Serum isoprostanes levels in patients after abdominal hysterectomy

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Abstract

Purpose: Reactive oxygen species (ROS), continuously generated in tissues, are involved in signal transduction under physiological conditions. The amount of ROS increases in response to surgical trauma. Isoprostanes are novel sensitive and specific markers of lipid peroxidation in vivo. Plasma concentration of isoprostanes increases in patients with various diseases associated with oxidative stress. In the present study we investigated the effect of abdominal hysterectomy on serum isoprostanes concentration.

Material and methods: The study was performed in 20 women (aged 45-63, average 50.3) who had undergone total abdominal hysterectomy with salpingooophorectomy, operated for benign diseases in the 1st Department of Gynaecology of Lublin Medical University. Isoprostanes were assayed by enzyme immunoassay (EIA) using 8-isoprostane EIA kit (Cayman Chemical, Ann Arbor, MI, USA).

Results: Serum concentration of isoprostanes before the surgery had value 38.9±10.7 pg/ml and it decreased at 8, 24 and 96 h after the operation.

Conclusions: Measurement of serum isoprostanes in small group of patients after hysterectomy did not brought the clear answer if the assessment of isoprostanes levels is a valuable method for evaluation of oxidative stress after a surgery.

Key words: abdominal hysterectomy, isoprostanes, reactive oxygen species.

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Introduction

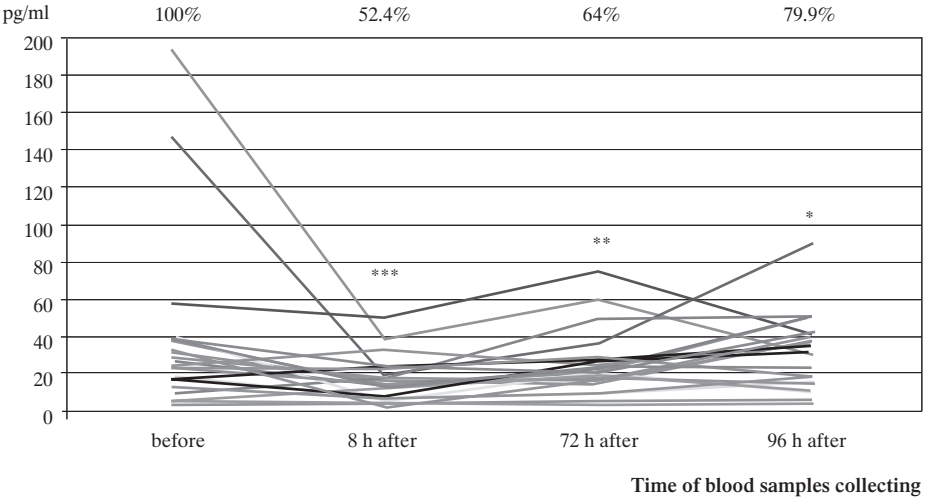
Reactive oxygen species (ROS), continuously generated in tissues, are involved in signal transduction under physiological conditions. Increased amount of ROS leads to damage of biologically relevant macromolecules including lipids, proteins and nucleic acids [1]. The amount of ROS increases in response to surgical trauma [2]. The level of lipid peroxidation products is often used as a marker of oxidative stress [1,3]. However, in most studies addressing the effect of surgery on oxidant antioxidant balance the nonspecific markers of lipid peroxidation such as thiobarbituric acid reactive substances (TBARS) or malonyldialdehyde (MDA) are measured. Thus we have recently demonstrated that the concentration of malonyldialdehyde + 4-hydroxyalkenals (MDA+4-HDA) as well as lipid hydroperoxides in serum have been increased after abdominal hysterectomy [4]. Isoprostanes are novel sensitive and specific markers of lipid peroxidation in vivo. These compounds are formed during ROS-mediated nonenzymatic peroxidation of arachidonic acid and other polyunsaturated fatty acids [5-9]. Plasma concentration of isoprostanes increases in patients with various diseases associated with oxidative stress [5,6,8].

Nevertheless, to our knowledge the level of isoprostanes after gynecological surgery has not been studied so far. In the present study we investigated the effect of abdominal hysterectomy on serum isoprostanes concentration to perform the evaluation of oxidative stress level in different methods of gynaecological operations in future.

Material and methods

The study was performed in 20 women (aged 45-63, average 50.3) who had undergone total abdominal hysterectomy with salpingooophorectomy, operated for benign diseases in the 1st Department of Gynaecology of Lublin Medical University. Average BMI of patients was 29.2. Peripheral blood samples were collected from patients before surgery as well as at 8, 24

Figure 1. **Serum isoprostanes levels**



* p<0.05; ** p<0.01; *** p<0.001 versus before the operation

and 96 hours after the operation. Blood samples were allowed to clot for 1 hour at a room temperature and centrifuged at 2000 x g for 10 minutes to separate serum. Serum samples were frozen and stored at -70°C until analysis.

Isoprostanes were assayed by enzyme immunoassay (EIA) using 8-isoprostane EIA kit (Cayman Chemical, Ann Arbor, MI, USA). Serum samples were mixed with ethanol and centrifuged to remove particulate matter. Ethanol was evaporated from the supernatant under a stream of nitrogen. Then, supernatant was acidified with acetate buffer to pH 4.0 and isoprostanes were extracted using C-18 SPE cartridges (Waters Corporation, Milford, MA). Cartridges were activated by rinsing with 5 ml methanol and 5 ml H_2O, and then the sample was passed through the cartridge. The cartridge was rinsed with 5 ml of water, dried, and rinsed with 5 ml of HPLC grade hexane. 8-isoprostanes were eluted with 5 ml ethyl acetate containing 1% methanol. The solvent was evaporated to dryness; the sample was dissolved in 450 µl of EIA buffer and used for the analysis. All samples were assayed in duplicate; before purification one set of samples was spiked with 8-isoprostane standard contained in the kit to correct for individual recovery. The recovery averaged 76%. The limit of the sensitivity of the assay was 5 pg/ml and the intraassay coefficient of variation was 8%. The nonparametric Wilcoxon's test was used to compare the data at different time points. The relationships between variables were evaluated by Spearman's rank correlation test. $p<0.05$ was considered significant. The Bioethical Committee at the Medical University in Lublin approved the investigation and all patients gave their informed consent to participate in it.

Results

Serum concentration of isoprostanes before the surgery had value 38.9±10.7 pg/ml and it decreased by 47.6% at 8 hours after the operation. Subsequently, isoprostane concentration progressively increased, however, it was still significantly reduced in comparison to preoperative values at 24 h (-36.0%) and at 96 h (-21.1%) after the operation (*Fig. 1*).

Table 1. **The characteristics of morphotic elements and biochemical parameters of patients' blood**

Morphotic blood elements		
Haemoglobin (g/dl)	Before operation	13.4
	24 h after the operation	12.2
Haematocrit (%)	Before operation	39.97
	24 h after the operation	36.44
Leukocyte count (K/µl)	Before operation	7.049
	24 h after the operation	10.41
Erythrocyte count (M/µl)	Before operation	4.707
	24 h after the operation	4.28
Thrombocyte count (K/µl)	Before operation	301.7
	24 h after the operation	278
Biochemical parameters of blood before operation		
Protein level (g/dl)	7.295	
Total cholesterol level (mg/dl)	213.2	
HDL level (mg/dl)	55.6	
LDL level (mg/dl)	133.5	
Triglicerides (mg/dl)	128.8	

The changes of blood parameters of patients before and after the operation are shown in *Tab. 1*. The granulocyte count increased by 47.7% after the surgery. The erythrocyte count decreased by 8.1%, thrombocyte count by 7.9% and haematocrit by 8.8% 24 hours after the operation. Patients were hospitalised for 5-8 days (average 6.6) after the operation.

Discussion

The generation of ROS increases after surgery as a result of tissue ischemia associated with anesthesia-induced hypotension as well as due to acute phase response which stimulates oxidative burst of phagocytes [3]. The increase of granulocyte count, observed after surgery, may suggest the induction of acute phase response, observed in our present and former investigations. Consistently with this, we have recently observed increase in plasma concentration of 'classical' lipid peroxidation products such as MDA+4-HDA and lipid hydroperoxides after abdominal hysterectomy [4]. Serum concentration of isoprostanes increases in patients with diseases associated with oxidative stress such as diabetes mellitus, hypercholesterolemia, hyperhomocysteinemia and connective tissue diseases [5-8]. Unexpectedly, in the present study we observed decrease in serum isoprostanes between 8 and 96 hours after surgery. The reason why MDA+4-HDA and isoprostanes change in the opposite directions is unclear at present. When we pass over the scattering of isoprostanes concentrations before the experiment we have to look for different explanation of this phenomenon. MDA and 4-HDA are low molecular weight compounds which can freely diffuse from tissues to plasma. It may be speculated that oxidative stress after surgery occurs mainly in injured tissues and most MDA originates in extravascular compartment. Thus, tissue oxidative stress will be only partially reflected by plasma lipid peroxidation markers. In contrast, isoprostanes are fatty acid derivatives and most of them is associated with triglycerides and phospholipids. Therefore, isoprostanes will very slowly exchange between tissue and plasma pools. Serum isoprostanes originate mainly during peroxidation of plasma lipoproteins and therefore may not adequately reflect the local oxidative stress, especially in the short run. Secondly, plasma lipoprotein level decreases after surgery as a result of hemodilution and acute phase response [10,11], which could lead to decrease in isoprostanes due to reduced availability of substrates for peroxidation. Finally, we have previously demonstrated that the activity of paraoxonase-1 (PON1) – an antioxidant enzyme contained in high-density lipoproteins – decreases following the surgery [12]. PON1 hydrolyses oxidised phospholipids and releases free isoprostanes [13,14]. Because in the method used by us mainly free non-estrified isoprostanes are detected, it is possible that reduced PON1 activity could contribute to fall in isoprostanes following the operation. Interestingly, two studies reported increase in plasma isoprostanes following coronary artery bypass grafting (CABG), however, the increase was short-lasting and observed only until less than 1 hour from the beginning of the operation [15,16]. Although we did not analysed blood samples obtained so early, one should take into account that it will be expected CABG causes much more marked oxidative stress.

Conclusion

In our initial studies we have observed decrease of serum isoprostanes levels after abdominal hysterectomy. These data suggest that measurement of serum isoprostanes in small group of patients after hysterectomy did not brought the clear answer if the assessment of isoprostanes levels is a valuable method for measurement of oxidative stress after operations.

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