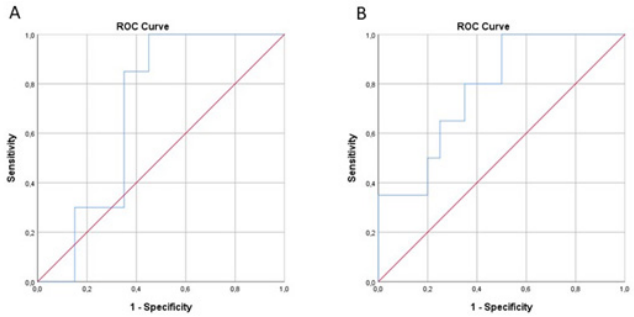

Fig. 4. Receiver operating characteristic (ROC) curve for gal-3 values in male and female patients. A - ROC in male patients after skin-grafting surgery. Sensitivity is 0.695 (CI 0.531-0.859, p<0.05). B - ROC in female patients before skin-grafting surgery. Sensitivity is 0.780 (CI 0.635-0.925, p<0.01).

Table 2. Correlation analysis on the values of OS parameters and gal-3 using Spearman's correlation coefficient.

Gal-3		
All patients		
O_2^-	R= - 0.019	p = 0.870
H_2O_2	R= - 0.209	p = 0.062
TBARS	R= - 0.018	p = 0.288
NO_2^-	R= - 0.127	p = 0.260
SOD	R= 0.070	p = 0.538
CAT	R= - 0.099	p = 0.383
GSH	R= - 0.018	p = 0.871
Male patients		
O_2^-	R= 0.105	p = 0.519
H_2O_2	R= 0.053	p = 0.745
TBARS	R= -0.258	p = 0.108
NO_2^-	R= - 0.202	p = 0.211
SOD	R= 0.240	p = 0.136
CAT	R= 0.181	p = 0.265
GSH	R= 0.167	p = 0.304
Female patients		
O_2^-	**R= - 0.349**	**p = 0.027**
H_2O_2	R= - 0.159	p = 0.328
TBARS	R= - 0.228	p = 0.158
NO_2^-	R= 0.037	p = 0.821
SOD	R= - 0.087	p = 0.592
CAT	**R= - 0.420**	**p = 0.007**
GSH	**R= - 0.482**	**p = 0.002**

tive correlation between gal-3 and GSH were observed (R=0.482; p=0.002).

3.5. Correlation between parameters of OS and gal-3 in the women group according to menopausal status

As shown in Table 3, significant negative correlation between gal-3 and O_2^- (R=-0.557; p=0.016) was registered in the premenopausal group of women, while there were no significant correlations found between any other parameters observed. Additionally, significant negative correlation between gal-3 and hydrogen peroxide (R=-0.172; p=0.005), and a significant negative correlation between gal-3 and CAT as well as GSH were observed (R=0.602; p=0.003 and R=0.567; p=0.006, respectively).

Table 3. Correlation analysis on the values of OS parameters and gal-3 in women group according to the menopausal status using Spearman's correlation coefficient.

Gal-3		
Premenopausal women		
O_2^-	**R= -0.557**	**p = 0.016**
H_2O_2	R= -0.145	p = 0.565
TBARS	R= -0.333	p = 0.177
NO_2^-	R= 0.068	p = 0.788
SOD	R= 0.065	p = 0.799
CAT	R= 0.462	p = 0.053
GSH	R= 0.416	p = 0.086
Postmenopausal women		
O_2^-	R= 0.204	p = 0.362
H_2O_2	**R= - 0.172**	**p = 0.005**
TBARS	R= 0.173	p = 0.442
NO_2^-	R= 0.105	p = 0.641
SOD	R= 0.135	p = 0.550
CAT	**R= - 0.602**	**p = 0.003**
GSH	**R= - 0.567**	**p = 0.006**

4. Discussion

Reactive oxygen species (ROS) generation is essential in some physiological processes, allowing and ensuring the cells and tissues functioning in a homeostatic milieu. However, the accumulation of ROS and/or decrease in the antioxidative defense system triggers oxidative stress (OS). OS has been shown to play a central role in the pathogenesis of many different pathologies [21]. It was previously shown that oxidative stress levels vary between genders, and hormone levels differences could be responsible for these alterations. Testosterone may have pro-oxid-ant and antioxidant [22] effects while estrogen possesses antioxidant potential [23] and can scavenge free radicals. In addition, anatomical and physiological differences between males and females could affect OS levels; women have higher proportion of body fat, which could generate ROS. It was shown that sex difference in OS is mediated not by alterations in endogenous antioxidant enzymes nor by estrogen levels differences in the plasma between men and women. However, cellular respiration in the mitochondria, which occurs in healthy conditions, is the main generator of ROS. Accordingly, a higher degree of oxidative stress in men in the current study may be caused by a higher baseline metabolic rate in males as compared to females [24]. Although the protective role of estrogen in the manner of its antioxidant potential could be responsible for those differences [25], the previously mentioned study did not reveal a significant correlation between TBARS and estradiol levels, while the activities of SOD and CAT were similar in men and women. It is important to note that the extent of gender differences in OS levels and their health implications is still an area of active research. Moreover, it is suggested that different forms of trauma itself may lead to OS occurrence in patients, as well as acute trauma animal models [26] from experimental studies. Even surgical incision could release reactive oxygen species generation and aggravate OS, often leading to occurrence of post-operative complications, pain or delay in the healing process [27]. As known, gal-3 represents a novel marker of fibrosis with described proinflammatory properties; it is enrolled in different physiological and pathophysiological processes in the organism [28, 29]. Moreover, the levels of gal-3 vary between genders [11], and relate to different pathophysiological mechanisms of diseases [30], even in critically ill patients with severe trauma and/or severe sepsis [31]. The biological interactions between OS and gal-3 in health and disease and not understood yet and the interplay of various factors makes it challenging to draw definitive conclusions. However, the focus of the present study was to examine if there is any alteration of OS markers and gal-3 in patients with hand injury before skin grafting and one week after the procedure, with an emphasis on gender differences. In addition, our aim is to determine a possible correlation between OS markers and gal-3 level in both genders. The results of the study showed that there was a significant increase in superoxide anion radical levels, as well as CAT activity and GSH levels in females before skin transplantation, compared to males. The surgical treatment resulted in a significant increment of pro-oxidants levels, such as superoxide anion radical and hydrogen peroxide, in the male gender, as well as the activity of antioxidative enzymes - SOD and CAT. In the female gender, biomarkers of OS such as superoxide anion radical and TBARS increased after surgical care and transplantation, as well as the activity of CAT. Regarding gal-3 levels, there were no significant effects of factor gender and factor time, while their interaction was significant. Levels of gal-3 inversely correlated with superoxide anion concentration, activity of CAT, and GSH levels in females, but not in males gender.

As shown in Table 1, there was no significant difference in the age of patients regarding their gender. Moreover, routine laboratory analysis performed before and after surgery revealed there was no difference in the observed group of patients, putting light on the homogeneity of the population examined in the study. The absence of any significant alteration of leukocyte number implied that there was no associated infection in the treatment course regarding the 7 days of patient monitoring. This observation highlights the significance of some other parameters, such as OS markers or gal-3, as investigated in our study, in better understanding the mechanisms and processes associated with clinical complications after different surgical procedures. As noted in Table 1, there was no difference in erythrocyte count number, and values of haematocrit and haemoglobin between groups, which mainly suggests that patients did not lose any significant volume of blood at the time of injury and after hospital care. According to the values of coagulation parameters, all investigated groups of

patients expressed homogeneity as well. Kidney function in all patients, assessed by urea and creatinine levels in serum, did not differ between groups also. Considering all previously mentioned observations, with the employment of strictly applied exclusion criteria of the study, we got a clearer insight in gender difference-related alterations of the observed biochemical parameters, mainly excluding confounding factors affected by potential comorbidities.

It was described that females are less susceptible to OS [32], which may be related to the protective role of oestrogen and its antioxidative properties. However, in our study, female patients had greater levels of superoxide anion radical and lower hydrogen peroxide levels compared to male patients prior to surgery. Moreover, female gender expressed the greater antioxidant potential [33], as shown in our study, by significantly greater values of CAT and GSH in female patients before and after the skin grafting procedure. Higher antioxidative potential in females is shown mostly in premenopausal women, probably due to the protective effects of oestrogen, or differences in NADPH-oxidase mechanisms between males and females [32]. Furthermore, it is interesting to note that mitochondria derived from female rats exhibit several post-translational modifications in mitochondrial enzymes that play a crucial role in modulation of ROS and oxidative metabolism. As a result of these modifications, females demonstrate a diminished level of ROS generation upon reoxygenation [34].

Superoxide anion and hydrogen peroxide are the two primary products of leakage of the respiratory transport chain in the process of oxidative phosphorylation in mitochondria. In physiological conditions, the cells have the ability to combat against alarming increase in the levels of these molecules by two antioxidant enzymes, SOD and CAT. One of the principal ROS created by numerous enzymatic and non-enzymatic processes that utilize oxygen molecules as an electron acceptor is superoxide, so it is responsible for other ROS generation [35]. Even though it does not induce severe damage itself, if not removed on time and properly, superoxide could lead to oxidative tissue injury and cell death. On the other hand, superoxide could be transformed into hydrogen peroxide by the enzymatic action of SOD. Hydrogen peroxide is more stable and less toxic, contrary to its further product – hydroxyl radical, which is extremely toxic, but with a very short life span. Moreover, hydrogen peroxide has a longer life span, and thereby it could damage the cell of origin or even act in intracellular communication. Moreover, due to its electroneutral nature, hydrogen peroxide is virtually not restricted to the local environment and thus reaches more sites than superoxide to express its toxicity [36]. However, the lack of studies investigating specific molecules such as superoxide anion radical and hydrogen peroxide led us to explore their sex differences, prior and after skin transplantation surgery. The results of our research could be compared to the study of Lacy and coworkers [37], who demonstrated higher levels of plasma hydrogen peroxide production in men compared to women, as we registered in preoperative, as well as in postoperative conditions (Fig. 1B). In accordance with our results, literature data showed that men had two times greater production of hydrogen peroxide by mitochondria compared to women [38]. Regarding superoxide anion radical, studies in rats demonstrated gender differences in its generation by the aorta endothelium [39], with greater production in male rats as compared to females, contrary to our results. The species differences and different origins of this pro-oxidant molecule in the evaluated samples could be responsible for the registered contradictory results. The existence of gender differences in the activities of superoxide dismutase, catalase, and glutathione peroxidase in human samples was demonstrated [40], with the specific observation that age did not influence CAT activity, despite interindividual variability. These results correspond to significantly higher CAT levels in female patients in our study. Because oestrogen chemical structure posed a phenolic hydroxyl group, it is capable of being a scavenger for the free radicals and act as an antioxidant [41], and women showed greater antioxidant activity, as in our study. GSH levels were also higher in females before skin grafting, as well as CAT activity, supporting the fact that females posed greater antioxidant potential.

After 7 days of skin transplantation for hand injury, superoxide anion and hydrogen peroxide concentration, as well as their counteracting SOD and CAT as antioxidative enzymes, increased in male gender. In females, 7 days after the skin-grafting procedure superoxide anion and TBARS levels as well as CAT activity significantly increased. The significant decrease of hydrogen peroxide in female patients compared to males after surgery could be observed by significantly higher CAT levels in the same group (Figure 2B). Such an increase in CAT activity (by 140%) led to the transformation of hydrogen peroxide into water and thereby decreased its concentration in the plasma. Considering the higher CAT activity in the 7th postoperative day in females compared to males, it could be explained by the higher hydrogen peroxide levels in the male group, contrary to female patients. In the female patients, the increased CAT activity was responsible for the mirror-like decrement of the hydrogen peroxide levels. It is important to mention an increase in SOD levels postoperatively in male gender, as well as superoxide concentrations. What is the main trigger in this relationship remains to be elucidated in further studies. There are exciting questions and need to be addressed in rationally designed clinical trials. On the other hand, gender differences in postoperative conditions regarding antioxidant capacity remained the same as in preoperative (increased in females), except for SOD (unaltered). The explanation for such diminished SOD alteration could be found in its depletion in order to eliminate the highest concentration of superoxide in the post-surgery groups.

TBARS levels did not differ between genders before skin grafting, although there was non-significant increase in male patients compared to females observed. Our results are in accordance with a previous large-scale study investigating TBARS levels in the general population [42]. Study of Ide et al. demonstrated greater levels of TBARS in healthy young males compared to premenopausal women [24]. While our patients were older than in the mentioned study, and with traumatic hand injury, the results could not be compared with healthy patients; the protective role of oestrogen in women can be assumed. However, the index of lipid peroxidation significantly increased in women 7 days after the skin transplantation procedure. It was shown that the type of surgical technique used for the treatment of the same pathologies could alter TBARS levels in patients [43]. Regardless of the technique, surgical treatment al-

ways results in tissue injury and the organism's subsequent response by increasing OS [44]. Mild traumatic head injury related to an increase of lipid peroxidation during the 7 days of post-injured period [45, 46]. Selective localized injury results in oxidative damage in the distant parts of the body [47], which could be considered as the factor responsible for recovery and potential complications. In the study of Olszewska-Słonina, MDA (as a marker of lipid peroxidation) was evaluated in patients of both genders with osteoarthritis who underwent surgical treatment for endoprosthesis implantation [48]. As the authors noted, MDA levels remained increased only in the elderly group (age>70), while in younger patients MDA levels decreased to control levels 10 days after surgery. These alterations were significant in male but not in female gender.

GSH is consumed by OS to regenerate vitamin E and ascorbic acid and protects the cell from damage, as well as by direct ROS scavenging activities. Its own oxidation, as well as the formation of adducts with electrophilic compounds, serve as mediation for OS in the cell, so the decrease in GSH strongly implies its depletion by antioxidative actions. Our results showed a significant increase in GSH levels in female patients, prior to skin transplantation as well as 7 days after the procedure. Our results are in concordance with the results of the study in the general population regarding GSH level deference between genders [42]. As known, sex-specific differences and prompt response indicated by higher values of GSH and an increase after 7 days in female gender could serve as an explanation of the obtained results.

Baseline gal-3 levels in this study were not affected by gender. Moreover, factor time was also not significant regarding alteration of gal-3 levels. However, it was registered significant interaction between two observed factors, which put into light importance of present study. The gender differences regarding the level of this marker in human blood were described in earlier studies as higher values were found in female population [11]. Increase in the level of gal-3 in female gender could be related to its modulation by sex hormones. However, it remains unclear how these regulatory mechanisms functioned. At least, it was observed reduced gal-3 expression in the eutopic endometrium of endometriosis, potentially explaining impaired receptive endometrial formation, while hormones were identified as the primary regulators of gal-3 in endometrial epithelial cells. In addition the same authors showed that gal-3 expression is specifically increased during the secretory phase of the menstrual cycle, indicating that gal-3 may be regulated by sex hormones [49]. Many studies have shown that gal-3 expression is seen in a wide range of disease states, including the development of tumours, autoimmune disease, cardiac disease, kidney disease, diabetes mellitus, viral infection, and neurodegenerative disorders. Gal-3 is not a disease-specific marker by itself because its levels are affected by a variety of clinical parameters depending on the underlying pathological conditions in patients. However, gal-3 is a promising biomarker mainly in context of early stages of different diseases [50]. Hand injury in patients who underwent STSG transplantation in our study altered serum gal-3 levels. Our results further support that galectin-3 is potentially sex-specific marker with regard to at least first seven days post-surgery in our investigation. As a novel, multifaceted marker of various diseases, gal-3 represents an extraordinary parameter and a step closer to better insight into pathophysiological mechanism of mechanical hand injury and surgical procedures providing better healing process in this type of study.

Correlation analysis of different OS markers with gal-3 revealed the existence of significant negative correlation of O_2^-, CAT, and GSH with gal-3 (R=0.349, 0.420, 0.482, respectively) but only in female patients. In the sense of registered relationships, it could be underlined that the evaluation of gal-3 undoubtedly has significance in this patient population. It was previously shown that OS could increase expression of gal-3 by activation pathways that upregulate gal-3 expression. On the other hand, gal-3 contributes to OS by promoting generation of ROS, activating NADPH oxidase [51]. Moreover, overexpression of gal-3 reduced SOD and CAT in the Spinal cord injury rat model by activating NLRP3 signaling pathway [52]. Regarding antioxidant defense system, the correlation between gal-3 and CAT as well as GSH, which was present in the women group, was registered in the postmenopausal women group also. However, such a correlation was not persisted in the premenopausal group of women. It could be interpreted as the higher estrogen levels in the premenopausal period exert as antioxidant protection and the profound fall of estrogen, occurring in menopause may be responsible for the observed difference. Regarding pro-oxidant molecules, superoxide anion radical was inversely correlated to gal-3 levels in premenopausal group of women, as seen in the women group. However, such a correlation disappeared in the postmenopausal group and we registered only correlation between hydrogen peroxide and gal-3. It was known that estrogen could increase expression of Gpx, an enzyme that degrades hydrogen peroxide [53], and that it is capable of counteracting hydrogen peroxide toxicity [54]. The onset of menopause induces notable alterations in antioxidant gene expression, impacting the overall redox state. Hormone replacement therapy with estrogens effectively mitigates and prevents these changes by regulating key antioxidant gene expression. It was known that sex hormones play a significant role in controlling antioxidant genes, potentially influencing the gender-based variations in disease pathophysiology through redox biology [55]. However, the mechanism underlying the existence of negative correlation with gal-3 in postmenopausal women, and with superoxide anion in premenopausal women remained to be elucidated in further studies.

So far, no other authors have compared OS parameters and gal-3 levels between male and female patients, before and after skin grafting in hand injuries. To the best of our knowledge, this is the first study exploring the OS markers and gal-3 values in both genders of patients undergoing STSG surgery. Nevertheless, the future analyses are needed in order to gather cellular and molecular evidence.. However, the significant correlations were registered between gal-3 levels and superoxide anion radical levels, as well as CAT and GSH in the female patients. It seems to be casual correlation, although gal-3 posed the ability to produce an oxidant environment [56] that can affect mitochondrial activity. SOD is the primary enzyme responsible for decreasing O_2^- concentrations in biological systems. However, SOD activity was not significantly correlated to O_2^- levels. Apart from SOD, other enzymes indirectly contribute to the reduction of O_2^- levels. For example, CAT converts H_2O_2 into water and oxygen, while gluta-

thione peroxidase uses glutathione as a cofactor to convert H_2O_2 and organic hydroperoxides into their corresponding alcohols. CAT activity correlates significantly to gal-3, which may give clues about the involvement of different signaling pathways for amelioration of oxidative stress by decreasing O_2^- concentrations. Based on the exisiting evidence, relevant to mention that these enzymes play a crucial role in reducing O_2^- levels, they also act in coordination with other antioxidant systems in cells to maintain redox balance and prevent oxidative-stress detrimental effects.. Further studies are needed to explore specific network between oxidative stress biomarkers and gal-3 in these conditions.

The ROC curve analysis conducted in this study aimed to evaluate the diagnostic utility of gal-3 in male and female patients undergoing skin-grafting surgery. The results indicate that, at a cutoff value of ≥ 2358.75 pg/ml for male patients, gal-3 demonstrated good predictive capability with an area under the curve (AUC) of 0.7 and a highly significant p-value ($p<0.05$). However, it's noteworthy that post-surgery, the AUC in male patients dropped below 0.5, suggesting a potential change in predictive accuracy. In contrast, for female patients, at a cutoff value of ≥ 3035.26 pg/ml, gal-3 exhibited good predictive performance with an AUC of 0.8 and a highly significant p-value ($p<0.01$). Interestingly, similar to the male group, the AUC for female patients was lower than 0.5 before surgery. Intriguingly, when considering the entire patient group (both genders), the AUC was also lower than 0.5, indicating a limited overall predictive capacity. These findings underscore the gender-specific variations in gal-3 as a predictive biomarker, emphasizing the importance of gender-stratified analyses in the context of skin-grafting surgery.

The relatively small population analysed and the inclusion of patients from a single clinic is the limitations of the current work. Based on their significant variability, the study groups' wide age range may partially account for the study limitation. The results may be affected by certain dietary practices, such as extensive vitamin/mineral supplementation (before to a traumatic incident). Notwithstanding these drawbacks, our results show the complexity of factors influencing circulating pro-oxidant factors, antioxidant status and gal-3 levels in the patients with traumatic hand injury and STSG coverage procedure. The connection between these parameters and gender as well as the time course after STSG defect coverage highlight the need for additional research. Our key finding is that any research done on the antioxidant capacity of trauma patients should consider both the site and timing of the trauma. These findings may also be helpful in defining the standards for the inclusion of antioxidant supplements. However, when discussing diseases for antioxidant therapy, it's crucial to distinguish between those where oxidative damage is a central mechanism in their pathogenesis and those where oxidative damage occurs as a later consequence of primary mechanisms. This differentiation enables the identification of diseases with a higher likelihood of success in antioxidant therapy [57].

5. Conclusion

It can be concluded that OS as well as gal-3 play important roles at least in the first 7 days of STSG postoperative period. In both genders, the release of pro-oxidants and mobilization of anti-oxidants were higher after skin transplantation. In addition, concerning that gal-3 correlated with some of the OS parameters in females, it seems that females are more prone to these biochemical changes that could interfere with transplantation success. Future research addressing the importance of OS in the prognosis of hand injury and STSG transplantation needs to be carried out and the design of a therapeutic antioxidant approach to improve the recovery in patients remains reasonable.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki (revision 2013), the principals principles of Good clinical practice and regulations, and it was approved by the Ethical Committee of University Clinical Center Kragujevac (No.01/18/1992).

Informed consent

Before all study procedures, all patients voluntarily gave their written informed consent for participation in the study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Conceptualization, D.V. and V.J.; methodology, V.Z, J.J.J. .; validation, M.V., S.B., V.P.F.; formal analysis, M.A, N.L., N.A, A.D.M; investigation, K.A., V.Z., J.J.J; writing—original draft preparation, K.A.; writing—review and editing, M.A, N.L., N.A, A.D.M; supervision, M.V., S.B., V.P.F.; project administration, V.J., D.V. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia (Junior Project No 12/18).

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