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Gallstone Patients with Enhanced Oxidative Stress Biomarker Superoxide Dismutase (SOD1) Plasma Levels Have Significantly Lower Number of Postoperative Analgesic Oxycodone Doses: A Prospective Study with Special Reference to Cancer Patients

JARI KÄRKKÄINEN[1*], IINA SAIMANEN[1*], TUOMAS SELANDER[2], SAMULI ASPINEN[1], JUKKA HARJU[3], PETRI JUVONEN[1] and MATTI ESKELINEN[1]

[1]Department of Surgery and [2]Science Service Center, Kuopio University Hospital, Kuopio, Finland; [3]Abdominal Centre, Helsinki University Hospital, Helsinki, Finland

Abstract. *Background/Aim: Oxidative stress biomarker superoxide dismutase (SOD1) plasma levels in operated gallstone patients versus cancer patients are unknown. In addition, the number of analgesic doses during the first 24 h postoperatively (NAD_{24}) in gallstone patients operated with laparoscopic cholecystectomy (LC) or minicholecystectomy (MC) is unreported. The aim of the study was to determine a correlation between the plasma SOD1 levels in the LC and MC patients versus cancer patients. Patients and Methods: Initially, 114 patients with symptomatic gallstone disease were randomized into LC (n=54) or MC (n=60) groups. The plasma levels of the SOD1 marker were measured just before, immediately after (POP1) and 6 h after the operation (POP2). Results: The median plasma SOD1 levels preoperatively and following surgery in the LC and MC patients versus cancer patients were statistically insignificant (p=0.90, p=0.88, p=0.21, respectively). The median plasma levels of SOD1 increased immediately after operation (POP1) and the postoperative elevation between the preoperative (PRE) and the POP1 values in the SOD1 marker were statistically significant (p=0.027). Then the median plasma levels of SOD1 marker decreased 6 h postoperatively (POP2) and the decrease between the POP1 and POP2 values in the SOD1 marker were statistically highly significant (p<0.001). There is a highly significant inverse correlation between the individual values of the NAD_{24} and plasma SOD1 values postoperatively in LC and MC patients (r=–0.335, p=0.011). Conclusion: The plasma SOD1 levels preoperatively and following surgery in the LC and MC patients versus cancer patients were quite similar. Cholecystectomy patients with enhanced levels of SOD1 appeared to have significantly lower number of analgesic oxycodone doses during the first 24 h postoperatively (NAD_{24}).*

Gallstone disease is common among the western population and the incidence in general varies between 5-22 % (1). The treatment of symptomatic gallstone disease is mainly surgical and a complication of cholelithiasis is indication for early cholecystectomy. The classical open cholecystectomy has been replaced by mini-invasive procedures, such as minicholecystectomy (MC) and laparoscopic cholecystectomy (LC). The LC is the golden standard operative technique, although the MC has been shown to be as safe and effective as the LC (2-4).

"The oxidative stress concept" is a biological condition in which the generation of reactive oxygen species (ROS) is higher than the capacity of detoxifying system. ROS are mainly the result of oxygen metabolism (5). Oxidative stress is associated with several acute and chronic diseases from Alzheimer's disease to diabetes (6). A good glycemic control in diabetes has been associated with decreased oxidative stress (7), while hyperglycemia has been shown to cause increased oxidative stress (8). Yiannakopolou *et al.* (9) reviewed clinical trials that investigated the oxidative stress in open surgery *versus* laparoscopic surgery. They found four randomized clinical trials that investigated the effect of laparoscopy cholecystectomy on oxidative stress (10-13). We have earlier investigated the role of oxidative stress in open and laparoscopic surgery (14-17) and concluded that midline

*These Authors equally contributed to this work.

Correspondence to: Matti Eskelinen, MD, Ph.D., School of Medicine, University of Eastern Finland, P.O. Box 100, FI-70029 KYS, Finland. Tel: +358 17173311, Fax: +358 17172611, GSM: +358 400969444, e-mail: matti.eskelinen@kuh.fi

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laparotomy may cause a reduction in oxidative stress biomarkers 8-OHdG (15) and GPX1 (16) plasma levels resulting in biochemical evidence of oxidative stress. However, the assessment of the oxidative stress biomarker superoxide dismutase (SOD1) plasma levels in gallstone patients *versus* cancer patients is unknown. The plasma levels of the SOD1 marker were measured just before, immediately after (POP1) and 6 h after operation (POP2). Firstly, the endpoint of this study was to determine a correlation between the plasma SOD1 levels in the LC and MC patients *versus* cancer patients and secondly, to determine a correlation between the plasma SOD1 levels and analgesic doses during the first 24 h postoperatively (NAD_{24}) in gallstone patients undergoing cholecystectomy.

Patients and Methods

The study was approved by the Ethics Committee of Helsinki and Uusimaa University District, Helsinki, Finland (DNRO 120/13/02/02/2010, May 12, 2010), it was registered in the ClinicalTrials.gov database (ClinicalTrials.gov Identifier: NCT01723540, Consort diagram, Figure 1), and it was conducted in accordance with the Declaration of Helsinki. Design of the study was a prospective, randomized, multicentre clinical trial consisting of 114 patients with confirmed symptomatic cholelithiasis. Written consent was collected from participants, after receiving verbal and written information about the study. Patients were randomized to undergo either laparoscopic cholecystectomy (n=54) or minicholecystectomy (n=60) in two separate hospitals in Finland: Kuopio University Hospital, Kuopio (n=86) and Helsinki University Central Hospital, Helsinki (n=28) between March 2013 and May 2015. Design of the study is presented in Figure 1. After patient enrolment, randomization was done with a sealed envelope method either to LC or MC groups. The cancer patients, recruited from our previous study, included ten patients with gastrointestinal cancer and 19 patients with gynecological cancer (18).

The surgical techniques used were standardized in both groups. The LC procedure was performed using the four-trocar technique (two 10-mm and two 5-mm trocars) and intra-abdominal pressure was set at 12 mmHg. The ultrasonic scissors (Harmonic ACE®, Ethicon Endo-Surgery, Cincinnati, OH, USA) were used both in the MC and LC procedure. The gallbladder was dissected from the liver with ultrasonic scissors. The cystic artery was sealed with ultrasonic scissor and two metal clips were inserted to the cystic duct.

The MC technique is mini-invasive open technique with very short wound; mean (SD) length of skin incision, 4.8 (1.6) cm, and the rectus muscle was not cut, but split. Skin incision length more than 7 cm or cutting of rectus muscle was considered as a conversion to conventional open operation. (3). Scin incisions were infiltrated with local anaesthetic (20 ml ropivacaine 7.5 mg/ml) at the end of the operation. Both study groups were standardized regarding endotracheal anaesthesia and postoperative care including the overall pain surveyed and filed on a 11-point numeric rating scale following surgery (NRS; 0=no pain; 10=worst pain).

For postoperative rescue analgesia, the patients were given oxycodone 3 mg *i.v.* if the pain at rest was NRS 3/10 or higher or during cough, and/or movement the NRS was 5/10 or higher. The total number of oxycodone 3 mg doses during the first 24 h postoperatively (NAD_{24}) for cholecystectomy patients was very carefully recorded in the patient records. The study protocol was fully described in our earlier original work (19).

EDTA-blood samples were taken at the prespecified time-points and centrifuged at 1,000 ×g (2,900 rpm) for 15 min. Plasma was separated and stored frozen at –70˚C until analyzed. The plasma

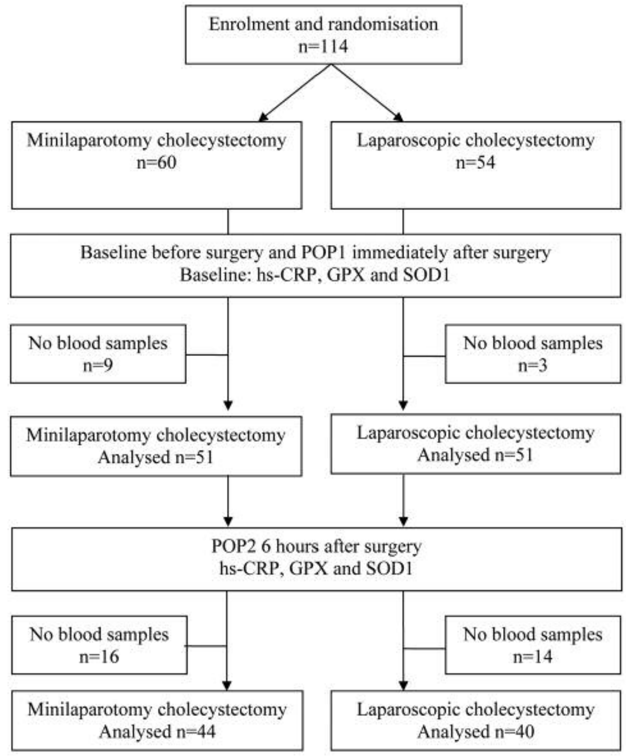

Figure 1. *Design of the study as flowchart.*

Table I. *Baseline demographic characteristics and surgical data for the two study groups. Values are mean (standard deviation) or *number of cases. T-test and Fisher's exact test* were used.*

Variable	Minilaparotomy n=60	Laparoscopy n=54	*p*-Value
Age (years)	50.7 (13.2)	53.2 (13.1)	0.316
Gender (male/female)*	11/49	18/36	0.066
Height (cm)	167.4 (7.6)	168.9 (9.9)	0.355
Weight (kg)	77.3 (14.4)	83.0 (17.4)	0.057
BMI (kg/m^2)	27.6 (4.4)	29.1 (5.6)	0.111
Operative time (min)	70.0 (26.9)	70.1 (35.3)	0.976
Time in the operation theatre (min)	120.4 (28.5)	127.1 (35.8)	0.287
Perioperative bleed (ml)	41 (59)	31 (39)	0.290
Conversion rate (n)*	3	3	1.000
Length of the skin incision(s) (mm)	49.7 (12.1)	78.3 (22.7)	<0.001

Table II. *Changes in plasma levels of hs-CRP, GPX1 and SOD1 measured at three time points; before the operation (PRE), immediately after the operation (POP1) and 6 h after the operation (POP2) in minicholecystectomy (MC) and laparoscopic cholecystectomy (LC) patients versus cancer patients. Values are median (interquartile range).*

Marker	MC	LC	Cancer	*p*-Value
Hs-CRP (mg/l)				
PRE	1.45 (0.49-3.1)	1.70 (0.56-3.65)	5.5 (1.0-63)	0.09
POP1	1.50 (0.50-3.0)	1.63 (0.6-3.45)	8.61 (0.8-59)	0.08
POP2	3.00 (1.4-5.7)	2.55 (0.98-4.38)	209.8 (101-375)	0.06
GPX1 (pg/ml)				
PRE	8.2 (5.7-13.8)	9.1 (5.5-16.8)	10 (6.3-19)	0.41
POP1	8.7 (6.3-14.4)	8.0 (5.4-13.5)	10.7 (7.4-22.4)	0.40
POP2	10.4 (5.1-14.6)	10.2 (6.6-15.6)	8.1 (5.2-12.3)	0.61
SOD1 (pg/ml)				
PRE	150 (99-204)	149 (83-222)	160 (107-211)	0.90
POP1	164 (118-241)	195 (108-260)	202 (136-338)	0.88
POP2	130 (92-168)	150 (116-231)	112 (86-196)	0.21

GPX1 assays were performed using sandwich-type ELISA methods from BioVendor GPX1 ELISA Kit (62100 Brno, Czech Republic, www.biovendor.com). Plasma hs-CRP was analyzed with a Cobas 6000-analyzer (Hitachi, Tokyo, Japan) using the method by Roche Diagnostics (Mannheim, Germany). The plasma SOD1 assays were performed using sandwich-type ELISA methods from BioVendor Cu/Zn SOD ELISA Kit (62100 Brno, Czech Republic, www.biovendor.com). In our own analysis (n=25) of the intra-assay CV% and the inter-assay CV% were 4.9% and 10.7%, respectively.

The data were entered and analyzed with a statistical software program (IBM SPSS Statistics 24.0, IBM, Somers, IL, USA). Baseline characteristics between groups in Table I were tested by Fisher's exact test and in case of continuous data the analysis was performed by analysis of variance (ANOVA). Group differences in three time points were tested by the Mann-Whitney *U*-test in Table II and the Wilcoxon signed rank test was used in Table III. The results of the laboratory measurements are presented as median with interquartile range as distributions were right skewed. A two-sided *p*-value of less than 0.05 was considered statistically significant. The results of the individual GPX1 and SOD1 values postoperatively (POP1) for LC and MC patients are shown as jitter plots with Spearman's correlation coefficients in Figure 2. The results of the individual postoperative (POP2) SOD1 values *versus* number of analgesic doses during the first 24 h postoperatively (NAD_{24}) for cholecystectomy patients are shown as jitter plots with Spearman's correlation coefficients in Figure 3.

Results

The demographic variables and surgical data were quite similar in the study groups. There was a trend for higher mean body weight in the LC *versus* the MC patients (83.0 kg *vs*. 77.3 kg, *p*=0.057, Table I). However, there was no statistically significant difference in the mean body mass index values (BMI) between the LC and MC patients (29.1 kg/m^2 *vs*. 27.6 kg/m^2, *p*=0.111, Table I). Interestingly, the mean length of the skin incision was significantly longer in the LC patients than in the MC patients (78.3 mm *vs*. 49.7 mm, *p*<0.001, Table I).

The median plasma hs-CRP levels preoperatively and following surgery in the LC and MC patients were quite similar (*p*=0.42, *p*=0.43, *p*=0.63, respectively, *p*-value between LC and MC groups, Table II). Also, the median plasma hs-CRP levels preoperatively and following surgery in the LC and MC patients *versus* cancer patients were statistically insignificant (*p*=0.09, *p*=0.08, *p*=0.06, respectively, Table II), although there was a trend for higher postoperative plasma hs-CRP levels in cancer patients (Table II). The median plasma GPX1 levels preoperatively and following surgery in the LC and MC patients *versus* cancer patients were statistically insignificant (*p*=0.41, *p*=0.40, *p*=0.61, respectively, Table II). Also, the median plasma SOD1 levels preoperatively and following surgery in the LC and MC patients *versus* cancer patients were statistically insignificant (*p*=0.90, *p*=0.88, *p*=0.21, respectively, Table II).

The median plasma level of hs-CRP increased by 7.4 % immediately after surgery and this increase was statistically significant (*p*=0.021, Table III). The median hs-CRP level then increased by 62.1 % at 6 h postoperatively (*p*<0.001, Table III). The median plasma oxidative stress marker SOD1 levels preoperatively and following surgery in the LC and MC patients were quite similar (*p*=0.891, *p*=0.875, *p*=0.207, respectively, Table II). The median plasma level of SOD1 increased by 14.1 % immediately after surgery and this increase was statistically significant (*p*=0.027, Table III). The median SOD1 level then decreased by 21.3 % at 6 h postoperatively (*p*<0.001, Table III).

There was a highly significant correlation between SOD1 and GPX1 values immediately after operation (r=0.638, *p*<0.001, Figure 2). Interestingly, there was a highly significant inverse correlation between the individual values of the NAD_{24} and plasma SOD1 values postoperatively in LC and MC patients (r=–0.335, *p*=0.011, Figure 3).

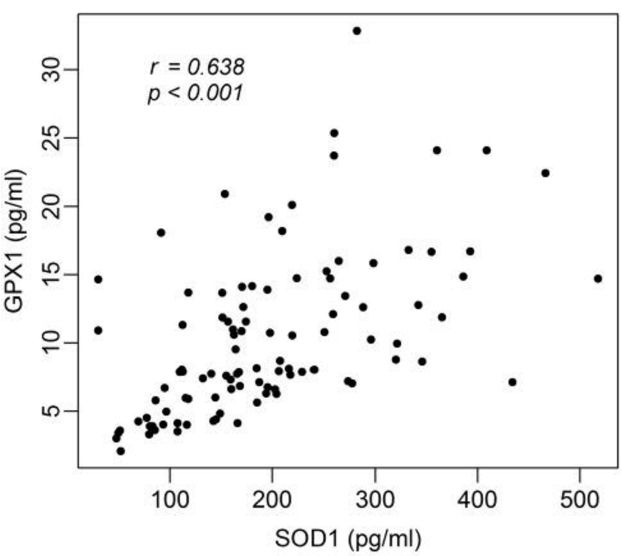

Figure 2. *Scatter plots of the post-operative (POP1) plasma superoxide dismutase (SOD1) values versus glutathione peroxidase (GPX1) in cholecystectomy patients (r=0.638, p<0.001).*

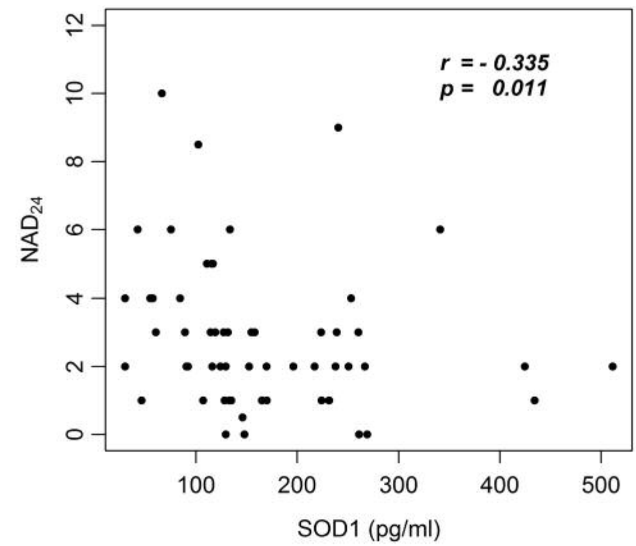

Figure 3. *Scatter plots of the post-operative (POP2) plasma superoxide dismutase (SOD1) values versus number of analgesic doses during the first 24 h postoperatively (NAD_{24}) in cholecystectomy patients (r=–0.335, p=0.011).*

Discussion

Many methods and markers have been used in early detection of oxidative stress and several issues need to be taken in consideration in oxidative stress research. One of the problems encountered with the oxidative stress biomarker analysis is the sample preparation. The blood sample from study patients should be standardized and quickly stabilized. The stabilization of the sample needs to be done by deep freezing after centrifugation. On this basis, in this study the EDTA-blood samples were taken at the prespecified time-points and centrifuged at 1000 g for 15 min. Plasma was separated and stored frozen at –70˚C until analyzed. Therefore, it is unlikely that there is a study bias from the blood sampling and/or the blood sample preparation and it is possible to compare the study group, even though, research laboratories are facing problems reproducing the data from sample to sample and from study to study (20).

Oxidative stress has an important role in the pathogenesis of toxic liver disease (21), atherogenesis (22) and is the cause of several acute and chronic conditions from oxidative stress-mediated biomolecular damage and inflammation in tumorigenesis (23) to oxidative stress during chronic alcoholism (24). Although, in the critical care patient's low plasma levels of antioxidant micronutrients have been shown, these low plasma levels are difficult to interpret since in CCU patients the multi-organ failure causes redistribution of micronutrients by cytokine mediated mechanisms (25).

Table III. *The postoperative alteration of plasma hs-CRP and SOD1 marker levels in the groups combined (all patients). Plasma levels were measured before operation (PRE), immediately after operation (POP1) and 6 h after operation (POP2). Median (interquartile range) values are shown. The Wilcoxon signed rank test was used.*

Marker	All patients	Alteration	*p*-Value
Hs-CRP (mg/l)			
PRE	1.35 (0.49-3.15)	PRE *vs*. POP1	0.021
POP1	1.45 (0.50-3.03)	POP1 *vs*. POP2	<0.001
POP2	2.35 (0.98-4.65)		
SOD1(pg/ml)			
PRE	149.97 (97.2-209.6)	PRE *vs*. POP1	0.027
POP1	171.2 (114.7-256.7)	POP1 *vs*. POP2	<0.001
POP2	134.5 (97.9-216.5)		

LC is the gold standard for the treatment of symptomatic gallstone disease, although MC has been shown to have a similar early recovery after surgery (26-35). Several markers and methods have been used in the early detection of systemic oxidative stress. Polat *et al*. (10) assessed the effect of different increased intra-abdominal pressure (IAP) on lipid peroxidation and protein oxidation status during laparoscopic cholecystectomy. 24 patients who underwent LC with either 10 or 15 mmHg of IAP were randomized into two groups. No difference was found between groups on lipid peroxidation or protein oxidation status.

Koksal *et al*. (11) compared the effects of sevoflurane and desflurane on lipid peroxidation and protein oxidation status during laparoscopic cholecystectomy. Patients were randomly allocated to be in sevoflurane (n=20) or desflurane (n=20) study groups. No significant differences were found in SOD1 in the samples from bronchoalveolar lavage. Yagmurdur *et al*. (12) studied the effect of etomidate (group 1, n=12), thiopentoal (group 2, n=12) and propofol (group 3, n=12) on hypoperfusion-reperfusion phenomenon in the LC patients. There was significant increase in plasma malondialdehyde (MDA) levels in group 1 and MDA plasma levels were significantly decreased in group 3, suggesting that propofol could offer many advantages inhibiting lipid peroxidation and ROS formation.

Bickel *et al*. (13) assessed the reduction of the oxidative stress by intermittent pneumatic compression device (ISPC) in LC patients. IAP during LC may lead to decreased cardiac output and visceral perfusion and possible hypoperfusion-reperfusion effect. 20 LC patients were randomized into 2 groups; control group without ISPC (n=10) and study group with ISPC (n=10). There was no significant difference between study groups in liver enzymes, bilirubin or in hemodynamic patterns. In the control group increased lipid peroxidation level were noted post-operatively in comparison to preoperative levels, while in the study group such changes were not shown (p=0.002).

Earlier, we reported the pain at rest and the NAD_{24} in the LC *versus* MC patients and our results suggest relatively similar results between these two groups (19). Firstly, the aim of this study was to investigate the plasma SOD1 levels in LC and MC patients *versus* cancer patients and secondly, to determine a correlation between the plasma SOD1 levels and analgesic doses during the first 24 h postoperatively (NAD_{24}) in gallstone patients undergoing cholecystectomy. The SOD1 levels increased by 14.1 % immediately after surgery and then decreased by 21.3 % at 6 h postoperatively. However, the plasma SOD1 levels preoperatively and following surgery in the LC *versus* MC patients were quite similar supporting the quite similar oxidative stress concept between these two cholecystectomy techniques. Interestingly, a highly significant inverse correlation between the individual NAD_{24} values and plasma SOD1 values postoperatively in operated patients was found. This could suggest that enhanced oxidative balance following surgery may have a protective role against postoperative pain.

The significant enhancement in the SOD levels, but not in the GPX levels after cholecystectomy is a new finding, although the rise in the SOD levels following surgery is in line with the results of Turker *et al*. (36) in patients undergoing open heart surgery with cardiopulmonary bypass. Woźniak *et al*. (37) found the SOD plasma levels to alter one week postoperatively, while the GPX levels stayed markedly lower than before surgery.

In conclusion, the cholecystectomy does significantly alter the oxidative stress marker SOD1 plasma levels immediately after operation, but the levels drop back 6 h following surgery. The plasma SOD1 levels preoperatively and following surgery in the LC and MC patients *versus* cancer patients were quite similar. Interestingly, cholecystectomy patients with higher levels of SOD1 appeared to have lower number of analgesic oxycone doses during the first 24 h postoperatively (NAD_{24}), which could suggest that enhanced oxidative balance following surgery may have a protective role against postoperative pain.

Conflicts of Interest

The Authors report no conflicts of interest or financial ties to disclose. The Authors alone are responsible for the content and writing of this article.

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