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Evaluation of total oxidative and antioxidative status in pediatric patients undergoing laparoscopic surgery

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Abstract

Objective: Reactive oxygen species (ROS) induced by several diseases can trigger oxidative stress. During laparoscopy, increased intraabdominal pressure caused by pneumoperitoneum may lead to splanchnic ischemia followed by reperfusion because of deflation. Because ischemia reperfusion creates oxidative stress, in this study, we aimed to investigate the oxidative-antioxidative status of the pediatric patients with laparoscopic surgery.

Methods: The children underwent laparoscopic procedures under general anesthesia, and they were mechanically ventilated. Blood samples were obtained after induction of anesthesia, at the end of the surgery, and were centrifuged at 3000 revolutions per minute for 10 minutes to separate plasma. The plasma total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were determined.

Results: Plasma TOS and OSI levels were significantly higher at the end of the surgery than after induction of anesthesia ($P < .05$ and $P < .01$, respectively). On the other hand, plasma TAS levels were lower in the end of the surgery than after induction of anesthesia ($P < .05$).

Conclusions: These results suggest that ROS are generated during the laparoscopic procedure, possibly as a result of an ischemia-reperfusion phenomenon induced by the inflation and deflation of the pneumoperitoneum, which causes oxidative stress and consume plasma antioxidants.

Laparoscopic surgery, compared to conventional surgery, has many advantages for the patient, including smaller incisions, lesser postoperative pain, earlier oral intake, quicker mobilization, faster discharge, and a better cosmetic effect [1-3]. Nowadays, there has been a considerable improvement in laparoscopic surgical techniques and equipments. This has led to an increasing number of diagnostic and surgical procedures being done laparoscopically in pediatric patients [1]. In the near future, it can be expected that more difficult and lengthy operations will be performed, even in pediatric patients, by this method.

Reactive oxygen species (ROS) is produced in metabolic and physiologic processes, and harmful oxidative reactions may occur in organisms that remove them via enzymatic and nonenzymatic antioxidative mechanisms. Under certain conditions, the increase in oxidants and decrease in

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antioxidants cannot be prevented and the oxidative or antioxidative balance shifts toward the oxidative status [4]. Most common causes of oxidative status include ischemia-reperfusion injury, sepsis, and brain injury [5]. Trauma and surgical injury are associated with increased production of ROS and use of antioxidant systems, in particular when associated with relative tissue ischemia followed by reperfusion (condition of oxidative stress denoted as ischemia/reperfusion injury).

Blood contains many antioxidant molecules that prevent and/or inhibit harmful effects of ROS. The effects of antioxidant components of plasma are additive, the measurement of total antioxidant status reflects the antioxidative status of plasma. The cooperation of these various antioxidants in human plasma provides protection of the organism against oxidative stress. Laparoscopic procedures are regarded as minimally invasive procedures avoiding much of the tissue injury that is associated with traditional open laparotomy. However, increased intraabdominal pressure caused by pneumoperitoneum during laparoscopic procedures can cause splanchnic ischemia [6-8]. After deflation of the abdomen, intraabdominal pressure and splanchnic blood flow normalize, representing reperfusion. The clinical consequences and severity of oxidative stress of ischemia and reperfusion during laparoscopic procedures have not yet been elucidated [9]. It could be postulated that less trauma and decreased tissue lesions during laparoscopic surgery can cause less oxidative stress, but the impact of the ischemia-reperfusion condition during laparoscopic surgery on ROS production is still unknown. The exact role of ROS on the systemic stress response also remains to be understood, although lipid peroxidation as the consequence of excessive ROS production could have important effects on several metabolic pathways and inflammatory response to tissue damage [6].

The aim of the present study was to evaluate the consequences of oxidative stress in pediatric patients undergoing laparoscopic operations. To this end, a prospective clinical trial was established, and plasma total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were determined.

1. Methods

The study protocol was approved by the institutional review board. All parents gave their verbal and written informed consent. Thirty patients (mean age, 5 ± 2.52 years) with American Society of Anesthesiology I physical status, who were scheduled for elective laparoscopic appendectomy, were enrolled in the study. Patients with any metabolic, endocrine, hepatic, or renal diseases were excluded from the study.

The patients were premedicated with midazolam, 0.15 mg kg^{-1}, 30 minutes before the induction of anesthesia intravenously. After arrival in the operating room, patients were placed in the supine position. For reestablishment of the fluid balance, 8 mL kg^{-1} h^{-1} of Ringer's solution was infused and continued at a rate of 5 mL kg^{-1} h^{-1} during the surgery. Anesthesia was induced with propofol, 2 to 2.5 mg kg^{-1}; alfentanil, 5 to 10 μg kg^{-1}; and rocuronium bromur, 0.5 mg kg^{-1}, together with 100% oxygen. After tracheal intubation, the fresh gas-flow rate was set to 4 L min^{-1} in all patients. After 5 minutes, the total fresh gas flow was reduced to 2 L min^{-1} until the end of surgery. Anesthesia maintenance was adjusted to maintain air 50% in oxygen, intravenous alfentanil, 3 to 5 μg kg^{-1}, and rocuronium bromur, 0.2 mg kg^{-1} 30 minutes periodically. Volatile anesthetic concentrations were adjusted to maintain sevoflurane 2 minimum alveolar concentration (MAC). Ventilation was controlled with a tidal volume of 10 mL kg^{-1}, and respiratory rate was adjusted to maintain an end-tidal carbon dioxide ($Etco_2$) value between 35 and 45 mm Hg. Datex Ohmeda ADU anesthesia system (Datex Ohmeda, Helsinki, Finland) was used for anesthesia machine. All patients were monitored by electrocardiography, noninvasive blood pressure, peripheral oxygen saturation (Spo_2), and $Etco_2$ measurement (Datex Ohmeda, ADU, S/5, Helsinki, Finland). During laparoscopic procedures, the abdominal pressure was displayed and automatically maintained at 8 to 10 mm Hg by using a Storz (Gentec Ltd., Tuttlingen, Germany) electronic laparoflator. A nasogastric tube and urinary catheter were placed in all patients. Carbon dioxide was used to insufflate the peritoneum. All patients lay supine, with no tilt of the table.

Blood samples were obtained after induction of anesthesia and at the end of the surgery. The samples centrifugated at 3000 revolutions per minute for 10 minutes to separate plasma and then blood samples were stored at −80°C until analysis.

Plasma TAS levels were determined using a commercially available kit developed by Erel [9] (REL assay diagnostics, Mega Tip, Gaziantep, Turkey). In this method, hydroxyl radical, which is the most potent radical, is produced via Fenton reaction. In the classical Fenton reaction, the hydroxyl radical is produced by mixing of ferrous ion solution and hydrogen peroxide solution. In the most recently developed assay by Erel [9], same reaction is used. In the assay, ferrous ion solution, which is present in the reagent 1, is mixed by hydrogen peroxide, which is present in the reagent 2. The sequentially produced radicals such as brown-colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. In this assay, antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has got excellent precision values, which are lower than 3%. The results are expressed as millimoles of Trolox (Sigma-Aldrich Chemical Co., Deutschland, Germany) equivalent per liter.

Plasma TOS levels were determined using a commercially available kit, developed by Erel [10] (REL assay diagnostics, Mega Tip, Gaziantep, Turkey). In this method, oxidants present in the sample oxidize the ferrous ion-*o*-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv/L).

Hydrogen peroxide and other derivatives of peroxides, produced physiologically in organisms and occurring in higher concentrations under some pathologic conditions, diffuse into plasma. The level of total peroxide was measured and expressed as TOS in this study. The percentage of ratio of the TOS to the TAS gave the OSI, an indicator of the degree of oxidative stress [9,10]. To perform the calculation, the result unit of TAS, micromole of Trolox equivalent per liter, was converted to micromole equivalent per liter, and the OSI value was calculated as the formula OSI = [(TOS, μmol/L)/(TAS, mmol Trolox equivalent/L) × 100].

The comparisons of parameters were performed with Student's *t* test by using the Statistical Package for Social Sciences (SPSS 11.5, SPSS Inc, Chicago, Ill). Paired-samples *t* test was used for detection of differences between blood samples. All data were expressed as mean ± SD. A *P* value of less than .05 was accepted as statistically significant.

2. Results

Demographic data that were matched for age, height, weight, duration of operation, and duration of anesthesia are shown in Table 1.

All patients were hemodynamically stable throughout the procedure and completed the study. There were no any significant worsening in heart rate, mean arterial pressure, end-tidal carbon dioxide, peripheral oxygen saturation, and respiratory rates (data not shown).

Plasma TOS and OSI levels were significantly higher at the end of the surgery than after induction of anesthesia. Plasma TAS levels, however, were significantly decreased at the end of the surgery (Table 2).

3. Discussion

Pediatric laparoscopic surgeries are increasingly performed in the world. The possible unwanted side effect of these procedures remains unclear. This is the first study has been focused on the effects of laparoscopic procedures on the oxidative-antioxidative status of pediatric patients. Despite the limitation of our study to conduct a homogenous group including patients who underwent laparoscopic surgery, pediatric patients who had laparoscopic surgery for appendectomy operation have been included in the present study and the effects of laparoscopic surgery on these patients have been investigated measuring oxidant and antioxidant parameters in their blood before and after the surgical operation. We found that the oxidative/antioxidative balance shifted toward oxidative side, called increased oxidative stress, was present in the pediatric patients who underwent laparoscopic surgery.

Table 1 Demographic data concerning with patients

Age (y)	5 ± 2.5
Height (cm)	103 ± 13
Weight (kg)	22 ± 7
Operation time (min)	44 ± 8
Anesthesia time (min)	53 ± 7

Table 2 Oxidative stress parameters of the patients

Parameters	After induction of anesthesia	End of the surgery	*P*
TOS (μmol H_2O_2/L)	9.46 ± 5.57	16.92 ± 9.24 *	.019
TAS (mmol Trolox equiv/L)	1.84 ± 0.31	1.47 ± 0.33 *	.012
OSI (arbitrary unit)	5.18 ± 2.89	12.35 ± 8.14 *	.006

The values represent the mean ± SD.
* Statistically differences compared to "after induction of anesthesia."

It is well known that the induction of the pneumoperitoneum for laparoscopic surgery causes intraabdominal pressure levels of 10 to 15 mm Hg, which is higher than normal pressures of the portal system (7-10 mm Hg). Previous studies in humans [5,11] have shown that the use of a pneumoperitoneum during laparoscopic surgery causes significant reduction in gut perfusion and hepatic microcirculation. The deflation of the pneumoperitoneum reduces the intraabdominal pressure and increases splanchnic perfusion. Thus, laparoscopic surgery may represent an ischemia-reperfusion model. It is well known that the generation of ROS is the main cause of reperfusion-induced toxicity in various tissues [12].

The term *ischemia reperfusion* involves ischemic depletion of cellular energy sources and accumulation of free radical precursors because of subsequent reperfusion especially with high levels of circulating O_2. Although it is obvious that tissue ischemia initiates a series of events that can ultimately lead to cellular dysfunction and necrosis, resumption of blood flow can paradoxically create more tissue injury, possibly because of production of oxygen-derived cytotoxic products. Oxygen free radicals, produced on reperfusion, play a critical role in the injury caused by ischemia reperfusion. Reactive oxygen radicals lead to an inflammatory response and tissue damage by activating some mediators. It can also directly damage cell components.

In the present study, a significant increase in total peroxide levels was noted after laparoscopic surgery. Hydrogen peroxide and other derivatives of peroxides, produced physiologically in organisms and occurring in higher concentrations under some pathologic conditions, diffuse into plasma [10]. According to the best of our knowledge, this is the first time such an observation has been recorded in pediatric patients. The ratio of total peroxide to total antioxidant potential is the OSI, an indicator of the degree of oxidative stress. The oxidative stress index was significantly higher at the end of the surgery than after induction of anesthesia value although we used sevoflurane as a volatile anesthetic that was shown as not changing lipid peroxidation and antioxidant defenses unlike halothane [13]. If free radicals of any kind are indeed generated during laparoscopic procedures and are able to attack lipid molecules, it is certain that they also react with the low-molecular-weight antioxidants contained in plasma. Consequently, a decrease in TAS should be observed in the plasma under these conditions. We observed a significant difference between after induction of anesthesia and at the end of the surgery TAS levels in the patients. These results provided an evidence for the consuming of plasma antioxidants by the generated ROS.

In this study, we have used novel measurement methods to evaluate the extent of oxidative stress in pediatric patients with laparoscopic operations. It provides a useful method for the rapid evaluation of the TAS and TOS, which are valuable not only in the diagnosis of this condition but also for other disorders involving oxidative stress. In laparoscopic procedures, routine measurement of the TAS and TOS may provide a useful tool to aid in the assessment of the patient's oxidative and antioxidative status and in the determination of an appropriate protection from oxidative damage and treatment management plan. Because laparoscopic surgical techniques have been improving and an increasing number of diagnostic and surgical procedures are being done laparoscopically in pediatric patients, screening of TAS, TOS, and even OSI may be useful as a routine biochemical parameter at least to prevent oxidative cell and tissue injuries during the surgical procedures and to help the physicians to have much more better results from the process. Adding supplemental antioxidants to the treatment seems to be harmless but quite useful for these kinds of patients.

In the light of these findings, it is possible to conclude that laparoscopic surgery may cause oxidative stress in pediatric patients. Routine measurement of TAS, TOS, and OSI with the other routine clinical parameters might be useful to determine a better surgery and treatment procedure. In the near future, patients of all ages having a wide spectrum of benign and malignant diseases will be operated on laparoscopically. To address this eventuality, further studies will be needed to establish the severity of the reperfusion injury in patients who undergo lengthy procedures, in patients with cardiorespiratory and metabolic problems, and in those with hepatic failure and malignant diseases. For mentioned reasons above, to prevent deleterious effects of the oxidative stress, the potential protective antioxidant therapy may be necessary in these cases.

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