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Glutathione and glutathione dependent enzymes in different pathophysiological states

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Abstract

Background: Oxidative stress (OS) has been implicated in pathogenesis of different human disorders. Antioxidant system, which is composed of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidases (GPX), glutathione reductase (GR) and antioxidant reduced glutathione (GSH) plays an important role in the protection of cells against enhanced OS. The aim of this study was to assess the enzyme activities of GPX1, GR and levels of GSH in different pathophysiological states.

Materials and methods: The activities of erythrocyte GPX1, GR and GSH were analysed in 35 women with depressive disorder (DD), 40 patients with metabolic syndrome (MetS), 30 septic patients (S) followed up in the course of sepsis; 15 non-septic critically ill patients (NC), 13 patients with acute pancreatitis (AP), 50 with chronic pancreatitis (CP) and 50 patients with pancreatic cancer compared to age- and sex-matched healthy controls (CON)..Activities of GPX1 and GR and levels of GSH were determined spectrophotometrically in red blood cells.

Results: The erythrocyte activities of GPX1 has been found to be decreased in DD patients, AP, S as well as in CP and PC patients, whereas no significant differences in GPX1 activities were observed in MetS patients compared with CON. In the contrast to GPX1 activity, higher GR activity has been observed in DD, MetS and S compared to CON and S in comparison with NC. Whereas GR activity was found unaffected in the course of sepsis and AP, the decrease in GR activity has been observed in CP and PC patients. In all aforementioned pathophysiologic states the levels of GSH were decreased.

Conclusion: Deficiency of antioxidant defence system results in increased OS, which is implicated in the pathogenesis above mentioned diseases.

Key words: oxidative stress, antioxidant enzymes, depressive disorder, sepsis, metabolic syndrome, acute and chronic pancreatitis, pancreatic cancer

Abstrakt

Úvod: Oxidační stres hraje významnou úlohu v patogenezi různých onemocnění. V ochraně buněk proti zvýšenému OS a volným radikálům hraje důležitou roli antioxidační systém tvořený enzymy superoxiddismutasou, katalasou, glutathionperoxidasou (GPX), glutathionreduktasou (GR) a redukovaným glutathionem (GSH), hlavním intracelulárním neenzymovým antioxidantem. Cílem této disertační práce bylo změřit aktivity antioxidačních enzymů GPX1, GR a hladiny GSH za různých patofyziologických stavů.

Materiál a metody: Aktivity GPX1, GR a hladina GSH, stejně jako koncentrace markerů OS byly sledovány u 35 žen s depresivní poruchou (DD), 40 pacientů s metabolickým syndromem (MetS), 30 septických pacientů (S) sledovaných v průběhu sepse; 15 kriticky nemocných neseptických pacientů (NC), 13 pacientů s akutní pankreatitidou (AP), 50 s chronickou pankreatitidou (CP) a 50 pacientů s rakovinou slinivky břišní a každá skupina byla porovnána s kontrolní skupinou spárovanou na základě věku a pohlaví (CON). Aktivity GPX1, GR a koncentrace GSH v erytrocytech byly stanoveny spektrofotometricky.

Výsledky: U pacientů s DD, AP, S v průběhu sepse, stejně jako u CP a PC pacientů byly pozorovány snížené aktivity GPX1 v erythrocytech v porovnání s CON. Nepozorovali jsme žádné významné rozdíly v aktivitě GPX1 mezi pacienty s Met S a CON. Naopak aktivita GR byla zvýšená u žen s DD a pacientů s MetS ve srovnání se zdravými lidmi. Vyšší aktivita GR byla pozorována také u S oproti NC, zatímco v průběhu S a AP se aktivita GR významně nelišila. Pacienti s CP a PC měli významně snížené aktivity GR oproti kontrolám. Snížené hladiny GSH byly zjištěny u všech výše uvedených patofyziologických stavů.

Závěr: Prokázali jsme oslabený antioxidační systém u pacientů s různými onemocněními, v jejichž rozvoji hraje významnou roli oxidační stres.

Klíčová slova: oxidační stres, antioxidační systém, sepse, deprese, metabolický syndrom, akutní a chronická pankreatitida, karcinom pankreatu

1. Introduction

Reactive oxygen and nitrogen species (RONS) may play a dual role in biological systems because their effect can be beneficial or deleterious [1, 2]. Reactive oxygen and nitrogen species include molecules like superoxide, hydrogen peroxide, hydroxyl and peroxyl radical, hydroperoxyl radical, hypochlorous acid, nitric oxide and peroxynitrite [2, 3]. Enhanced levels of RONS and /or insufficient activity of antioxidant defence system result in imbalance and increased oxidative stress (OS) which has been implicated in pathogenesis of many diseases.

In the protection of cells against RONS play an important role antioxidant defence system, which is composed of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPX) and glutathione reductase (GR), Antioxidant function also includes several biologically important non - enzymatic molecules such as reduced glutathione (GSH), vitamin C (ascorbic acid), vitamin E (α- tocopherol), vitamin A, bilirubin, uric acid [1, 2].

Glutathione peroxidase catalyses the reduction of H_2O_2 and polyunsaturated fatty acids hydroperoxides to water and related alcohols, respectively. The enzyme with indirect antioxidant function is the flavoprotein GR which regenerates reduced glutathione from oxidized glutathione which is formed in the reaction catalysed by GPX [4] . In addition to these above mentioned antioxidant enzymes, antioxidant defence system includes also non-enzymatic antioxidants e.g. GSH, which is the major low-molecular-weight thiol that maintains redox homeostasis in cells [5, 6, 7].

2. Aims and Scopes

The aim of the doctoral thesis study was to ascertain the importance of antioxidant enzymes - glutathione peroxidase, glutathione reductase and non-enzymatic antioxidant reduced glutathione in relation to oxidative stress markers in different pathophysiologic states - depressive disorder, metabolic syndrome, sepsis, chronic and acute pancreatitis and pancreatic cancer compared with healthy controls.

Antioxidant status together with oxidative stress markers have been followed up in acute phase of sepsis, septic shock and acute pancreatitis, in the course of these diseases and after clinical recovery phase.

This doctoral thesis is focused on the state of antioxidant defense system in various pathophysiologic states. We assume that oxidative stress plays a key role in pathophysiology of above mentioned diseases.

3. Materials and Methods

Patients

Activities of GPX1, GR and levels of GSH as well as markers of oxidative stress were measured in six different pathophysiologic states – in 35 patients with depressive disorder (DD), 40 patients with metabolic syndrome (MetS), in 30 patients with sepsis (S), 15 non-septic critically ill patients (NC), 13 patients with acute (AP) and 50 with chronic pancreatitis (CP) and 50 with pancreatic cancer (PC). All patients were compared with sex- and age-matched healthy controls.

Blood samples

Blood samples were obtained after overnight fasting. Activities of GPX1 and GR were estimated in haemolysed erythrocytes, levels of GSH were measured in washed erythrocytes. Blood samples were collected into plastic K_2EDTA tubes. Erythrocytes were washed free times in physiological saline (0.9% sodium chloride) solution. Suspension of washed erythrocytes for GSH measurement was mixed

with 100 μL of diluted acetic acid in water (6%, v/v) and 400 μL of 5-sulphosalicylic acid 10% (w/v) was immediately added. After centrifugation, supernatant solution was collected for analysis. Samples were stored at -80 °C until assay

Measurement of antioxidant enzymes activities

Glutathione peroxidase 1: The glutathione peroxidase activity was measured by the modified method of Paglia and Valentine using tert-butyl hydroperoxide as a substrate [8]. Briefly, 580 μL of 172.4 mM tris–HCl buffer containing 0.86 mM EDTA, pH = 8.0; 100 μL of 20 mM GSH, 100 μL of 10 U/mL GR, 100 μL of 2 mM NADPH and 100 μL of diluted sample were pipetted into the cuvettes. The reaction was started after 10 min of incubation at 37 °C by the addition of 20 μL of 9.99 mM tert-butyl hydroperoxide. The rate of NADPH degradation was monitored spectrophotometrically at 340 nm.. Activity of GPX1 was calculated using the molar extinction coefficient of NADPH 6220 M^{-1} cm^{-1} and expressed as U/g haemoglobin

Glutathione reductase: The activity was measured according to the method of Goldberg [9]. Briefly, 700 μL of 0.127 M potassium phosphate buffer containing 0.633 mM $Na_2EDTA{\cdot}2H_2O$, pH = 7.2 was added to cuvettes followed by 100 μL of 22 mM oxidized glutathione (GSSG) and 100 μL of diluted hemolysate. Prepared hemolysate was diluted before measurement 30 times with phosphate buffer. The reaction was started after 10 min of incubation at 37 °C by addition of 100 μL of 1.7 mM NADPH. The rate of NADPH degradation was monitored spectrophotometrically at 340 nm. Activity of GR was calculated using the molar extinction coefficient of NADPH 6220 M^{-1} cm^{-1} and expressed as U/g haemoglobin.

Concentration of reduced glutathione: Reduced glutathione was measured by the modified spectrophotometric method described earlier [10]. This method is based on the determination of relatively stable

product of reduction of 5.5′ dithiobis-2-nitrobenzoic acid (DTNB) reduction by sulfhydryl compounds to yellow product. Briefly, 50 μL of 0.125 M potassium phosphate buffer containing 6.3 mmol/L $Na_2EDTA{\cdot}2H_2O$, pH = 7.5 was added to micro-cuvettes followed by 37.5 μL of the sample and 12.5 μL of 6 mmol/L DTNB. The absorbance of the yellow product (reduced chromogen) was measured at 412 nm. Concentration was calculated by means of calibration curve and was expressed as mg/g haemoglobin.

Statistical analysis: All statistical analyses were performed using version 8.0, 9.0 and 10.0 of Statistica programme (StatSoft software CZ). $P < 0.05$ was considered to be statistically significant.

4. Results

Activities of antioxidant enzymes GPX and GR and levels of GSH in erythrocytes as well as markers of oxidative stress CD/LDL and ox-LDL, vitamins A and E and selenium has been measured in six different pathophysiologic states in which pathophysiology we assumed increased oxidative stress and weakened antioxidant defense system.

The erythrocyte activities of GPX1 has been found to be decreased in DD patients, AP, S as well as in CP and PC patients, whereas no significant differences in GPX1 activities were observed in MetS patients compared with CON. In the contrast to GPX1 activity, higher GR activity has been observed in DD, MetS and S compared to CON. Whereas GR activity was found unaffected in the course of sepsis and AP, the decrease in GR activity has been observed in CP and PC patients compared to CON. In all aforementioned pathophysiologic states the levels of GSH were decreased.

Table 1. Activities of antioxidant enzymes and levels of reduced glutathione

	GPX 1 (U/g Hb)	GR (U/g Hb)	GSH (mg/g Hb)
Depressive disorder N (F) = 35	53.7 (42.7 – 65.7)*	7.95 (6.84 – 8.62)*	0.569 (0.388 – 3.484)*
Controls N (F) = 35	64.0 (52.9 – 70.7)	7.0 (6.19 – 8.30)	2.375 (0.515 – 5.668)
Metabolic syndrome N = 40; M/F (20/20)	59.4 ± 15.8	8.19 ± 1.54***	0.570 (0.380 – 2.731)*
Controls N= 40; M/F (20/20)	59.1 ± 17.7	6.88 ± 1.66	1.460 (0.414 – 5.221)
Septic patients N = 30; M/F (18/12)	43.2 ± 12.5*	9.25 ± 2.57*	-
Controls N = 30; M/F (18/12)	52.1 ± 16.0	7.2 ± 1.99	-
Acute pancreatitis N = 13; M/F (9/4)	47.8 ± 14.5**	8.76 ± 2.23	5.719 (2.677 – 8.545)
Controls N = 13; M/F (9/4)	62.4 ± 14.9	8.26 ± 1.62	6.441 (2.305 – 6.685)
Pancreatic cancer N= 50; M/F (40/10)	43.9 ± 13.2***	7.22 ± 1.45*	2.392 (0.147 – 5.386) $^{+;}$ ***
Chronic pancreatitis N= 50; M/F (40/10)	41.0 ± 12.2***	7.03 ± 1.66**	4.015 (0.768 – 7.877)
Controls N= 50; M/F (40/10)	58.0 ± 15.1	7.98 ±1.35	6.021 (2.451 – 7.913)

Abreviations used: GPX1 – glutathione peroxidase1; GR- glutathione reductase; GSH – reduced glutathione. * Patients with different diseases vs. healthy controls; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $^{+}$ PC vs.CP; $^{+}$ $p < 0.05$.

5. Discussion

We have studied activities of main antioxidant enzymes such as GPX1 and GR as well as non-enzymatic, low molecular-weight antioxidant GSH in six different pathophysiologic states - depressive disorder, metabolic syndrome, sepsis, acute and chronic pancreatitis and pancreatic cancer. In all aforementioned pathophysiologic states, levels of CD/LDL were also measured. In patients with S, CP, PC and AP we also analysed levels of ox-LDL. Furthermore, serum concentrations of Se and serum levels of vitamin A and E were determined in some studies. Our results demonstrated increased levels of OS markers in all aforementioned studies.

5.1 Glutathione peroxidase

We have studied activities of erythrocyte GPX1 in patients with depressive disorder in comparison with healthy controls. Decrease in GPX1 activities has been found in patients with major depression than those in healthy controls [11]. Major depression is characterized by decreased levels of a number of important antioxidants and by lowered antioxidant status. Moreover lowered GPX1 activity is supposed to be one of the characteristic features for depression and play a role in pathogenesis of depression [12]. Similarly to our findings Maes *et al.* observed that whole blood GPX1 was significantly decreased in patients with major depressive disorder (MDD) compared to controls [13]. In the contrast according to study of Bilici *et al.* patients with major depression had increased erythrocyte GPX1 activity, especially patients with melancholia [14]. They suggested that depressive disorder is associated with overproduction of RONS. Another studies on patients with MDD reported that activity of GPX1 did not differ between patients with MDD and healthy subjects [15, 16].

Metabolic syndrome is associated with a number of pathophysiologic processes such as increased OS, activation of inflammatory cytokines and prothrombic mediators. The erythrocyte GPX1 activity in our study was not altered in MetS patients in comparison with healthy controls [17]. In accordance with our findings, in other studies also observed no significant changes in GPX1 in erythrocytes of patients with MetS compared to healthy controls [18, 19, 20, 21]. On the contrary, Cardona *et al.* found lower activities of GPX1 in a group of subjects with hypertriglyceridemia, a component of MetS, that is associated with increased OS, and the decrease of its activity was almost to 75 % of the control group [22, 23]. Similarly in the study of Koziróg *et al.* and Chen *et al.* found that patients with MetS had significantly lower GPX1 activities compared to healthy controls [24, 25]. In the contrast Ferro *et al.* investigated activities of erythrocyte GPX1 in obese women with metabolic syndrome and compared to healthy controls. They found higher GPX1 activity in obese women with MetS than in controls [26].

The key role of OS has been shown in pathogenesis of sepsis [27]. In the part of study dealing with sepsis, we compared patients in the course of sepsis with healthy controls. We have shown decreased GPX1 activities in erythrocytes at the onset of sepsis in the comparison with healthy controls. This decrease in activity of GPX1 persisted still after recovery. Decreased GPX1 activity was accompanied by depletion in Se levels at the onset of sepsis as well as after recovery in comparison with healthy controls. When we compared septic patients with non – septic critically ill patients and healthy controls, septic patients exhibited decreased GPX1 activity in comparison with critically ill and control subjects, whereas there was no difference in erythrocyte GPX1 activity between critically ill patients and controls. Decrease in Se levels has been observed in both septic and critically ill patients compared to healthy controls. Moreover, septic patients had lower selenium levels

than did critically ill. It is known that GPX1 requires GSH as a substrate in the milimolar range, which is at intracellular space. The decrease in GPX1 activity can be possibly explained by two main reasons: low level of GSH observed in erythrocytes of septic patients by others [28] and/or decreased concentration of selenium that is bound at the active site of the enzyme in the form of SeCys residue and is essential for its activity [29, 30].

By contrast to our findings, Mishra *et al.* compared patients with systemic inflammatory response syndrome (SIRS) with those of severe sepsis. Erythrocyte GPX1 activities did not differ between these groups [31]. No significant difference has been observed in erythrocyte GPX1 activity between severe septic patients with high dose and normal selenium supplementation [31], whereas in the study of Valenta *et al.* septic patients with high dose selenium supplementation had increased whole blood GPX1 activity than did septic patients with normal selenium dose, except baseline levels [32]. Further, Forceville *et al.* studied patients with septic shock or severe systemic SIRS with organ failure and patients without sepsis or SIRS, there were found no significant differences in plasma GPX activity between septic patients, non-SIRS or healthy controls [33], whereas Manzanares *et al.* observed decreased plasma GPX activity in patients with SIRS compared to patients without SIRS and controls [34]. Critically ill patients without SIRS had similar plasma GPX activity compared to healthy controls [34].

Supplementation with selenium improved antioxidant capacity, as demonstrated by increased GPX activity [35].

The aim of our study focused on acute pancreatitis was to observe changes in the antioxidant system during the course of the acute pancreatitis, which is rapidly developing inflammatory process associated with significant metabolic changes and clinical response. Oxidative stress plays an important role in progression of AP and its

intensity correlates with the severity of disease. Our study showed that the activity of GPX1 was lower at the onset of acute pancreatitis and persisted lowered in all AP samplings (after 72 hours, five days after onset of signs and finally 10 days after admission) in comparison with healthy controls [36]. Decrease in GPX1 activity has also been observed in post-acute pancreatitis patients [36]. Decreased GPX1 activity was accompanied by lower plasma levels of selenium in patients with AP and PAP in comparison with healthy controls [36]. In accordance with our results, Musil *et al.* measured antioxidants such as GPX1 activity and selenium in the course of severe and mild acute pancreatitis compared to healthy controls [37]. They found a significant decrease in erythrocyte GPX 1 in patients who had severe AP from admission to day 8 of the study, same findings has been obtained for selenium concentrations [37]. Another study focused on patients with AP determined the concentration of GPX activity and selenium levels in serum with respect to AP severity [38]. They observed significantly lower serum GPX in patients with severe form of AP during the 10 days observation period. Furthermore, decreased serum GPX concentration in acute pancreatitis was found in other studies [39, 40], whereas in the study of Szuster- Ciesielska *et al.*, the serum level of GPX was comparable to that of controls [41].

Furthermore, there are several studies showing that chronic pancreatitis is associated with a decrease in plasma selenium concentration [42, 43, 44]. Other antioxidants that have been measured were vitamins A and E. Vitamin E plays an essential role in the protection of cell membranes against free radical damage and affects the response of cells to OS. Vitamin A is also known to have antioxidant capacity [45]. We have found decreased vitamin A and E levels in patients with AP compared to controls. The decrease in levels of vitamin A and E during acute pancreatitis has been demonstrated in the study of Curran *et al.* [46]. Decreased levels of vitamin A have also been observed in the study of AP patients by Musil *et al.*, while the

concentration of vitamin E did not differ [37,47]. Plasma levels of vitamins A and E also significantly differed among patients with chronic pancreatitis, lower levels of vitamin A and E has been found in CP patients compared to controls [48, 43, 44].

Decrease in erythrocyte GPX1 activity we have been also observed in pancreatic cancer and in patients with chronic pancreatitis compared to healthy controls [49]. Similarly to our findings, Girish *et al.* found lower erythrocyte GPX1 activity in patients with tropical and alcoholic chronic pancreatitis as compared with healthy controls [50]. In the contrast to our study, Quillot *et al.* showed that erythrocyte GPX1 activity did not differ significantly between patients with CP and healthy controls [43, 44]. Published results in serum and plasma GPX activities are inconsistent. Szuster-Ciesielska *et al.* and Van Gossum *et al.* found significantly decreased GPX concentration in patients with chronic pancreatitis in serum and plasma [41, 48, 51], while in other studies observed no significant difference [42,43,44]. Lowered GPX1 activity may be explained by the depletion in selenium levels in both groups and/or decreased concentrations of GSH found in PC patients.

Depression, sepsis, acute and chronic pancreatitis are inflammatory disorders. It is known that inflammatory response leads to the increased production of RONS and induction of OS. Insufficient protection against RONS due to lowered GPX1 activity may lead to oxidative damage of membrane lipids (lipid peroxidation) and DNA. Depletion in GPX1 activity in aforementioned diseases may be explained by decreased GSH levels in these pathophysiologic states.

5.2 Glutathione reductase

We have found increased GR erythrocyte activities in depressive women than in healthy controls [11]. Similarly to our findings, Bilici *et al.* also found increased plasma GR activities in MDD with melancholia compared to controls [14]. They suggested that major depression is

associated with elevated antioxidant enzymes activities [14]. Andrezza *et al*. observed significant increase in the late stage of bipolar disorder in comparison with controls [52]. Gibson *et al.* assayed GR protein expression in cultured fibroblasts under glucose conditions in patients with MDD and showed increased GR protein expression in MDD patients group than in controls [53].

In patients with metabolic syndrome we have found elevated GR activities in comparison with healthy controls [17]. On the other hand, Cardona *et al.* observed a significant depletion in GR activity in patients with hypertriglyceridemia with and without MetS compared to control subjects and also in other study in patients with MetS compared to controls [22, 23]. Increased activity of GR could be attributed to a compensatory protective mechanism of the cells against RONS.

We have measured GR activities in septic patients, non-septic critically ill patients and healthy controls. In the course of sepsis there were no significant difference in all samplings of septic patients in comparison with healthy controls, but at the onset there is a trend to increased GR activities in septic patients in comparison with healthy controls. On the other hand, there was an increase in GR activity in comparison with critically ill and healthy controls. GR activities did not differ between critically ill and controls. There are no studies on GR activity in septic patients, whereas it has been shown a higher activity of GR in liver of septic rats compared to pair-fed rats [54]. This is in accordance with the results of Hunter and Grimble who also observed that rats treated with tumor necrosis factor alpha had increased GR activity [55]. This response of liver to an inflammatory challenge may lead to the maintenance of a high GSH/GSSG ratio.

In patients with acute pancreatitis we have not found significantly different activities of GR in the course of AP compared with healthy controls [36]. Our findings are in accordance with only once study of

Czeczot *et al.* where activity of GR did not differ in patients with acute pancreatitis and controls [39].

We have shown decreased erythrocyte GR activity in patients with pancreatic cancer and chronic pancreatitis in comparison with healthy controls [49]. To our knowledge there are no studies focused on analysis of GR activity in patients with pancreatic cancer. Decrease in GR activity may lead to a reduction in a GSH content, which we have found in patients with pancreatic cancer.

Increase in GR activities could be a compensatory mechanism to reduce further oxidative damage and progression of illness during oxidative stress.

5.3 Reduced glutathione

Reduced glutathione represents a major intracellular defence system against oxidative stress. Reduced glutathione is the major scavenger of ROS in the brain. We have described disturbed GSH metabolism in depressive patients [11]. We have shown decreased levels of reduced glutathione in patients with depressive disorder compared to controls. In the study of Samuelsson *et al.*, they did not found differences in total GSH in plasma or blood GSH levels of depressive patients before and after electronvulsive therapy [56]. Gawryluk *et al.* examined post-mortem brain tissues of patients with bipolar disorder, MDD, schizophrenia and compared with non-psychiatric, non-neurological control group [57]. Supporting our findings GSH levels were also reduced in psychiatric illness in this study. Decreased levels of GSH have been measured in animal models with stress induced depression [58, 59].

We have found significant depletion of erythrocyte GSH levels in patients with MetS [17]. Decreased concentrations of GSH with opposite changes in GSSG levels were also found in MetS subjects in the study of Cardona *et al.*, where patients with hypertriglyceridemia with or without

MetS had lower GSH levels than control group [22, 23]. Furthermore, in other study also observed decreased levels of GSH in subjects with different cardiovascular risk factors such as hypertension (HT) with and without MetS, familial hypercholesterolemia (FH) and familial combined hyperlipidemia (FCH), where patients with HT had the lowest GSH concentration among FH, FCH and control groups [60]. Furthermore, our expected increase in the GSSG/GSH ratio due to lower levels of GSH may stimulate compensatory increase in GR activity to reduce increased levels of GSSG in GSH.

The erythrocyte GSH concentration of patients with AP was increased during the first 3 days of hospitalization compared to other days and controls and the most pronounced was 3rd day. Other samplings of AP patients did not differ significantly from the values found in erythrocytes of control subjects [36]. In the study of Bansal *et al.* measured levels of antioxidants in patients with severe acute pancreatitis that were randomly assigned to antioxidant treatment group (received vitamin C, E and A) or to a control group [61]. Levels of reduced glutathione did not significantly differ at baseline in both the groups, increase in GSH levels were observed after 7 days in both the groups, but these changes from baseline were not spastically significant [61]. In the contrast to our study, significantly reduced GSH levels have been found in patients with mild and severe form of acute pancreatitis compared with healthy controls [62, 63]. Also in serum has been observed decreased GSH levels in AP patients in comparison with control subjects [39].

It is known that deficiency of glutathione may lead to progression of many pathologic states [64]. We have observed in patients with pancreatic cancer decreased levels of GSH compared to chronic pancreatitis and healthy controls, whereas we have found no significant difference in patients with CP compared to controls [49]. In the contrast to our findings, Girish *et al.* found decreased concentration of GSH in

patients with tropical and alcoholic chronic pancreatitis in comparison with controls; moreover patients with alcoholic form of CP had lower GSH levels than patients with tropical form [50, 65]. Similarly, Czeczot *et al.* observed lower GSH concentration in CP patients than in control group [39, 48]. To our knowledge, there are no studies on concentration of reduced glutathione and pancreatic cancer.

Increased oxidation could be explained by the impaired GSH function and weakened GSH redox efficiency. Increase in GSH levels could be a part of an adaptive response to elevated oxidative stress.

6. Conclusion

- In women with depressive disorder were observed decreased erythrocyte activities of GPX1 and levels of GSH, while activities of GR were elevated in comparison with healthy controls.
- Patients with metabolic syndrome had increased activities of GR, but decreased concentrations of GSH in erythrocytes compared to healthy controls. We have found no significant difference in erythrocyte activities of GPX1 between patients with metabolic syndrome and controls.
- The decrease in GPX1 activity has been found in septic patients in the course of sepsis (persisted in all three samplings) in comparison with controls. In the contrast, no significant difference in GR activity in the course of sepsis has been observed between individual S samplings and controls. The decrease in activities of GPX1 has been also found among septic patients, critically ill non-septic patients and control subjects. In the contrast, GR activity was increased in sepsis compared to critically ill patients and CON. No significant changes in activities of GPX1 and GR were found between critically ill patients and healthy controls.
- Patients with chronic pancreatitis and pancreatic cancer had lower GPX1 activities than did controls. Similarly, activities of GR were decreased in pancreatic cancer and chronic pancreatitis in comparison with controls. No significant differences have been found between pancreatic cancer and chronic pancreatitis. Moreover, patients with pancreatic cancer had lower GSH concentration than those with chronic pancreatitis and controls. No significant differences were found in GSH concentration between patients with chronic pancreatitis and healthy controls.
- In the course of acute pancreatitis activities of GPX1 and GR did not differ among individual samplings. However, patients with acute

pancreatitis had decrease in GPX1 activity in all individual samplings in comparison with healthy controls. Furthermore, no difference in GR activities among individual AP samplings and controls. Decreased levels of GSH were found in patient with acute pancreatitis at baseline in comparison with AP3 sampling where the concentration of GSH was the highest.

In conclusion, our findings indicate that the cumulative effect of continuous oxidative stress results in the imbalance of oxidant/antioxidant system. Increased oxidative stress leads to decrease and exhaustion of antioxidant defence system. These findings show that oxidative stress may have pathophysiologic role in aforementioned diseases.

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List of publications

1) Publications with background for doctoral thesis

a) with IF:

1. **Kodydková J**, Vávrová L, Staňková B, Macášek J, Krechler T, Žák A. Changes in antioxidants and oxidative stress markers in pancreatic diseases. Pancreas. 2013;42(4):614-621. **IF = 2.386**
2. Vávrová L, **Kodydková J**, Zeman M, Dušejovská M, Macášek J, Staňková B, Tvrzická E, Žák A. Altered Activities of Antioxidant Enzymes in Patients with Metabolic Syndrome. Obesity Facts. 2013;6(1):39-47. **IF = 1.856**
3. **Kodydková J**, Vávrová L, Zeman M, Jirák R, Macášek J, Staňková B, Tvrzická E, Žák A.: Antioxidative enzymes and increased oxidative stress in depressive women. Clin Biochem. 2009; 42(13-14):1368-74. **IF = 2.019**

b) without IF:

1. Vávrová L, **Kodydková J**, Macášek J, Ulrych J, Žák A. Oxidační stres v průběhu akutní pankreatitidy. Klin Biochem Metab. 2012; 20(41): 188-193.
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2) Publications without connection to doctoral thesis

a) with IF:

1. Vecka M, Dušejovská M, Staňková B, Zeman M, Vávrová L, **Kodydková J**, Slabý A, Žák A. N-3 polyunsaturated fatty acids in the treatment of atherogenic dyslipidemia. Neuro Endocrinol Lett. 2012;33 (Suppl 2):87-92. **IF=1.296**
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b) publications without IF:

1. Kocík M, Zimovjanová M, Petruželka L, **Kodydková J**, Vávrová L, Žák A. Oxidative stress after anthracycline therapy in patients with solid tumors. Cas Lek Cesk. 2012;151(10):463-7.
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