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PHD THESIS

ABSTRACT

**MARKERS OF POSTOPERATIVE STRESS
ASSESSMENT**

**SCIENTIFIC COORDINATOR
PROF. UNIV. DR. FLOREA PURCARU**

**PHD STUDENT
STĂNCULESCU ANDREEA DORIANA
(PRELIPCEAN)**

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Key words: malign diseases, laparoscopic surgery, stress oxidative markers, anesthetics drugs, postoperative delirium.

GENERAL DATA. THE STAGE OF KNOWLEDGE

I.1. Malign urological surgical diseases

I.1.1. Renal Cell Carcinoma

The first time renal tumors were described in 1826, with the name renal adenocarcinoma or renal cell carcinoma (RCC) being adopted. [1]

Renal carcinomas represent 2-3% of human cancers. [2]. Incidence has risen in the past two decades by about 2%. [3]. Men predominate in a ratio of 1.5: 1, with a higher incidence in the age group of 60-70 years.

I.2. Malign gynecological surgical diseases

I.2.1. Endometrial Cancer

Endometrial cancer is one of the most common types of genital cancers in developing countries, with the predominance of endometrial adenocarcinoma, and the incidence is steadily increasing. [4]

I.2.2. Ovarian Cancer

Ovarian cancer is the most common cause of death in all gynecological cancers in the United States.

I.2.3. Cervix Cancer

The incidence is increased in women with sexual life because of the mechanical irritation caused by sexual intercourse, as well as by the action of sperm on the cervix. [5]

Laparoscopic surgery is performed with great success in these urological or gynecological malignancies [6,7]

I.3. Oxidative stress

I.3.1. Definition

Oxidative stress is a physiological phenomenon that occurs throughout the body. Under normal conditions of good functioning of the body, free oxygen radicals (intracellular reactive oxygen species - ROS) are maintained at a low level by a varied system of enzymes involved in redox homeostasis in vivo. Therefore, oxidative stress should be seen as an imbalance between pro-oxidant and antioxidant factors.

I.3.2. Oxidative stress

The deleterious effect of oxygen free radicals and RNS that can cause biological damage is oxidative stress and nitrosative stress [8-10]. This is evident at the level of the biological systems where there is excessive production of ROS / RNS and / or an enzymatic or non-enzymatic antioxidant deficiency. Redox stress / oxidative stress is a complex process. Its impact on the body depends on the type of oxidant, the site and intensity of its production, the composition and activity of different antioxidants, and the ability of repair systems [11].

I.3.3. Oxidative stress evaluation

The concentration of various reductive-oxidant markers is considered an important parameter in assessing pro-oxidant status of body tissues [12]. There are numerous indicators of redox status in vivo, including the GSH ratio in GSSG, NADPH, NADH and the ratio of reduced to oxidized thioredoxin. Of these redox pairs, the GSH: GSSG ratio is considered to be one of the most abundant redox buffer systems in mammals [11]. A decrease in this ratio indicates a relative shift from the reduced form to the oxidized form of GSH, suggesting the presence of cellular or tissue oxidative stress.

I.4. Anesthesia in laparoscopic surgery

General anesthesia is not limited to the use of inhalation agents, even if general anesthesia can be maintained with a total intravenous technique. Thus, anesthesia may be complete intravenous anesthesia (TIVA) or general inhaled anesthesia [13]

I.5. Postoperative delirium

Delirium is the absolute and total conviction of a person who cannot be contradicted by clear, logical ideas and does not succumb to his own ideas contrary to arguments and evidence. It is an extremely common mental disorder in elderly hospitalized patients (over 60 years of age) and is the most common postoperative complication in elderly patients and in patients undergoing major surgical interventions. [14]

PERSONAL CONTRIBUTIONS

II.1. Aim and objectives

The present study comprises two studies.

In the first study, we aimed to evaluate oxidative stress markers in cancer patients undergoing surgery laparoscopic large scale in conjunction with anesthetic substances used to demonstrate the effectiveness of certain drugs used during surgery to reduce as much as possible oxidative stress.

The aim is assessing the effects of sevoflurane or propofol on oxidative stress markers, malondialdehyde as oxidant factor and the factors antioxidant, superoxide dismutase and glutathione peroxidase in patients with urological and gynecological malignancies undergoing laparoscopic surgery.

In the second study, we aimed to evaluate oxidative stress markers in patients diagnosed in the early stages with different renal malignancies undergoing surgery or gynecological laparoscopic large scale in correlation with postoperative delirium appearance.

The aim is to identify an indicator of oxidative stress to predict the appearance of postoperative delirium in elderly patients with urological and gynecological malignancies undergoing laparoscopic surgery and the possible correlation between anesthetic drugs and the emergence of postoperative delirium.

II.2. Materials and Method

The present study was conducted in the Urology and Gynecology Clinics of the County Emergency Clinical Hospital Craiova during 2014-2016. All 91 patients hospitalized during this period were studied in the two Surgical Clinics with malignant urological and gynecological pathologies. Of the 91 patients diagnosed with malignant pathologies, only 54 were proposed for radical surgery by laparoscopic technique.

The final study included 44 patients with urological or gynecologic malign pathology admitted to the Urology and Gynecology Clinic of the Craiova County Emergency Clinical Hospital. 10 patients were excluded for various reasons: they did not meet all the criteria for inclusion, the laparoscopic surgery had classically converted, metastases were found or they were discharged at the request of the family prior to surgery.

Patients were included in the study according to inclusion criteria:

- over 18 years of age,
- anesthetic risk I-III AAS,
- renal or gynecologic malign pathologies diagnosed at an early stage of the disease,
- undergoing elective laparoscopic renal or gynecological surgery.

The exclusion criteria for patients were as follows:

- history of allergy to propofol,
- history of allergy to sevoflurane,
- severe other diseases such as severe heart disease, chronic obstructive pulmonary disease, severe liver or kidney disease.

In the second study, in addition to the inclusion criteria mentioned, two more criteria were introduced: the age over 65 and no pre-existing cognitive disturbance. Thus, out of the 44 patients, 29 patients enrolled in the second study met all the inclusion criteria.

The patients included in the first study were randomized into two groups: the Sevoflurane (S) group, which included 21 patients and the Propofol (P) group, which included 23 patients.

In the second study, patients were randomized into two groups: the Sevoflurane (S) group, which included 14 patients and the Propofol (P) group, which included 15 patients.

All patients received general anesthesia with tracheal intubation, either inhaled or total intravenous.

Blood samples were collected from a preoperative peripheral vein prior to anesthetic induction immediately after the end of the operation and 24 hours postoperatively for plasma levels of malondialdehyde, superoxide dismutase and glutathione peroxidase.

For evaluation of postoperative patients in order to establish the diagnosis of delirium, the ICDSC algorithm was used.

Methods of data analysis

The statistical analysis was performed for the parameters of the patients in the two groups.

Clinical and biological data were recorded in a database using the MS Excel platform, which helped to perform primary data analysis, common descriptive statistics, calculation of confidence intervals, and other baseline indicators (mean, standard deviations, etc.).

Statistical processing was performed using the MedCalc, SPSS software packages that implement specific statistical analysis algorithms needed for the study, comparison tests, correlation analysis, etc. Comparison of the mean values of the studied parameters was performed using parametric comparison tests (t-Student). The sensitivity and specificity of these parameters were evaluated for the evaluation of oxidative stress in the studied patient groups.

II.3. Results

Regarding serum markers of oxidative stress in the first study we studied patients included malondialdehyde as marker oxidant, and superoxide dismutase and glutathione peroxidase as markers antioxidants compared to patients who have undergone inhalational anesthesia with sevoflurane and those who When propofol was administered for total intravenous anesthesia.

The values of serum malondialdehyde in patients who have received volatile anesthetic immediately after surgery was significantly increased ($7.17 \pm 1.17 \mu\text{mol} / \text{L}$) compared to preoperative values obtained before induction, $3.13 \pm 0.89 \mu\text{mol} / \text{l}$. They were maintained and grown at 24 hours after the operation, gradually decreasing but not significantly, $5.72 \pm 1.36 \mu\text{mol} / \text{l}$. The patients in the Propofol, malondialdehyde preoperative values prior to induction of anesthesia were $3.02 \pm 0.78 \mu\text{mol} / \text{l}$ increased significantly immediately after surgery $6.31 \pm 1.07 \mu\text{mol} / \text{l}$ and reaching 24 Hours postoperatively at $4.85 \pm 0.98 \mu\text{mol} / \text{l}$. From the statistical analysis, there are no statistically significant differences between MDA values obtained preoperatively in patients in the two groups studied. However there are significant differences between the values obtained immediately postoperative serum MDA in patients in the sevoflurane compared to propofol group. Similar results are obtained in the case of values obtained 24 hours after the end of the surgery ($p < 0.05$). Our results indicate that patients with

different malignancies renal and gynecological undergoing laparoscopic surgery were markers of increased oxidative stress during surgery, namely malondialdehyde serum is exposed to as postoperative increased oxidative stress, and patients who have undergone anesthesia intravenous propofol Are less exposed to the effects of oxidative stress compared to patients who have been inhaled with sevoflurane.

Regarding the results of superoxide dismutase in patients in the Sevoflurane group, its values measured preoperatively prior to induction of anesthetic induction are 2052.14 ± 239.56 U / g Hb. These values decreased significantly in this category of patients immediately postoperatively (1776.57 ± 177.80 U / g Hb), increasing subsequently but insignificant at 24 hours postoperatively, 1858.19 ± 171.72 U / g Hb. Preoperatively determined superoxide dismutase values in patients in the Propofol group were similar to those in the Sevoflurane group measured at the same perioperative time, 2040.43 ± 226.96 U / g Hb, then these values decreased less immediately postoperatively compared to patients in the Group S (1893.83 ± 195.63 U / g Hb), increasing to 24 hours postoperatively, 1980.91 ± 193.97 U / g Hb. Based on the statistical analysis of the results obtained with superoxide dismutase, it was found that there were no statistically significant differences between the two preoperative groups. However, the values obtained immediately after surgery in patients in group S decreased significantly compared to the values determined in patients in group P, the difference being important also after 24 hours postoperatively. ($P < 0.05$) The results obtained in our study indicate that patients operated for various renal or gynecological malignancies and who received total intravenous propofol anesthesia had higher antioxidant markers compared to patients who received inhaled sevoflurane anesthesia. This once again indicates the antioxidant protective role of propofol.

Regarding the results of glutathione peroxidase in patients in the group receiving inhaled anesthesia with sevoflurane at the three set-up times, the values were as follows: Preoperatively before the anesthetic induction 5300.05 ± 1698.33 U / l, then the values Significantly decreased immediately after surgery (3560.10 ± 531.07 U / l), then increased insignificantly 24 hours postoperatively, reaching 3960.10 ± 465.52 U / l. In the group of patients undergoing total intravenous propofol anesthesia, the glutathione peroxidase values were different compared to the Sevoflurane group. Thus, the glutathione peroxidase values obtained preoperatively at harvest prior to anesthetic induction were 5386.61 ± 1698.33 U / l and at the end of the surgical procedure significantly decreased, 4229.52 ± 1307.49 U / l, subsequently at 24 Of postoperative hours to significantly increase, 4460.96 ± 1018.62 U / l. Following statistical analysis of serum glutathione peroxidase values obtained at the three times studied, it was demonstrated that the preoperative values determined in patients in the Sevoflurane group were not significantly different compared to patients in the Propofol group. Instead, GPX values obtained immediately after surgery in patients in the Sevoflurane group were significantly reduced compared to those obtained in patients in the Propofol group. ($P < 0.05$, t-Student test) Similar results were obtained at 24 hours postoperatively, when glutathione peroxidase values increased significantly in patients in the Propofol group compared to patients in the Sevoflurane group. ($P < 0.05$, t-Student test) The results obtained in the current study indicate that, following total intravenous propofol anesthesia, patients are less subject to the perioperative oxidative stress effect in patients at risk compared to patients receiving inhaled anesthesia with Sevoflurane, which demonstrates that propofol has antioxidant properties, as other studies that have investigated this hypothesis have shown.

Results of Oxidative Stress Investigation in the second study in patients diagnosed with various kidney malignancies, renal or gynecological tumors, cervical cancer, uterine cancer or ovarian cancer and undergoing laparoscopic surgery were the analysis of serum malondialdehyde, serum superoxide dismutase and serum glutathione peroxidase, values obtained at various during surgery moments compared with the occurrence of postoperative delirium.

Serum malondialdehyde (MDA) values in patients in the Sevoflurane group were significantly increased in both patients who developed delusion and those who were not diagnosed with postoperative delirium ($7.3 \pm 0.8 \mu\text{mol} / \text{l}$ and $8, 1 \pm 0.8 \mu\text{mol} / \text{l}$) compared to preoperative values obtained prior to anesthetic induction ($3.3 \pm 1.0 \mu\text{mol} / \text{l}$ and $3.0 \pm 0.6 \mu\text{mol} / \text{l}$) respectively. The values of this oxidative marker decreased gradually but insignificantly 24 hours postoperatively in both groups, the values reaching $6.3 \pm 1.1 \mu\text{mol} / \text{L}$ and $6.0 \pm 0.5 \mu\text{mol} / \text{l}$ respectively.

In the Propofol group, malondialdehyde values in the non-preoperative non-delirium group were $3.2 \pm 0.6 \mu\text{mol} / \text{l}$, increasing to $5.9 \pm 0.7 \mu\text{mol} / \text{l}$ immediately at the end of surgery, then dropping significantly to $4, 5 \pm 0.5 \mu\text{mol} / \text{L}$ 24 hours postoperatively. In the Propofol group, serum malondialdehyde values in patients who developed delirium were preoperatively $3.4 \pm 0.5 \mu\text{mol} / \text{l}$, increasing to $6.8 \pm 0.6 \mu\text{mol} / \text{l}$ immediately postoperatively, then declining significantly to 24 Hours postoperatively reaching the following values of $5.7 \pm 0.8 \mu\text{mol} / \text{l}$. From the results, no statistically significant differences are observed between the mean MDA values in patients in the two groups, Sevoflurane and Propofol, at the first oxidative biomarker collection time, namely preoperative prior to anesthetic induction. A significant increase in malondialdehyde values in the Sevoflurane group compared to the post-operative Propofol group ($p < 0.01$, t-Student test) and 24 hours after the end of surgery ($p < 0.01$, t-Student test) was obtained.

It was found that there are no statistically significant differences between malondialdehyde values obtained preoperatively from patients who developed delusion and those who did not develop from the Sevoflurane group. The results are similar to those obtained in patients in the Propofol group. ($P = \text{ns}$, t-Student test) Instead, significantly different malondialdehyde values were obtained in patients in the delirium group compared to those in the non-delirium group in both groups, Sevoflurane and Propofol, immediately postoperatively. ($P < 0.05$, t-Student test) The serum malondialdehyde values obtained 24 hours postoperatively were significantly elevated in patients in the Propofol group who developed delirium compared to those who did not develop this postoperative syndrome. ($P < 0.01$, t-Student test)

Superoxide dismutase (SOD) values in patients in the non-delirium group included in the Sevoflurane group, measured preoperatively prior to anesthetic induction, were $2242.1 \pm 94.7 \text{ U} / \text{g Hb}$. The values of this marker measured preoperatively in patients who developed delirium were lower, $2012.7 \pm 97.5 \text{ U} / \text{g Hb}$. Subsequently, superoxide dismutase values decreased in both groups, delirium and non-delirium, immediately after the surgery, reaching $1846.4 \pm 64.7 \text{ U} / \text{g Hb}$ and $1776.5 \pm 60.3 \text{ U} / \text{g Hb}$ respectively, then increasing At 24 hours postoperatively $2039.4 \pm 106.3 \text{ U} / \text{g Hb}$, respectively $2062.8 \pm 72.9 \text{ U} / \text{g Hb}$. Preoperative values of superoxide dismutase in patients who received total intravenous anesthesia with propofol that did not develop delirium were $2170.8 \pm 98.4 \text{ U} / \text{g Hb}$. Its SOD levels were significantly lower in the delirium group of the Propofol group, measured at the same time interval, $2004.0 \pm 130.0 \text{ U} / \text{g Hb}$. The values of this biomarker decreased slightly immediately postoperatively reaching values of $2064.4 \pm 56.9 \text{ U} / \text{g Hb}$, respectively $1932.0 \pm 103.1 \text{ U} / \text{g Hb}$. The serum superoxide

dismutase values increased 24 hours postoperatively in groups, delirium and non-delirium, with values of 2064.6 ± 71.7 U / g Hb, respectively of 2108.8 ± 79.5 U / g Hb.

The serum glutathione peroxidase (GPX) values preoperatively measured in the two delirium and non-delirium patient groups in the Sevoflurane group were 4682.0 ± 561.7 U / l and 5246.8 ± 1382.8 U / l respectively. The values of this biomarker immediately decreased postoperatively in both Sevoflurane patient groups reaching the following values: 3236.9 ± 188.5 U / l and 3517.5 ± 216.9 U / l respectively. The decrease in the values obtained between the two categories of patients is statistically significant. ($P < 0.05$, t-Student test)

Glutathione peroxidase values showed an insignificant increase 24 hours postoperatively in both delusional and delusional patients in the Sevoflurane group, such as 3871.6 ± 164.5 U / l and 3741.3 ± 166.4 U / l. The serum glutathione peroxidase values obtained preoperatively prior to anesthetic induction were as follows in the Propofol group for patients diagnosed with delirium 6031.0 ± 2718.7 U / l and those with non-delirium 4783.5 ± 1304.0 U / l. The biomarker values immediately decreased postoperatively in both patients, with or without delirium, in patients in the Propofol group, such as 3637.4 ± 241.5 U / l and 3326.7 ± 219.2 U / l, respectively. These decreases are statistically significant in both categories of patients. ($P < 0.05$, t-Student test) At 24 hours postoperatively, glutathione peroxidase values increased insignificantly, such as 3997.0 ± 254.9 U / l for patients with delirium and 3860.1 ± 160.6 U / l for patients without delirium in the same patient group (P) who underwent total intravenous general anesthesia with propofol.

Correlations between the markers of oxidative stress and the anesthetics used, as well as the occurrence of postoperative delirium, have also been performed.

II.4. Conclusions

1. Most of the patients with malignant pathology included in the study were female (60%), with an average age of 64 years old.
2. Renal carcinoma was the most frequently malign disease with 50% of cases, followed by cervical cancer (20.5%), uterine cancer (15.9%), and ovarian cancer with an incidence of 13.6%.
3. Patients enrolled in the study had an anesthetic risk between I-III ASA.
4. The mean duration of anesthesia in the Sevoflurane group of 190 minutes was significantly higher than in the Propofol group (180 minutes) probably due to the faster anesthetic wake up of Propofol. The mean duration of surgery did not differ significantly between the two batches.
5. The incidence of both intra and postoperative complications, both major and minor, was equally sensitive in the studied patient groups.
6. Although preoperative serum malondialdehyde (MDA) markers were similar in the two groups, patients who received total intravenous anesthesia with Propofol showed significantly lower increases in MDA compared to those in the Sevoflurane group.
7. Preoperative values of superoxide dismutase (SOD) and glutathione peroxidase (GPX), antioxidant markers, were similar in the two groups of patients. However, we found that these significantly lower values for patients in the Sevoflurane group both immediately postoperatively and 24 hours after surgery, confirming the antioxidant properties of Propofol.
8. By analyzing the correlations between the oxidative stress markers and the duration of the hospitalization, we noticed significant correlations with the postoperative SOD values (negative correlation, $r = -0.497$) and MDA (positive correlation, $r = 0.382$) confirming the influence of oxidative stress on postoperative recovery of patients.

9. The incidence of delirium in patients over 65 years of age was 38% (11 cases with delirium / 29 patients). In the Sevoflurane group, delirium occurred in 6/14 patients (42%) and in the Propofol group developed 5/15 (33%) patients.
10. In patients who developed delirium, the duration of anesthesia was significantly higher because patients woke up more severely from anesthesia, possibly influencing the occurrence of delirium.
11. During surgery end tidal CO₂ (EtCO₂) values were higher in delirium patients in both study groups, Sevoflurane and Propofol indicating that increased CO₂ during surgery increased the likelihood of delirium increasing the risk of this postoperative syndrome.
12. The hospitalization period was significantly higher for patients who developed delirium compared to those who did not develop this postoperative syndrome in both patient groups.
13. In patients in the delusion study, we noted a marked increase in MDA immediately postoperatively irrespective of the anesthetic substance used followed by a decrease in this markedly more marked marker for patients who received total intravenous anesthesia with Propofol. This is confirmed by the ROC analysis that has shown that patients with an MDA value above 6.7 $\mu\text{mol} / \text{l}$ are associated with a significant risk of post-operative delirium (81% sensitivity, 67% specificity).
14. The preoperative values of the SOD antioxidant marker in these patients were significantly lower in patients who subsequently developed delirium without being related to the type of anesthesia performed. In this case, the ROC analysis identified a sensitivity of 91% and a specificity of 83% for an SOD value below 2084 U / g Hb. Thus, we can state that preoperative SOD dosing may be a marker of the risk of post-operative delirium in elderly patients.
15. The results of post-operative SOD dosing were similar, with significantly lower values in patients who developed delirium, but with lower sensitivity and specificity (82% and 72% for SOD below 1857 U / g Hb). There was no significant difference in postoperative surgery.
16. Preoperative dosing GPX analysis did not reveal significant differences depending on the occurrence of delirium. As with SOD, immediate postoperative GPX values were significantly higher in patients with delirium (Sensitivity 81%, Specificity 72% for GPX value over 3425 U / l).
17. Possible correlations between the markers analyzed in delirium patients were analyzed and a significantly negative correlation between SOD and immediate postoperative MDA was noted ($r = - 0.747$), which confirms that in these patients the oxidative stress is involved in the delirium.

Final Conclusions

1. The use of Propofol can reduce oxidative stress in patients with localized cancer treated by laparoscopic surgery.
2. Preoperatively determined superoxide dismutase can be used as a marker indicating an increased risk of post-operative delirium in elderly patients.
3. The occurrence of postoperative delirium, although rarer in patients receiving Propofol anesthesia, is not significantly influenced by the type of anesthesia.

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