6

Selenium (Se), Blood Glutathione Peroxidases and DNA Damage in Chronic Kidney Disease Patients on Hemodialysis and After Kidney Transplantation - The Effect of Se Supplementation

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1. Introduction

1.1 Generation, role and destruction of reactive oxygen species

Chronic kidney disease (CKD) is characterized by complex changes in cell metabolism leading to increased production of oxygen radicals which, in turn, may play a key role in numerous clinical complications of this pathological condition. Several reports have focused on the identification of biological elements involved in the development of systemic biochemical alterations in CKD (Granata et al., 2009). Under normal conditions in living organisms oxygen metabolism (above all reduction) leads to the formation of highly reactive intermediates called reactive oxygen species (ROS) that are dangerous for the cell (Yu, 1994). As long as the balance between ROS production and ROS scavenging is maintained, no disorders are observed. ROS include superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), perhydroxyl radical ($HO_2^{\bullet}$) and singlet oxygen ($^1O_2$). According to some scientists, hydrogen peroxide (H_2O_2) also belongs to this group since it easily penetrates the membranes and in the presence of transition metals (copper or iron) it can be reduced to $OH^{\bullet}$ as in the example below (Yu, 1994):

$$H_2O_2 + Cu^{+} \rightarrow OH^{\bullet} + OH^{-} + Cu^{2+}$$

Hydroxyl radical is the strongest oxidant generated in biological systems because of its extremely short half-life (Yu, 1994; Fantel, 1996). Highly reactive hydroxyl radicals readily react with a variety of molecules. In general, the harmful effects of excess ROS on the cell most often include damage of DNA, oxidation of polyunsaturated fatty acids (PUFA) in lipids (lipid peroxidation), oxidation of amino acids in proteins and oxidatively inactivate specific enzymes by oxidation of co-factors (Brenneisen et al., 2005; Valko et al., 2007). ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically, which may result in significant damage to cell structures. This

accumulates into a situation known as oxidative stress. ROS are also generated by exogenous sources such as ionizing radiation. In recent years, a substantial body of evidence has accumulated supporting a key role for ROS in many fundamental cellular reactions and suggesting that oxidative stress may be important in the pathophysiology of common diseases including atherosclerosis, malignant diseases, chronic kidney disease and diabetes mellitus as well as in the aging process (Young & Woodside, 2001; Sies, 1991).

It has been shown that CKD patients are at substantially higher risk of cardiovascular diseases, atherosclerosis and cancer than age-matched subjects in the general population (Mandayam & Shahinian, 2008). In CKD patients, the efficiency of the antioxidative defense system declines with the progress of the disease and reaches its peak in the end-stage. The extent of oxidative stress can be slowed by increased efficiency of the natural antioxidant system (Meydani, 2002). Mammalian cells are protected against ROS by two lines: endogenous mechanisms (mainly enzymes) and exogenous low molecular weight compounds (free radical scavengers) (Joseph, 1995). The antioxidant enzymes are: superoxide dismutases (SOD), catalases (CAT) and glutathione peroxidases (GSH-Px) and probably selenoprotein P (Joseph, 1995; Zachara et al., 2006). The share of these three enzymes that work closely together in ROS neutralization is shown in Fig. 1.

SODs play a central role in catalyzing the spontaneous dismutation of superoxide producing O_2 and H_2O_2. Marklund (1984) studied SOD isoenzymes in human tissues and has shown that liver contains very little extracellular SOD and a lot of the other isoenzymes, whereas all isoenzymes are abundant in the kidney. Tissue homogenates from chronically rejected human renal allografts demonstrated decreased activity of MnSOD and increased expression of MnSOD protein (MacMillan-Crow et al., 1996). The molecular mechanisms involved in the induction of MnSOD during oxidative stress have yet to be elucidated.

Protection by SOD is incompletely achieved if H_2O_2 is not subsequently degraded. H_2O_2 accumulation, if not efficiently recycled, will lead to the appearance of the very aggressive $OH^{\bullet}$. Decomposition of H_2O_2 is the function of CAT, which generates oxygen and water (Joseph, 1995). However in many cells CAT activity is very low, and frequently unavailable for H_2O_2 dismutation. Thus, in most tissues, the degradation of hydroperoxides is effected by GSH-Px. This enzyme contains essential trace element Se at the active site and reduces H_2O_2 to water and organic hydroperoxides to alcohols. Two forms of GSH-Px have been identified in blood: cellular, called cytosolic or classical (GSH-Px 1), and extracellular (present in plasma; GSH-Px 3) (Arthur and Beckett, 1994). GSH-Px 3 is synthesized in the kidney and shows a significant diagnostic value in kidney diseases (Zachara et al., 2006).

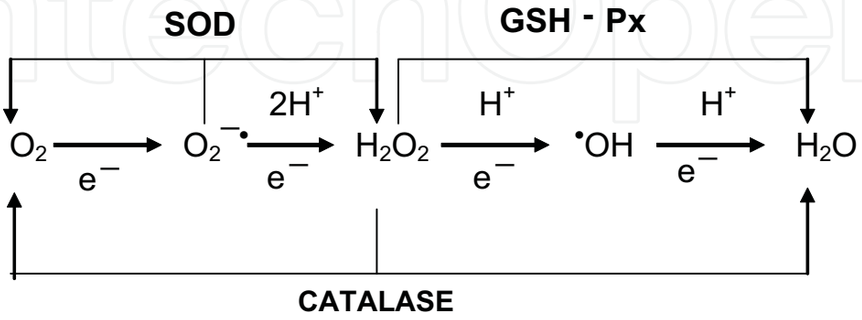


Fig. 1. Antioxidant enzymes. All three enzymes are not localized in the same compartments of the cells: SODs are either in the cytosol (Cu-Zn SOD) or in mitochondria (Mn SOD); GSH-Px is cytosolic; catalase is mainly found in peroxisomes (Adapted from Joseph, 1995).

Exogenous low molecular weight antioxidants include vitamins (A, C and E), carotenes, glutathione (GSH), uric acid, bilirubin and several trace elements (selenium, copper and zinc) (Young & Woodside, 2001, Joseph, 1995). The tripeptide GSH is generally considered an intracellular antioxidant and it plays a crucial role in the cells (Fantel, 1994). Being the most abundant antioxidant inside the cells, GSH can directly scavenge free radicals ($OH^{\bullet}$, $O_2^{\bullet-}$ and organic free radicals) (Yu, 1994) or act as a cosubstrate in the GSH-Px-catalyzed reduction of H_2O_2 and lipid hydroperoxides, a constituent of cell membranes (Loughrey et al., 1994; Zachara et al. 2005). Vitamin E is the most widely distributed antioxidant in nature. Among at least eight structural isomers of tocopherols, α-tocopherol is the best known and possesses the most potent antioxidant activity. It is highly effective in providing protection against membrane lipid peroxidation by reacting with lipid peroxyl radicals (Yu, 1994). In patients with CKD vitamin E level is reduced and supplementation suppresses oxidative stress. Similarly, vitamin C may be effective in reducing complication events in HD patients (Del Vecchio et al., 2011). In maintenance HD patients, plasma malondialdehyde (MDA), which is the terminal compound of oxidation of PUFA, is elevated (Boaz et al., 1999; Paul et al., 1993; De Vecchi et al., 2009). Loguhrey et al. (1994) have shown that in the serum of CKD patients MDA level is 42% higher, and in patients on HD – 76% higher, as compared with the control group. Hemodialysis did not change the elevated MDA concentration (Turi et al., 1991), however it is notable that HD combined with Vitamin E supplementation to patients prevented the breakdown of PUFA and reduced MDA level (Roozbeh et al., 2011). Vitamin E supplementation to patients on HD resulted in progressive increase in red blood cell vitamin E and a concomitant progressive decrease in MDA concentration, suggesting associated diminution in oxidative stress. However, vitamin E supplementation did not affect some other protective mechanisms, namely GSH and SOD (Cristol et al., 1997).

2. Selenium content and glutathione peroxidases activities in different stages of CKD

Numerous studies suggest that the formation of trace elements-containing compounds (mostly enzymes) and not the elements *per se* are critical to biological activities. Trace elements, Se, Cu and Zn play an essential role in the antioxidant defense system. They perform antioxidative functions through proteins in which they are incorporated (El-Bayoumy 2001). Se is a component of about 25 enzymes including the GSH-Px family, thioredoxin reductases and selenoprotein P which provide antioxidant protection against ROS-driven cancer initiation and promotion, as well as some others (Zachara et al., 2006; Brozmanova et al., 2010). The other two elements, Zn and Cu are incorporated *inter alia* into SOD. Cu is also a component of several other enzymes, for example, ceruloplasmine and cytochrome oxidase (L'Abbe and Friel, 1992). Cu deficiency leads to increased generation of free radicals and can promote lipid peroxidation (Lai et al., 1996). Zn is incorporated into many proteins, of which several hundred are enzymes (Valee, 2001). All mentioned enzymes participate in the metabolism of nucleic acids, proteins, carbohydrates and others.

In a healthy person the kidneys play an important role in the homeostasis of several trace elements, including Se (Wasowicz and Zachara, 1987). When Se is consumed at rates close to the human nutritional requirement, its highest levels are found in the kidneys and followed by the liver (Lockitch, 1989; Zachara et al., 2001c; Zachara et al., 2006). The Se level in tissues depends on its daily intake and the ingested chemical form. In people living in seleniferous regions the level is much higher than in those living in low Se areas e.g. New Zealand and

the Keshan region in China (Casey et al., 1982; Yang et al., 1989). Se is excreted from the organism mainly in the urine and feces, but urine contains 50-70% of the ingested element (Sanz Alaejos and Diaz Romero, 1993). Some authors (Yang et al.,1989) studied the relation between Se intake and urinary Se excretion in Chinese people living in different regions (low and high Se levels in the soil) and found a linear, significant correlation ($r = 0.886$; $P < 0.001$) between these two parameters. The amount of urine Se excretion increased progressively with increasing Se intake, but the form in which selenium was excreted in the urine remained unknown for a long time.

It was thought that the main form of Se excreted with urine is the metabolite called trimethylselenonium ion (TMSe) (Byard, 1969; Suzuki, 2005). In fact, this compound was isolated from rat urine and it represented 20-50% of Se excreted (Palmer et al.1970). Later on TMSe was identified in human urine (Francesconi and Pannier, 2004). Recent analytical advances based on HPLC separations coupled with atomic and molecular mass spectrometric detection have provided new insights into this area. New Se-containing carbohydrates (selenoshugars) are now known to be the major urinary metabolites in humans and in rats. The major metabolite is beta-methylseleno-N-acetyl-D-galactosamine (Suzuki et al, 2005). This selenosugar plateaus with a dose higher than 2.0 μg Se/mL water or g diet, and TMSe starts to increase in higher Se intakes, indicating that TMSe can be a biomarker of excessive and toxic doses of Se. TMSe is now considered to be a less significant metabolite (Francesconi, 2006). More recently it has been shown that after Se supplementation to mice the element is metabolized mainly in the liver and kidney (Suzuki et al, 2010). Excess Se was associated with selenosugar in the liver, transported to the kidney and the excess amount was excreted mainly as a selenosugar in urine. Quite recently French authors have identified, for the first time, a novel Se metabolite in human urine – Se-methylselenoneine (Klein et al., 2011). It cannot be ruled out that the use of other methods of analysis will discover more selenium compounds in urine.

2.1 Selenium concentration in the blood components of chronic kidney disease patients

Free radicals damage different tissues or organs, hence the trace and toxic elements behave differently in various organs. Available data suggested that in patients with CKD the levels of cadmium, chromium, copper, lead and vanadium are higher and the levels of selenium, zinc and manganese are lower than in healthy subjects (Tonelli et al., 2009). Among various important elements in CKD patients, Se is the focus of this study, therefore attention will be concentrated on this element and on GSH-Pxs present in blood.

Although some authors did not find any differences between Se concentration in the blood components between CKD patients and controls (Milly et al., 1992) many others found significantly lower values (for a review see Zachara et al., 2006). Several authors have shown a gradual decrease of Se level along with the progress of the diseases (Ceballos-Picot et al., 1996; Zachara et al., 2004). An example is shown in Fig. 2 (taken from Zachara et al., 2004a).

Several authors have shown that in non-dialyzed CKD patients the overall plasma Se concentration from incipient to the end stage of the disease is 12.5 to 44% lower than in healthy subjects (Ceballos-Picot et al., 1996; Richard et al., 1991; Zachara et at., 2001a). It has been shown that in the early stage of CKD, Se concentration in whole blood and plasma does not differ significantly from the values found in the control group (Ceballos-Picot et al., 1996; Zachara et al., 2001b). Along with the progression of the kidney impairment, Se concentration decreases in whole blood and plasma. This was particularly evident in the end-stage of the

disease, where Se concentration in whole blood and plasma was lower by 47 and 50%, respectively ($P < 0.0001$) as compared with the control group (Zachara *et al.*, 2000b).

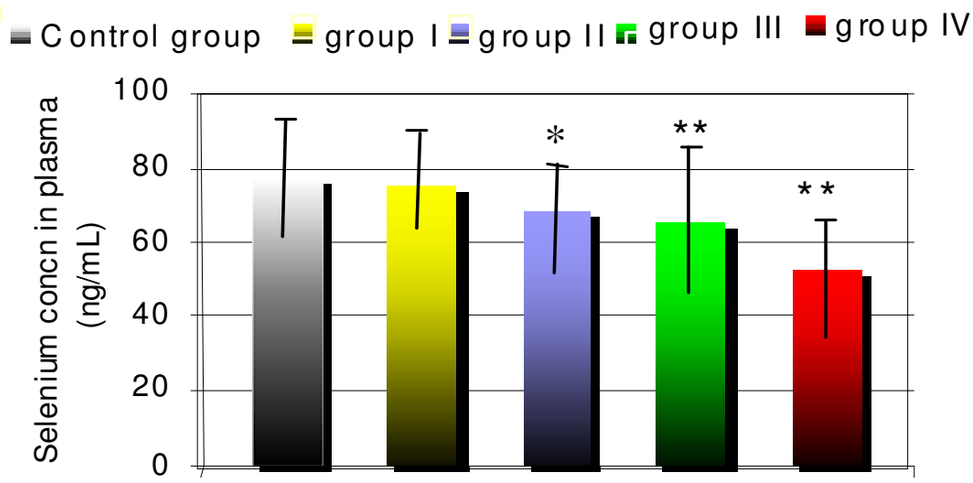


Fig. 2. Selenium concentration in plasma in controls and in different stages of CKD patients; (*p < 0.01; **p < 0.001 vs ctr.).

Methods of assessing the degree of renal damage in these patients include serum creatinine level, creatinine clearance and glomerular filtration rate (Krol and Rutkowski, 2008). The data presented in this paper are based mainly on the concentration of creatinine in plasma. Creatinine levels in CKD patients increase gradually with the development of the disease. Generally in CKD patients the concentration is several times higher than in healthy subjects (Zachara et al., 2004b; Rohun et al., 2011) and is highest in ESKD patients. In HD and in peritoneal dialysis patients the concentration is even higher (Zagrodzki et al. 2007; Mekki et al., 2010). Hemodialysis usually does not change the elevated concentration of creatinine (Mekki et al., 2010). Zachara et al. (2004a) have shown, in the entire group of CKD patients, a significant negative correlations between plasma selenium and plasma creatinine levels ($r = -0.380$; $P < 0.0001$; Fig. 3).

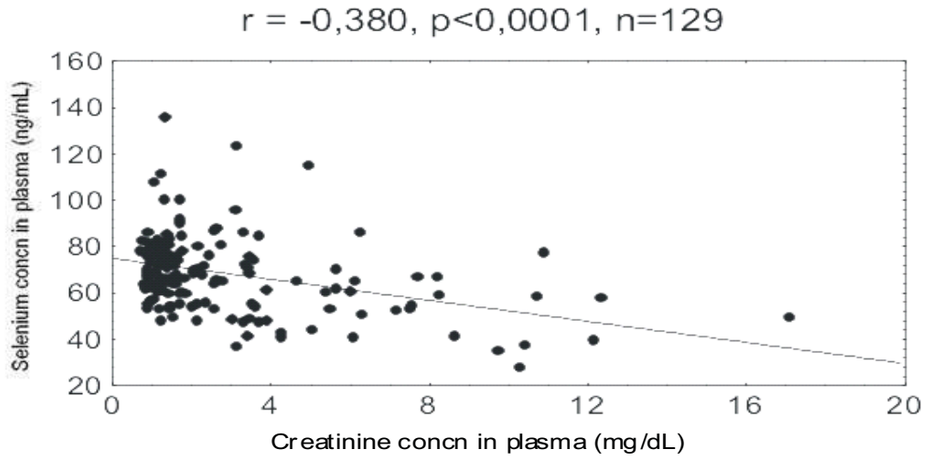


Fig. 3. Correlation between plasma selenium and ceatinine concentrations in control group and in different stages of chronic kidney disease patients (taken from Zachara et al., 2004a).

Schiavon et al. (1994a) studied 34 patients with different degrees of renal impairment, and found higher negative correlation between plasma selenium concentration and serum creatinine values ($r = -0.55$; $P < 0.001$). A negative correlation between Se concentration in plasma and creatinine clearance ($r = -0.47$, $P < 0.001$) was found by Ceballos-Picot et al. (1996). Ceballos-Picot et al. (1996) have shown that plasma Se concentration in patients on HD was more than 50% lower as compared with patients in advanced stage of the disease. However, many other authors found that the degree of Se reduction in dialyzed patients was similar to that observed in non-dialyzed patients who are in more advanced stages of the disease (for details see Zachara et al., 2006).

The biological significance of low Se concentrations in the blood is not fully understood, but severe deficiency leads to cardiomyopathy, as for example in the Keshan region in China (Ge et al., 1983). Lower concentrations of serum Se without severe deficiency have been associated with hypertension, heart disease and coronary disease in the general population, and with cardiomyopathy among dialysis patients. Mild Se deficiency appears to increase the susceptibility to oxidant stress, which may be relevant to hemodialysis patients in whom oxidative stress is markedly increased (Tonelli et al., 2009).

The question arises as to the cause of the low concentration of selenium in patients with CKD. Se levels in blood components are influenced by diet and food intakes, which are the principal source of this element. The lower Se levels in whole blood and plasma in uremic patients can be associated with the decreased intake of protein and increased protein loss with urine. Usually the patients are advised to consume limited amounts of protein to relieve uremic symptoms and to decrease the progression of kidney function (De Luis and Bustamante, 2008). Protein is the main source of Se, therefore the quantity of the element supplied is reduced (De Luis and Bustamante, 2008). Marchante-Gayon et al. (1996) indicated that the majority of Se (about 95%) seems to be bound to serum proteins. The most abundant selenoproteins in the blood components are selenoprotein P and GSH-Px (Ashton et al., 2009). Deagen et al. (1993) have shown that the Se is incorporated, in the form of selenocysteine (Sec), to selenoprotein P (≈ 50% of plasma selenium) and GSH-Px (which accounts for 10-30% of plasma selenium) or bound in the form of selenomethionine to albumin (≈ 23%). Very similar results have been obtained for blood plasma in healthy subjects by Koyama et al. (1999). They have shown a preference for supplemented Se to be taken up as selenoprotein P, the protein that incorporates up to 10 selenocysteine residues into the polypeptide chain (Persson-Moschos et al., 1995).

In CKD patients protein, and especially albumin concentration in plasma is lower than in healthy subjects (Dworkin et al., 1987; Zachara et al., 2004b; Zima et al., 1998). The precise cause remains unknown, but it is believed that among others the restricted diet, urinary or dialytic losses, impaired intestinal absorption, abnormal binding to Se transport proteins or drug therapy may all be responsible (Olson and Palmer, 1999; Vendrely et al., 2003; Bonomini and Albertazzi, 1995; Lockitch, 1989; Pasaoglu et al., 1996). Since protein foodstuffs contain the largest amount of Se, reduced protein intake seems to be a substantial cause of low Se concentration (Bonomini and Albertazzi, 1995). A strong positive correlation was noted between plasma total protein and albumin and Se concentration in healthy subjects and CKD patients (Dworkin et al., 1987; Zachara et al. 2004b). **The results of my group are presented in Fig. 4.**

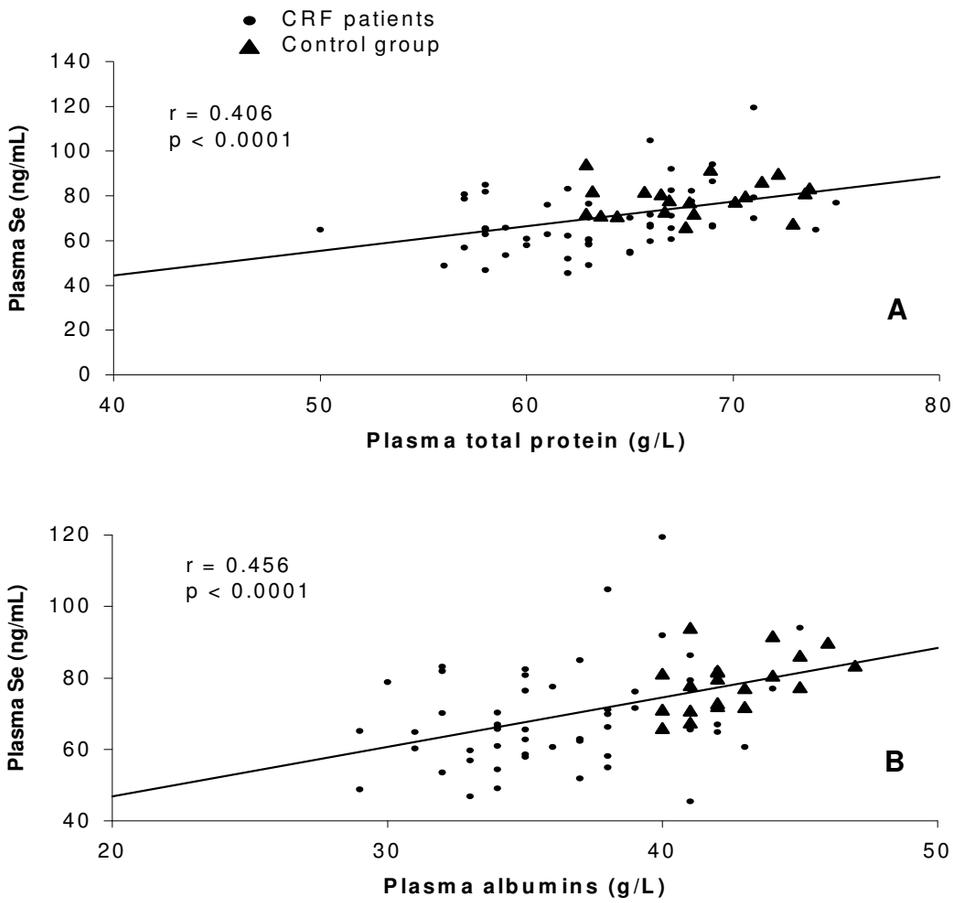


Fig. 4. The relationship between plasma total protein (A) and albumin (B) levels and plasma selenium concentrations in the control group (▲) and in patients at different stages of CKD patients (•) (taken from Zachara et al., 2004b).

3. Glutathione peroxidase activities in the blood of patients in different stages of chronic kidney disease and on hemodialysis

In human tissues, among about 25 selenoproteins (Papp et al., 2007), five are glutathione peroxidases: cytosolic (cellular, classical), (GSH-Px 1), gastrointestinal (GSH-Px 2), plasma (extracellular) (GSH-Px 3), phospholipid (GSH-Px 4) and sperm nuclei (GSH-Px 5) (Behne and Kyriakopoulos, 2001; Brigelius-Flohe, 2006). All of them can metabolize hydrogen peroxide and organic hydroxides (Arthur, 2000). Two of GSH-Pxs are present in the blood: GSH-Px 1 present in red blood cells and GSH-Px 3 present in plasma. Both have a tetrameric form and contain one selenium per subunit (or four gram atoms of Se per mole of enzyme) in the form of Sec (Maddipati and Marnett, 1987, Zachara, 1992). Se supply has been shown to induce the synthesis of these enzymes (Beilstein and Whanger, 1986). The relative position of the selenoproteins within the hierarchy is believed to reflect the relative biological importance. In the GSH-Pxs family the two extremes are represented by GSH-Px 1 and GSH-Px 3, which disappear rapidly in even moderate Se deficiency (Brigelius-Flohe, 2006).

Cellular GSH-Px is present in all tissues, and was detected by Mills (1957) as an enzyme involved in the protection of red blood cells against oxidative hemolysis. It was the first protein with selenium in the polypeptide chain to be identified independently by Flohe et al., (1973) and Rotruck et al. (1973). Plasma GSH-Px was recognized as a distinct from the red cell enzyme. Later on it was purified and characterized from human plasma (Broderick et al., 1987). This enzyme is structurally, enzymatically and antigenically different from that in erythrocytes and other cells (Zachara, 1992). GSH-Px 3 is widely used as the marker of Se status (Papp et al., 2007).

Studies on red blood cell GSH-Px activity in CKD patients produced inconsistent results. Some authors found significantly lower (Zachara et al., 2001a; 2004a; Bulucu et al., 2000; Martin-Mateo et al., 1999), some - significantly higher (Ceballos-Picot et al., 1996; Mimic-Oka et al., 1992) and still others did not observe any differences (Zachara et al., 2004b; Temple et al., 2000). It can be concluded that bone marrow in CKD patients is not damaged and the synthesis of this enzyme does not deviate from the norm. In patients on dialysis the activity of this enzyme is similar as in ESKD (Ceballos-Picot et al., 1996).

In CKD patients plasma GSH-Px seem to be much more important than red cell enzyme. Therefore it is worthwhile to concentrate primarily on plasma GSH-Px in CKD patients. Studies on this enzyme have shown that although GSH-Px 3 is synthesized in a range of tissues, the renal proximal tubular epithelial cells are the main source from which it is secreted into plasma (Avissar et al., 1994; Whitin et al., 1998). Many reports indicate that patients with CKD have very low plasma GSH-Px activity (Yoshimura et al., 1996, Zachara et al., 2004a, Ceballos-Picot et al., 1996) including those undergoing HD (Yoshimura et al., 1996, Roxborough et al., 1999, Ceballos-Picot et al., 1996, Zachara et al., 2001a). Reports by several authors have shown that plasma GSH-Px activity in uremic patients is reduced by 34-52% as compared with healthy controls (Richard et al., 1991; Schiavon et al., 1994a; Ceballos-Picot et al., 1996; Yoshimura et al., 1996; Zachara et al. 2004a). Some of the authors indicated a gradual decrease in the activity with advancing stage of the disease. An example is shown in Fig. 5 B (Zachara et al., 2004a).

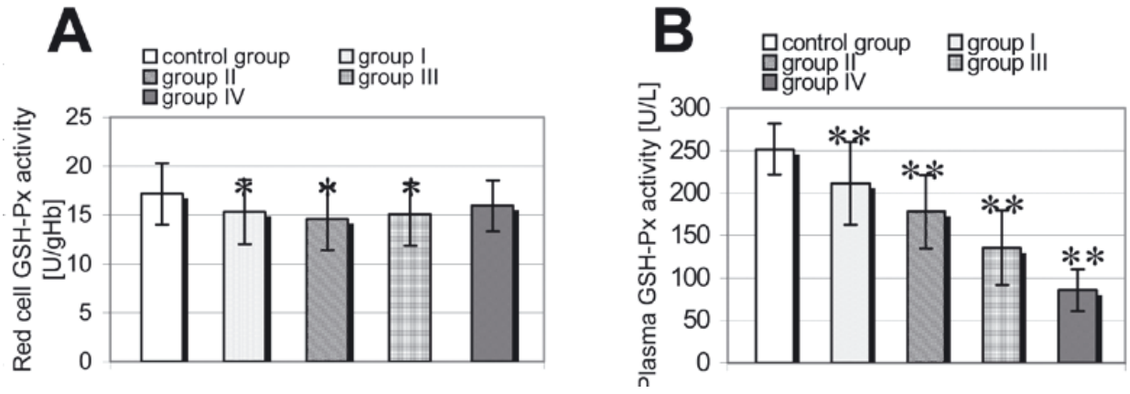


Fig. 5. Glutathione peroxidase activity in red blood cells (A) and plasma (B) in the control group and in patients with chronic kidney disease (CKD) in various stages of the disease. Patients were divided according to plasma creatinine (cr.) level: group I, incipient (n = 55) with cr. up to 1.36 mg/dL; group II, moderate (n = 40) with mean cr. level 1.72 mg/dL; group III, advanced (n = 40) with mean cr. level 3.96 mg/dL; group IV, end stage (n = 15) with mean cr. level 4.87 mg/dL. Statistics: *, $P < 0.01$ vs. control; **, $P < 0.0001$ vs. control (Adapted from Zachara et al., 2004b).

In contrast to the concentration of selenium, which does not differ in the initial stage of the disease from healthy subjects, the activity of GSH-Px already in the incipient stage was significantly lower ($P < 0.0001$) than in the control group. In the end stage of CKD, the activity of this enzyme decreased to one third of the value observed in the healthy subjects. A negative, statistically significant, correlation between plasma GSH-Px activity and creatinine concentration ($r = -0.657$) as well as between plasma GSH-Px activity and urea nitrogen ($r = -0.653$) in plasma was revealed (in both parameters $P < 0.0001$) (Fig. 6). A progressive decline in the activity of this enzyme is linked with the fact that GSH-Px 3 is primarily synthesized in the kidney and the progressing damage of this organ is reflected in diminished enzyme activity. Low GSH-Px 3 activity found in ESKD may depend on its synthesis in the residual, non damaged cells of the kidney and in other tissues/organs that synthesize small amounts of this enzyme. It has been shown that in humans this peroxidase is synthesized also in the liver, heart, lung and breast (Chu et al., 1992).

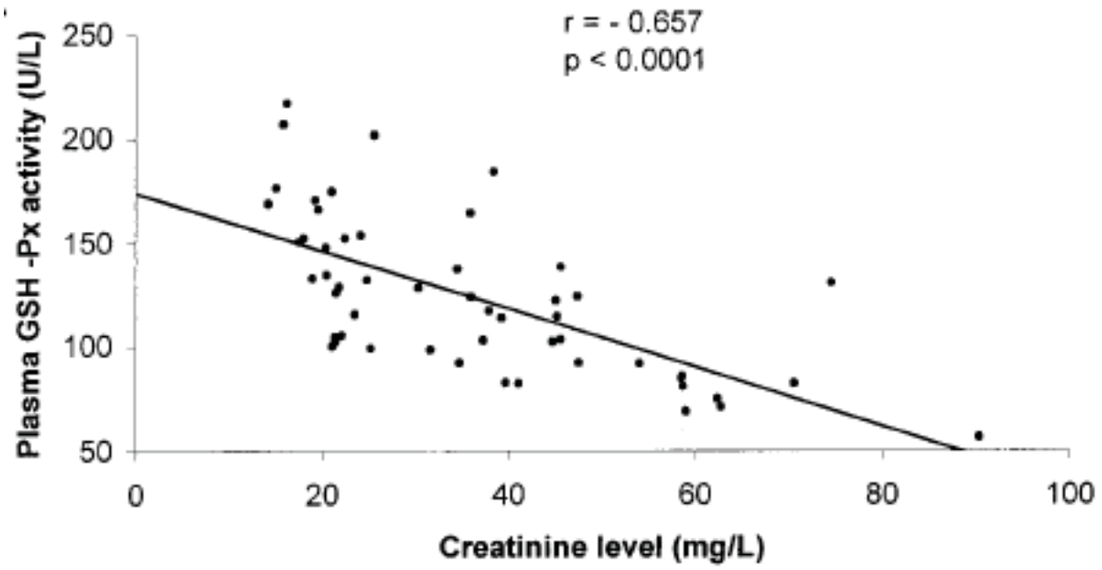


Fig. 6. Relation between plasma GSH-Px activity and creatinine level in CKD patients (taken from Zachara et al., 2004b).

4. The effect of selenium supplementation to CKD and HD patients on plasma GSH-Px activity and protein level

In healthy persons, Se supplementation leads to increased GSH-Px activity in red blood cells, plasma, other body fluids and tissues (Trafikowska et al., 1998; Iwanier and Zachara, 1995; Zachara et al., 1993). In plasma it reaches plateau value after 3-4 weeks of supplementation (Trafikowska et al., 1998; Iwanier and Zachara, 1995; Bonomini et al., 1995). In red blood cells, after Se supplementation, the GSH-Px activity increases much more slowly than in plasma (Trafikowska et al., 1998; Iwanier and Zachara, 1995) because of long life span of these cells. Thus plasma GSH-Px activity is a more sensitive index of short-term Se status, whereas red cell GSH-Px activity gives a long-term index status (Thomson and Robinson, 1980).

There is a dearth of publications on the effect of Se supplementation to CKD patients. Saint-Georges et al. (1989) were probably the first to supplement uremic, hemodialyzed patients

with sodium selenite administered orally. After supplementation (3 months with 500 μg/day and then for 3 months with 200 μg/day) plasma GSH-Px activity increased to a plateau after 16 weeks but remained about 30% lower than the control values. Studies of the effects of Se supply on plasma GSH-Px activity in patients on HD, carried out by various authors, have produced ambiguous results. Some authors show statistically significant increase in this enzyme activity after Se supplementation to dialyzed patients (Richard et al., 1991; Richard et al., 1993; Bellisola et al., 1994), others have shown slightly raised activity at the end of the HD session (Schiavon et. al., 1994a) or did not report any substantial differences (Zachara et al., 1994, 2001). Zachara et al. (2004b) supplemented CKD patients with Se in the incipient stage of CKD and in the end stage and have shown that in patients in the initial stage of uremia Se supplementation enhances plasma GSH-Px activity, whereas in the more advanced stages, the increase is much lower or does not occur at all. Schiavon et al. (1994a, 1994b) suggest that the measurement of plasma GSH-Px activity in CKD patients should be complemented by the determination of serum creatinine level and creatinine clearance, and may have a considerable diagnostic value, which means that this enzyme may be regarded as an additional marker useful in assessing the extent of CRF progress.

Yet there is no conclusive evidence whether CKD patients show a lowered plasma GSH-Px activity, or decreased synthesis of this enzyme. It is thought that endogenous, toxic compounds in the blood, which exert an inhibitory effect on this enzyme, are responsible for the decrease in GSH-Px activity. During dialysis, these compounds are removed so that the enzyme activity is enhanced. To explain whether this group of patients demonstrates a decrease in peroxidase activity or its synthesis, Yoshimura et al. (1996) determined simultaneously plasma activity and protein GSH-Px concentration in several non-dialyzed patients. In the study group, the enzyme activity was over 50% lower than in controls, and plasma concentration of GSH-Px protein was reduced or undetectable. The authors conclude that the decreased plasma activity of this enzyme in CKD patients was associated with its low concentration. The data reported by them (Yoshimura et al., 1996) show that the lowered plasma concentration of GSH-Px protein may be attributed to impaired biosynthesis of this enzyme in the kidney. The authors made other interesting observations. Since it had previously been thought that the decreased plasma GSH-Px activity in CKD patients may result from Se deficiency (Richard et al., 1991), they determined Se concentration in plasma and GSH-Px activity in red blood cells, and observed normal Se concentration and higher GSH-Px activity in red blood cells in patients than in the control group. The conclusion was that the lowered plasma GSH-Px activity cannot be attributed to blood Se deficiency. With regard to the concentration of GSH-Px protein, Roxborough et al. (1999) presented results completely opposite to those reported by Yoshimura et al. (1996). They obtained specific antibodies against GSH-Px of human plasma and determined plasma concentration of GSH-Px protein in the control group and in patients on dialysis. The significant findings were as follows: enzyme activity in patients before dialysis was over 50% lower than in healthy persons and it increased significantly after dialysis, but did not reach the value observed in the control group; secondly, before and after dialysis, plasma concentration of GSH-Px protein was at the same level, and, even more importantly, it did not differ from that observed in healthy persons.

Zachara et al. (2009) supplemented 30 patients on HD for 3 months with 200 μg Se/day (yeast Se) and have shown that plasma element concentration increased from 42 ng/mL (0 day) to 102 and 132 ng/mL after 1 and 3 months, respectively ($P < 0.0001$), but plasma GSH-Px protein level did not change significantly and was 11.4 μg/mL at the beginning (4.2 times

lower as compared with the control group – 48.4 μg/mL) and 11.8 μg/mL after 3 months. This level was similar to the group on placebo. These data show that Se supply to CKD patients on HD has no effect on the level of plasma GSH-Px protein (Fig. 7).

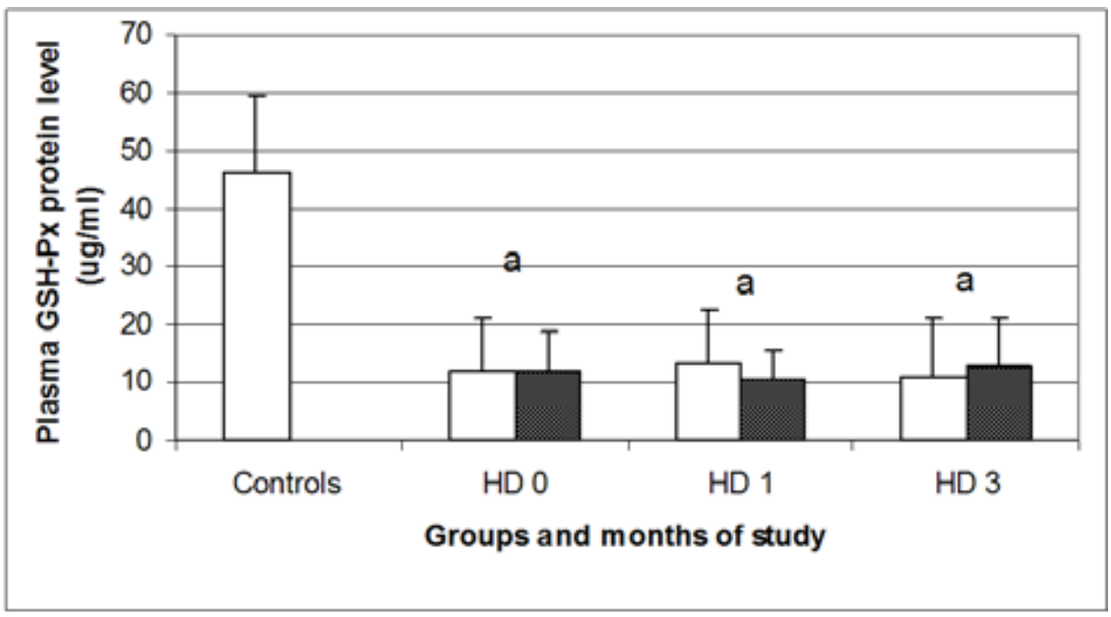


Fig. 7. Plasma GSH-Px protein level in healthy subjects and in CKD patients on HD supplemented with selenium and placebo. CKD patients on hemodialysis at the beginning of the study (HD 0) and after 1 and 3 months (HD 1 and HD 3, respectively) of Se or placebo supplementation. HD patients: white columns = placebo, black columns = + Se. Statistics: a, $P < 0.0001$ vs. controls (taken from Zachara et al., 2009).

Our results on the effect of Se supplementation to HD patients on GSH-Px protein level are the first published to date. Thus the question of the effect of Se on GSH-Px level in HD patients remains open and the matter requires further study.

5. Antioxidants in patients after kidney transplantation and the effect of Se supplementation

Kidney transplantation is one of the ways of kidney replacement therapy. Currently, kidney transplantation is the treatment of choice in patients with ESKD, showing higher survival rates than dialysis (Luque Galves et al., 2005). Kidney transplantation is now the only treatment that may restore plasma GSH-Px in patients suffering from ESKD. Data on Se concentration and GSH-Px activities, and some other parameters, in blood of patients after kidney transplantation is still rather scarce. To the best of my knowledge there were, except ours, two research centers reporting the results on the antioxidant status in patients in early stages after kidney transplantation: one in the United States (Avissar et al., 1994; Whitin et al., 1998) and one in Spain (De Vega et al., 2002; 2003). In this article I will focus on the GSH-Px.

In 1990 Avissar et al. (1994), searching for the source of plasma GSH-Px, studied the activity of this enzyme in the serum and plasma of anephric patients (cf. Zachara et al., 2006). They found that the activity was 22.6% of that noted in matched controls and was also significantly lower than plasma GSH-Px activity in HD patients. The authors also examined

GSH-Px activity and Se concentration in plasma of HD patients and controls. The patients' plasma Se levels were within the normal range, while GSH-Px activity of HD patients was 42% of the activity of the control group ($P < 0.001$). In this way the authors proved that the reduction in plasma GSH-Px activity could not be attributed to the lowered Se concentrations because: 1) the Se level in the patients' plasma was not significantly different from that in the controls; 2) when the patients were divided into two subgroups by Se levels (with the level below 96 ng/mL, and higher than 138 ng/mL), there was no significant difference in their plasma GSH-Px activity, and 3) the patients showed high (142% of the control value) red blood cell GSH-Px activity. In the sera of patients who had undergone transplantation, Se levels and GSH-Px activities were measured 22 and 30 days after surgery. Se level was the same as before transplantation, while plasma GSH-Px activity was eight times higher than before surgery and two times higher than in the controls. Several months after transplantation, plasma GSH-Px activity returned to normal values of healthy persons. Whitin et al. (1998), from the same center, transplanted kidneys in three groups of patients: 1) sixteen adults with renal disease who received a kidney transplant from related donors; 2) six adult patients who received cadaveric kidney transplants; and 3) three pediatric patients undergoing bilateral nephrectomy with subsequent kidney transplantation from related donors (cf., Zachara et al., 2006). Before transplantation, the HD patients had plasma GSH-Px activity of 34% (group 1) and 50% (group 2) of the control plasma, while the anephric individuals (group 3) had plasma GSH-Px activity ranging from 2 to 24% of that recorded in controls. After transplantation, plasma GSH-Px activity increased very rapidly: in group 1, the enzyme activity was two times higher three days after transplantation than before the operation, and 21 days post-transplant the averaged activities were within the normal values. In group 2, plasma GSH-Px activity increased rapidly over the first two weeks post kidney transplant. In six patients of this group, plasma GSH-Px activity reached normal levels after 9.8 days, and 27 days post-transplant the plasma enzyme activity reached a maximum level – 144% of the control value. The maximum level was temporary and after several weeks it decreased to a range similar to that of healthy individuals.

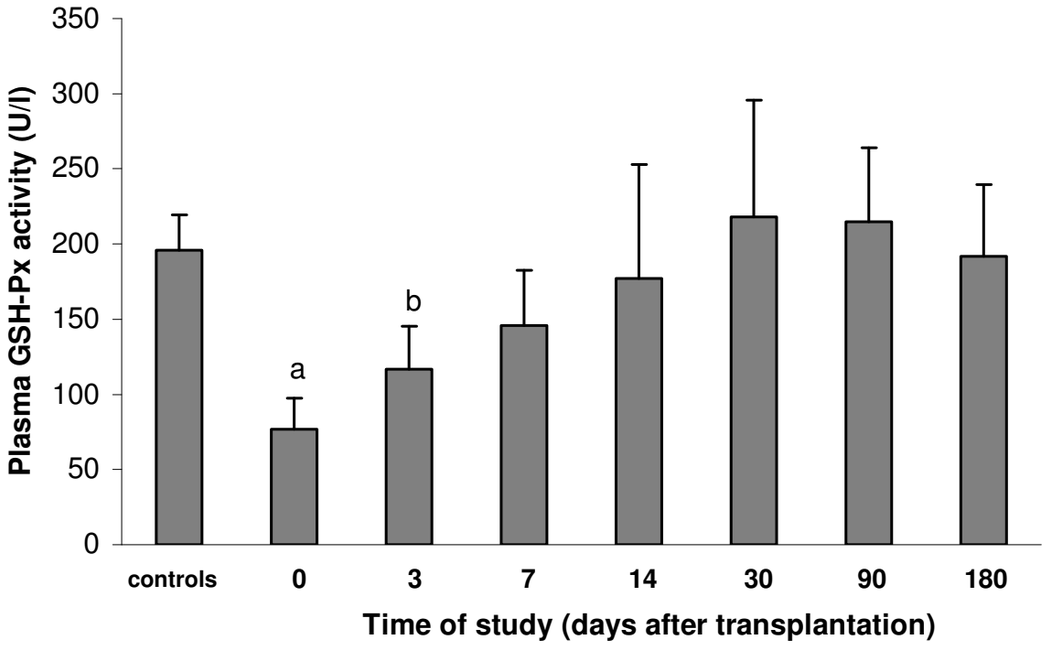
Our group studied the status of some antioxidants in the blood of patients before and in the early stages after kidney transplantation. I will focus my attention on some of the antioxidant parameters studied by us, namely on Se concentrations, red cell and plasma GSH-Px activities and some other parameters (Wlodarczyk et al., 2003). We found that whole blood and plasma Se concentration in patients before transplantation (79.2 and 64.3 ng/mL, respectively) was significantly lower as compared with healthy subjects (93.5 and 78.8 ng/mL, respectively; $P < 0.001$). The element concentration decreased significantly both in whole blood and in plasma ($P < 0.05$) within a week period after transplantation but returned to the values of the control group after 30 days. In the next two months there was a further, slight increase in Se concentration. Morris-Stiff et al. (2005) have shown that in 30, out of 40 patients before transplantation Se level in plasma was lower than in healthy subjects, but this was normalized in the majority of patients within 3 months. A decrease of Se in the first few days after surgery in our study is very likely associated with the restricted protein intake (the main source of Se) just after surgery. During the first 36-48 h after surgery, the patients received only fluids containing no protein or selenium. From the second/third day, a diet containing protein was introduced, so that the Se level increased slowly. GSH-Px activity in the red cells of patients before transplantation (15.3±3.6 U/g Hb) was the same as in controls (16.3±2.7 U/g Hb) and did not change

during the entire period of the study (it ranged from 15.3 to 16.3 U/g Hb). However, plasma GSH-Px activity of patients before surgery (76.7±20.6 U/L) was lower by 61% as compared with healthy controls (196±23.4 U/L $p<0.0001$). After transplantation, plasma GSH-Px activity increased very rapidly: by 53% after 3 days ($p<0.0001$), 90% after 7 days, and 2 weeks following surgery it almost reached the value of the control group (177 U/L). Two weeks later (at day 30 after transplantation) the activity exceeded the value of the control group. This higher activity was temporary and, similar to Whitin's study (1998), after several months it decreased to a range similar to that of healthy controls (Fig. 8).

Our results for the rapid increase in GSH-Px activity in plasma after kidney transplantation are in accord with the data of Whitin et al. (1998) who found that at 21 days post-transplant, the averaged plasma GSH-Px activity reached the normal value. De Vega et al. (2002, 2003) have shown that before transplantation plasma GSH-Px activity was – similar to our study – significantly lower than in the controls. The surprising results of these authors were that 48 hours following transplantation plasma GSH-Px activity decreased by 11% as compared to the pre-transplant value, whereas 7 and 14 days after surgery the enzyme activity increased by only 11 and 17% as compared to the initial value ($P > 0.05$). The cause of the decreased GSH-Px activity two days after transplantation and the very slow increase at a later stage remains unclear and open to speculation.

The plasma creatinine level, which in patients before surgery (6.93±2.29 mg/dL) was significantly higher ($p<0.0001$) than in the control group (0.8±0.1 mg/dL), decreased during the study and after 3 months it was 1.57 mg/dL. These data (plasma GSH-Px activity and creatinine concentration) indicate that the transplanted kidney takes up its function very rapidly. A highly significant negative correlation was found between creatinine level and plasma GSH-Px activity ($r = -0.588$, $p<0.001$).

According to my knowledge there are only few reports dealing with antioxidants in the blood of patients several months – several years following kidney transplantation. Simic-Ogrizovic et al. (1998) have shown that in recipients with stable kidney function 5 years post-transplant, plasma GSH-Px activity was the same as in the controls but in patients with chronic rejection, the enzyme activity was still significantly lower. Juskowa et al. (2001), studying the red cell GSH-Px activity several years after transplantation, found normal or decreased activity of this enzyme, depending of the medical treatment. Turan et al. (1992) studied, among other antioxidants, plasma GSH-Px activity in 30 kidney transplants 5 – 53 months (mean 19.9) after transplantation and found that the activity was significantly ($P < 0.001$) lower as compared with healthy individuals (3.40 vs. 10.43 U/L). Cristol et al. (1998) studied two similar groups of patients more than one year after transplantation and showed that in both groups plasma GSH-Px protein level was higher as compared with controls, but in the group with no clinical signs of chronic rejection the enzyme protein level was higher by 60% as compared with the group of histologically proven chronic rejection. Our results on rapid increase in plasma GSH-Px activity in post-transplant patients and the data on increased synthesis of plasma GSH-Px protein (Zachara et al., 2005, 2009) clearly show that the decreased plasma GSH-Px activity is most probably a consequence of impaired synthesis of this enzyme in the kidney. A significant negative correlation between plasma GSH-Px activity and creatinine concentration found in our study also support this supposition.



Statistics: a, p < 0.0001 vs controls; b, p < 0.0001 vs initial value

Fig. 8. Plasma GSH-Px activity in patients with ESKD before and after kidney transplantation and in healthy subjects. Please note a very rapid increase in the activity of this enzyme (taken from Wlodarczyk et al., 2003).

In a number of our patients hemodialysis was introduced almost immediately after surgery (Zachara et al., 2004c) because of the absence of kidney function. There were 15 men and 15 women aged 26-67 yrs (mean = 47.6 ± 11.3 yrs). The patients received grafts from 1 related living donor, and 29 kidneys from cadavers. All of them received the allografts for the first time. The mean serum creatinine concentration of patients was 7.72±2.35 mg/dl. The control group comprised 20 healthy volunteers (mean age, 43.0 ± 9.92 yr). It is interesting that in the group of patients who underwent two or more dialyses during the study, in the third day after transplantation (group 1; n = 19) plasma GSH-Px activity was significantly lower ($P < 0.02$) than in the group which only had one or did not require dialysis at all (group 2; n = 11). We examined those patients up to three months following surgery and the activity of this enzyme in the dialyzed group, during the entire period, was significantly lower ($0.05 < P < 0.01$) as compared with group 2 (Fig. 9, left part). On the contrary, creatinine concentration in group 1, from 3 to 30 days after transplantation, was significantly lower ($0.0001 < P < 0.01$) than in group 2. (Fig. 9, right part).

When calculating the relationship between plasma GSH-Px activity and creatinine level in the entire period of the study, we found a highly significance negative correlation ($r = -650$; $P < 0.0001$). This correlation was lower in subgroup 1 ($r= -472$; $P < 0.001$) and higher in subgroup 2 ($r= -0.676$; $P < 0.0001$).

While at present we have no convincing explanation for these disparate results, one can suspect that the main factor could be the immediate or delayed function of the transplanted kidney. In patients with immediate graft function and subsequent plasma creatinine level decline, plasma GSH-Px activity increases relatively fast and reaches the levels for healthy

subjects very soon after operation. On the other hand, when delayed graft function is noted, the increase in GSH-Px activity is much slower, as shown by different correlation factors between these two groups. A satisfactory explanation of this situation will require further studies.

The amount of Se in the diet in Poland and in some European countries is low and, consequently, the dietary element intake is below the recommended value. Diet is the main source of selenium and approximately 80% of dietary Se is absorbed depending on the type of food consumed (Navarro-Alarcon & Cabrera-Vique, 2008). Patients with CKD are advised to consume limited amounts of protein (the main source of Se). Thus, patients with ESKD have low Se levels in the blood. That is why our group wanted to check the effect of Se administration on blood GSH-Px activity in patients after kidney transplantation (Wlodarczyk et al., 2005).

To my knowledge there is only one publication on the effect of Se supplementation to patients after organ transplantation (Bost & Blouin, 2009). The authors have shown that in the plasma of patients before kidney and heart transplantation (month 0) Se levels were similar (94 and 96 μg/mL). The authors studied the effect of Se (as yeast-Se) supplementation on plasma element concentration in patients of kidney and heart recipients over a period of 3 years. They found that recipients who were supplied with 200 μg Se/day, after 12 and 24 months after surgery had the same level of Se in plasma (176 μg/L), and after 36 months the level was almost the same (182 μg/L plasma). This means that in those patients Se level reached a plateau after 12 months and was 1.9 times higher than at month 0 ($P < 0.0001$).

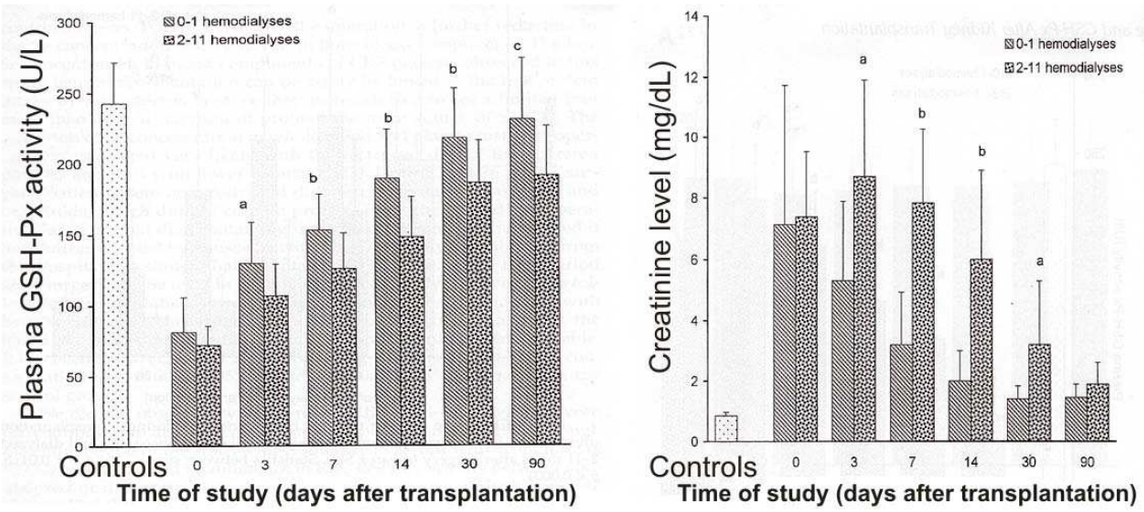


Fig. 9. (left part): Plasma GSH-Px activities before and after kidney transplantation in patients, who after surgery were not dialyzed (columns on the right) and those who had 2 – 11 HD sessions. Statistics between groups: a, $P < 0.02$; b, $P < 0.01$; c, $P < 0.05$. (right part): Creatinine levels in the same patients. Statistics between groups: a, $P < 0.01$; b, $P < 0.0001$.

Our study comprised thirty two patients: 17 were supplied with 200 μg Se/day (yeast-rich Se) and 15 patients were administered placebo. The study lasted 3 months. The data on plasma Se concentration and plasma GSH-Px activity are shown in Fig. 10. In the placebo group plasma Se concentration increased gradually from the 7th day and at day 90 it almost

reached the value of the control group, while in Se supplemented group at day 14 the level was the same as in controls and at day 90 it was higher than in controls by 39.0% ($P < 0.0001$). Kidney transplantation or Se supplementation to patients following surgery had no influence on red cell GSH-Px activity. During the entire period, in both groups (yeast-Se and placebo), this activity ranged from 14.5 to 15.7 U/g Hb and did not differ from the control group (16.7 U/g Hb; data not shown). In plasma, however, GSH-Px activity increased gradually in both groups, but after 30 and 90 days after transplantation in the Se supplied group the activity was significantly higher as compared to the placebo group. Se supplementation induces the synthesis of plasma GSH-Px in the transplanted kidney and, very likely, also in other tissues. It can thus be concluded that Se supplementation to kidney recipients has a positive effect on plasma GSH-Px activity and may be advisable in individuals affected with moderate impairment of kidney function.

6. The effect of selenium supplementation in hemodialyzed patients on the prevention of DNA damage in white blood cells

As stated in the introduction, oxidative stress, described as a disturbance in the prooxidant-antioxidant balance in favor of the former, leads to oxidative damage. It induces DNA damage, lipid peroxidation of PUFA in cell membranes, protein modification and others. Such effects are observed in CKD patients, especially in ESKD and in patients on HD. In those patients numerous diseases, such as cancer, cardiovascular complications, diabetes, atherosclerosis and neurological disorders are more frequent than in the age-matched subjects in the general population (Himmelfarb & Hakim, 2003). Many publications have shown that in patients undergoing HD some plasma antioxidant enzymes (mainly GSH-Px) activities are significantly lower (Zachara et al., 2000; Zachara et al., 2006; Yoshimura et al., 1996; Guo et al., 2009; Zwolinska et al., 2006). These enzymes, along with other antioxidants, protect the organism against ROS. ROS attack on DNA generates several modified DNA bases. The presence of these bases in cells may lead to mutagenesis. Such effects are observed in CKD patients, especially in ESKD and in patients on HD. Iseki et al. (1993) and Vamvakas et al. (1998) have shown that at the end-stage of CKD, the incidence of malignancies is higher than in the general population.. Kuroda et al. (1988) showed that in patients with diagnosed cancer of different sites (larynx, lung, stomach, liver, genitourinary tract) plasma Se concentration accounted for 99 ng/mL, whereas in the control group it was 145 ng/mL ($P < 0.001$). The evaluation of 15 extensive surveys indicated that in 10 surveys (72 484 patients at the ESKD) the cancer risk (the observed number of cancers compared with the expected number) was 7.6 (Vamvakas et al., 1998). In the majority of cases, tumors of kidney, prostate, liver and uterus were recorded. Although the pathogenic mechanisms of the enhanced incidence of cancer in CKD patients have not as yet been elucidated, Vamvakas et al. (1998) suggest that the impaired function of the immune system, reduced antioxidant capacity together with the increased ROS generation involved in DNA molecule damage and depression of DNA are the most essential factors. The diminished DNA antioxidative defense of the body leads to an intensified attack of free radicals on DNA molecules and finally to the development of malignant diseases (Ames, 1989). ROS induce various kinds of damage in DNA, e.g. oxidative damage and DNA strand cleavage (Wiseman & Halliwell, 1996). Hydroxyl radical reacts with DNA both with deoxyribose molecule, purine and pyrimidine bases (Fantel, 1996). The reaction of hydroxyl radicals with DNA results most frequently in damage to nitrogen bases, leading to the production of a

number of modified DNA bases, and finally to mutations. For example, 8-OH-guanine is a modified base. It may generate mutations contributing to a false reading of the modified base and its neighbouring bases. Transversion GC → AT is the most common change. Such a specificity of errors in 8-OH-guanine transcription plays a particular role in the mutation of gene that inhibits neoplastic changes (Fantel, 1996). Reactive oxygen species react both with nucleic and mitochondrial DNA (mtDNA). Human mtDNA is not shielded by histones, and thus it is easily damaged and the repair process is very slow (Shigenaga et al., 1994).

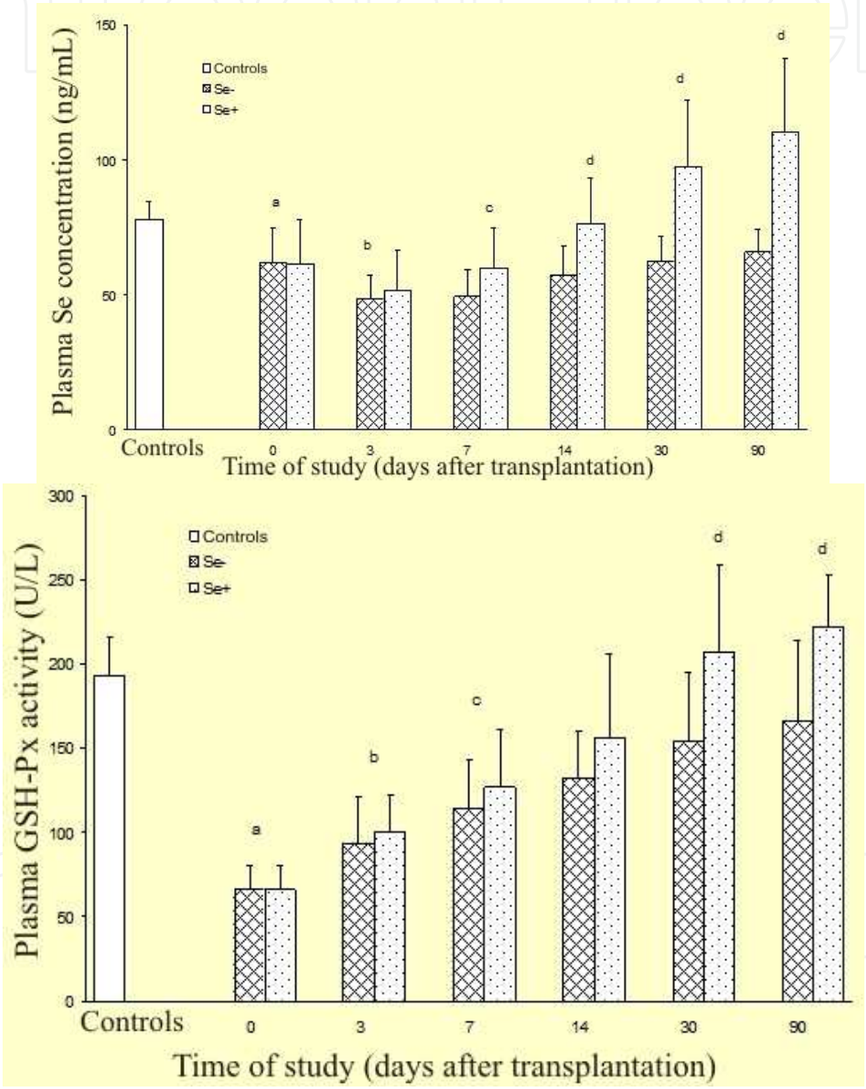


Fig. 10. Selenium concentration (left) and GSH-Px activity (right) in patients after kidney transplantation supplemented with 200 µg Se/day. Statistics: a, $P < 0.0001$ vs. controls; b, $P < 0.01$ vs. initial values; c (Se), $P < 0.02$ vs. placebo, and c (GSH-Px), $P < 0.0001$ vs. initial value; d, $P < 0.01$ vs. nonsupplemented group.

Except for the findings on frequent development of cancer in CKD patients listed above, reports on DNA damage in this group of patients are scanty in the literature. Lim et al.

(2000) investigated the frequency of mtDNA deletion in muscle sections taken from 22 CKD patients at the end-stage of the disease and in 22 healthy persons, free from any systemic diseases, matched by age and sex. Seventeen deletions (77%) were found in CKD patients and only five in the control group. These data show that mtDNA of skeletal muscles in CKD patients is extensively damaged and that these changes increase with age. Although the cause of DNA damage in CKD patients is still unknown, the authors (Lim et al., 2000) believe that accumulated uremic toxins, mostly of organic origin, are responsible for all the damage. These toxins stimulate ROS generation and damage cellular structures. ROS attack all structures, but mitochondrial genomes are thought to be most sensitive to the effect of free radicals.

The commonly used biomarkers of oxidative stress include measurement of oxidative damage to DNA (cf. Zachara et al., 2011). They can be assessed by determination of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) level in cellular DNA and the determination of urinary excretion of oxidatively modified bases/nucleosides (Dziaman et al., 2009). Worthy of attention is the fact that over the past decade another method – the comet assay – has become one of the standard methods for assessing DNA damage and repair (Collins, 2009). Being free of artifacts, it is one of the most sensitive and accurate methods, and it is also a valuable tool in assessing the role of oxidative stress in human diseases, and in monitoring the effects of dietary antioxidants (Collins, 2009). For that reason, we used the comet assay method which reflects the DNA content in the comet tail as well as the length of comet tail (Hartmann et al., 2003).

Nutrients constitute an important aspect of the antioxidant defense system with which humans have evolved (Ames, 2001). Selenium, as a nutrient, is the focus of this study and has, among others, two fundamental roles in cancer prevention: as a component of antioxidant defenses either as an agent able to scavenge free radicals or as an essential constituent of antioxidant enzymes such as GSH-Pxs (Combs, 1998; Schrauzer, 2003). Patients undergoing dialysis are at increased risk of many cancers, especially those of the kidney and urinary tract. Long periods of HD treatment are linked to DNA damage due to oxidative stress. In recent years, much attention has been paid to the role of selenium in the prevention of cancer of various organs (Valavanidis et. al., 2009; Reid et al., 2008; Gladyshev et al., 1998). Different forms of selenium compounds are administered to humans.

We did not find research in which selenium would be administered to persons with CKD on hemodialysis. Therefore we checked the effect of selenium supplementation in the prevention/repair of damaged DNA in patients with CKD on maintenance hemodialysis (Zachara et al., 2011). Forty-two CKD patients treated with regular HD were studied in a randomized, double-blind, placebo-control trials. Selenium (yeast-Se) was supplemented to 22 patients for 3 months with 200 μg Se/day, and placebo (beaker's yeast) was administered to 20 patients. The patients were dialyzed three times a week for 4 hours. The results were compared between the groups and with 30 healthy volunteers. Blood was taken from patients before the study and after 1 and 3 months, while from healthy subjects blood was taken only once. Several different parameters were analyzed, but here I will focus only on the results of DNA damage. DNA damage, including single-strand breaks (SSB) and alkali labile sites, were detected using alkaline single gel electrophoresis (SCGE; comet assay) according to the method of Singh et al. (1988) modified in our laboratory (Palus et al., 1999). The oxidative bases lesions in DNA were identified using formamidopyrimidine glycosylase

(FPG) enzyme which converts oxidized bases (Collins et al., 1997). The tail moment (tail length x tail % DNA/100) is the best indicator of DNA damage and will be presented here as the results achieved in the study.

Plasma Se concentration in patients at the beginning of the study (both groups taken together) was 23% lower compared with the controls (40.6 ng/mL vs. 52.7 ng/mL, respectively; $P < 0.0001$). In Se supplemented group it increased after 1 and 3 months to 94.6 and 115 ng/mL, respectively). In the placebo group there was no change in Se concentration throughout the study. Before the study the levels of single-strand breaks (expressed as the tail moment) were (in both groups taken together) before the study 3 times higher as compared with the control group (Fig. 11).

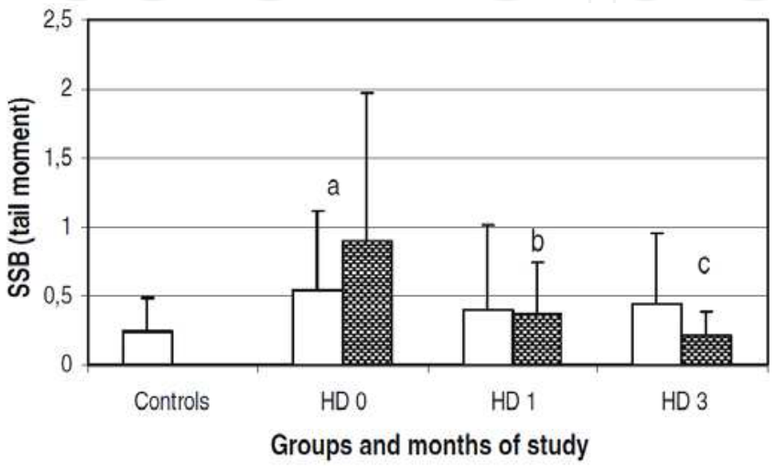


Fig. 11. Single-strand breaks (SSB) of DNA in white blood cells of healthy subjects and CKD patients on HD supplemented with placebo (white columns) and Se-yeast (black columns): HD 0 = start of the study, month 0; HD 1 and HD 3 = after 1 and after 3 months of tablets supply. Statistics: a, HD 0 both subgroups vs. controls, $P < 0.01$; b, HD 1 +Se vs. HD 0 +Se, $P < 0.02$; c, HD 3 +Se vs. HD 0 +Se, $P < 0.01$.

Almost the same values were obtained in relation to FPG: in patients they were 2.33 times higher than in the control group (Fig. 12). The higher, statistically significant levels of SSB and FPG in white blood cells of ESKD patients compared with healthy subjects at the beginning of the study shows that in those patients DNA is damaged, and that this may contribute to the development of cancer. The lower Se level in ESKD patients found in this study is probably responsible for DNA damage and may promote the development of cancer in such patients (Letavayova et al., 2006). Selenium supply in a dose of 200 μg/day led to the repair of damaged DNA. After 1 month the number of SSB was 2.4 times lower ($P < 0.02$) and after 3 months it was 4.3 times lower ($P < 0.01$). The FPG, after 3 months was 2.6 times lower compared with HD 0.

Epidemiological studies suggest that higher Se concentration reduces the risk of cancer (Letavayova et al., 2006) and increases DNA repair capacity in human fibroblasts damaged by H_2O_2 (Seo et al., 2002). Particular interest came from the clinical studies showing that dietary supply of organic Se, especially in the form of yeast enriched with Se, decreased the overall incidence of cancer twofold, above all of prostate, colorectal and lung cancers (Fisinin et al., 2009).

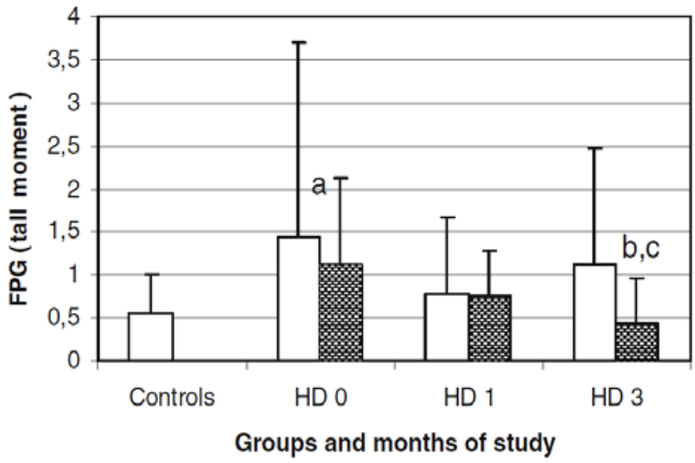


Fig. 12. Formamidopyrimidine glycosylase (FPG) in white blood cells of healthy subjects and CKD patients on HD supplied with placebo (white columns) and Se-yeast (black columns) at the beginning of the study (HD 0) and after 1 (HD 1) and 3 (HD 3)months. Statistics: a, HD 0, (both subgroups) vs. controls, $P < 0.01$; b, HD 3 +Se vs. HD 0 +Se, $P < 0.01$; c, HD 3 +Se vs. –Se, $P < 0.05$.

The data on the level of DNA damage in white blood cells of HD patients are in accord with the findings of Ribeiro et al. (2009) who have assessed the level of DNA damage by measuring the tail moment in various organs of Wistar rats in which the CKD was obtained by submitting the animals to 5/6 kidney mass ablation by ligation of kidney artery branches. The authors have also shown that CKD contributed to the damage of DNA not only in white blood cells but also in other tissues (liver, heart and kidney).

Interesting results concerning the protective effect of Se against oxidative damage to DNA in leukocytes of BRCA1 mutation carriers have recently been presented by Dziaman et al.(2009a). The authors demonstrated that BRCA1 deficiency contributes to 8-oxodG accumulation in cellular DNA which, in turn, may be a factor responsible for cancer development in women with mutations, and that the risk to developed breast cancer in BRCA1 mutation carriers may be significantly reduced in Se supplemented patients (300 µg/day) (Dziaman et al., 2009b).

The results of this study (Zachara et al., 2011) show that Se supplementation to HD patients reduced the level of DNA damage, as demonstrated by the progressive reduction of SSB and FPG in white blood cells. Longer term Se supplementation to patients on HD enhanced the protection of DNA against ROS, resulting in the reduction of the SSB and FPG levels. This dose (200 µg/day) and form (yeast enriched with Se) of the element was chosen because it had been shown that the inorganic form of Se is more toxic and less available than the organic form (Schrauzer, 2000). It is believed that the best form of Se for humans is the organic form – the selenomethionine (SeMet) (Schrauzer, 2001, 2003), and especially SeMet incorporated in yeast. It has been shown that SeMet enhances DNA repair and protects the cells against DNA damage (Laffon et al., 2010). Schrauzer (2009) has recently suggested that Se-yeast, in which the SeMet is protein-bound, shows a better effect than synthetic SeMet, since Se incorporated into protein is better protected from oxidation when exposed to air in the pure state. SeMet present in yeast is a protein-bound form, similar to the form normally present in food.

7. Conclusions

Patients with CKD and especially those treated by dialysis are at increased risk of many diseases and among others cancers, especially those of the kidney and urinary tract. Long periods of HD treatment are linked to DNA damage due to oxidative stress. It has been shown that in white blood cells of HD patients some biomarkers of oxidative damage to DNA [8-hydroxy-2′-deoxyguanosine content of leukocytes (8-OH-dG), and DNA strand breakage (alkaline comet assay)] are higher than in healthy controls. 8-OH-dG has been shown to have mutagenic properties. Reactions of ROS with DNA, proteins, and membrane lipids have been demonstrated and they can lead to mutations, inactivation of proteins and disruptions of cell integrity. Supplementation of Se to these patients resulted, as shown in this study (Zachara et al., 2011), in a significant decrease in DNA damage. The benefits of Se supply might be either through the prevention or repair of DNA damage, and they implicate at least one selenoprotein – GSH-Px 1 – in the process.

8. Acknowledgements

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9. References

Ames B.N. (1989). Endogenous oxidative DNA damage, aging, and cancer. *Free Radic. Biol. Med.* 7:121-128.

Ames B.N. (2001). DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. *Mutat. Res.* 475:7-20.

Arthur J.R. (2000). The glutathione peroxidases. *Cell. Mol. Life Sci.* 57:1825-1835.

Arthur J.R. & Beckett G.J. (2004). Newer aspects of micronutrients in at risk groups. New metabolic roles for selenium. *Proc. Nutr. Soc.* 53:615-624.

Ashton K., Hooper L., Harvey L.J., Hurst R., Casgrain A. & Fairweather-Tait S.J. (2009). Methods of assessment of selenium status in humans: a systematic review. *Am. J. Clin. Nutr.* 89:2025S-2039S.

Avissar N., Ornt D.B., Yagil Y., Horowitz S., Watkins R.H., Kerl E.A., Takahashi K., Palmer I.S. & Cohen H.J. (1994). Human kidney proximal tubules are the main source of plasma glutathione peroxidase. *Am. J. Physiol.* 266 (Pt 1):C367-C375.

Behne D. & Kyriakopoulos A. (2001). Mammalian selenium-containing proteins. *Annu. Rev. Nutr.* 21:453-473.

Beilstein MA., Whanger P.D. (1986). Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. J. Nutr. 116:1701-1710.

Boaz M., Matas Z., Biro A., Katzir Z., Green M., Fainaru M. & Smetana S. (1999). Serum malondialdehyde and prevalent cardiovascular disease in hemodialysis. *Kidney Int*. 56:1078-1083.

Bonomini M., Forster S., De Risio F., Rychly J., Nebe B., Manfrini V., Klinkmann H. & Albertazzi A. (1995). Effects of selenium supplementation on immune parameters in chronic uraemic patients on haemodialysis. Nephrol Dial Transplant. 10:1654-1661.

Bonomini M. & Albertazzi A. (1995). Selenium in uremia. Artif. Organs. 19:443-448.

Bost M. & Blouin E. (2009). Effect of supplementation with Se-enriched yeast and factors influencing Se concentration in plasma of transplant recipients. J. Trace Elem. Med. Biol. 23:36-42.

Bellisola G., Perona G., Galassini S., Moschini G. & Guidi G.C. (1993). Plasma selenium and glutathione peroxidase activities in individuals living in the Veneto region of Italy. J. Trace Elem. Electrolytes Health Dis. 7:242-244.

Brenneisen P., Steinbrenner H. & Sies H. (2005). Selenium, oxidative stress, and health aspects. Mol. Aspects Med. 26:256-267.

Brigelius-Flohe R. (2006). Glutathione peroxidases and redox-regulated transcription factors. *Biol. Chem*. 387:1329-1335.

Broderick D.J., Deagen J.T. & Whanger P.D. (1987). Properties of glutathione peroxidase isolated from human plasma. *J. Inorg. Biochem*. 30:299-308.

Brozmanova J., Manikova D., Vlckova V. & Chovanec M. (2010). Selenium: A double-edged sword for defense and offence in cancer. *Arch. Toxicol. Sept*. 25; doi 10.1007/s00204-010-0595-8.

Bulucu f., Vural A., Aydin A., & Sabal A. (2000). Oxidative stress status in adults with nephrotic syndrome. *Clin. Nephrol*. 53:169-173.

Byard J.L. (1969). Trimethyl selenide. A urinary metabolite of selenite. Arch. Biochem Biophys. 130:556-560.

Casey C.E., Guthrie B.E., Friend G.M. & Robinson M.F. (1982). Selenium in human tissues from New Zealand. *Arch. Environ. Health*. 37:133-135.

Ceballos-Picot I., Witko-Sarsat V., Merad-Boudia M., Nguyen A.T., Thevenin M., Jaudon M.C., Zingraff J., Verger C., Jungers P. & Descamps-Latscha B. (1996). Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure. *Free Rad. Biol. Med*. 21:845–853.

Chu F.F., Esworthy R.S., Doroshow J.H., Doan K. & Liu X.F. (1992). Expression of plasma glutathione peroxidase in human liver in addition to kidney, heart, lung, and breast in humans and rodents. *Blood*. 79:3233-2238.

Collins A.R. (2009). Investigating oxidative DNA damage and its repair using the comet assay. *Mutat. Res.* 681:24-32.

Collins A.R., Dobson V.L., Dusinska M., Kennedy G. & Stetina R. (1997). The comet assay: what can it really tell us? *Mutat.* Res. 375:183-193.

Combs G.F. Jr. & Gray W.P. (1998). Chemopreventive agents: selenium. *Pharmacol. Ther*. 79:179–192.

Cristol J.P., Bosc J.Y., Badiou S., Leblanc M., Lorrho R., Descomps B. & Canaud B. (1997). Erythropoietin and oxidative stress in haemodialysis: beneficial effects of vitamin E supplementation. *Nephrol. Dial. Transplant*. 12:2312-2317.

Cristol J.P., Vela C., Maggi M.F., Descomps B. & Mourad G. (1998). Oxidative stress and lipid abnormalities in renal transplant recipients with or without chronic rejection. *Transplantation*.65:1322-1328.

De Luis D. & Bustamante J. (2008). Nutritional aspects in renal failure. *Nefrologia* 28:333-342.

De Vecchi A.F., Bamonti F., Novembrino C., Ippolito S., Guerra L., Lonati S., Salini S., Aman C.S., Scurati-Manzoni E. & Cighetti G. (2009). Free and total plasma malondialdehyde in chronic renal insufficiency and in dialysis patients. *Nephrol. Dial. Transplant.* 24:2524-2529.

De Vega L., Férnandez R.P., Mateo M.C., Bustamante J.B., Herrero A.M. & Munguira E.B. (2002). Glutathione determination and a study of the activity of glutathione-peroxidase, glutathione-transferase, and glutathione-reductase in renal transplants. *Ren. Fail*. 24:421-232.

De Vega L., Fernandez R.P., Martin Mateo M.C., Bustamante J., Bustamante A., Herrero A.M. & Bustamante Munguira E. (2003). Study of the activity of glutathione-peroxidase, glutathione-transferase, and glutathione-reductase in renal transplants. *Transpl. Proc.* 35:1346–1350.

Deagen J.T., Butler J.A., Zachara B.A. & Whanger P.D. (1993). Determination of the distribution of selenium between glutathione peroxidase, selenoprotein P, and albumin in plasma. *Anal. Biochem.* 208:176-181.

Del Vecchio L., Locatelli F. & Carini M. (2011). What we know about oxidative stress in patients with chronic kidney disease on dialysis – clinical effects, potential treatment, and prevention. *Semin. Dial.*; doi: 10.1111/j.1525-39X.2010.00819.x.

Dworkin B., Weseley S., Rosenthal W.S., Schwartz E.M. & Weiss L. (1987). Diminished blood selenium levels in renal failure patients on dialysis: correlations with nutritional status. *Am. J. Med. Sci.* 293:6-12.

Dziaman T., Huzarski T., Gackowski D., Rozalski R., Siomek A., Szpila A., Guz J., Lubinski J. & Olinski R. (2009a). Elevated level of 8-oxo-7,8-dihydro-2'-deoxyguanosine in leukocytes of BRCA1 mutation carriers compared to healthy controls. *Int. J. Cancer*. 125:2209-2213.

Dziaman T., Huzarski T., Gackowski D., Rozalski R., Siomek. A, Szpila A., Guz J., Lubinski J., Wasowicz W., Roszkowski K. & Olinski R. (2009b). Selenium supplementation reduced oxidative DNA damage in adnexectomized BRCA1 mutations carriers. *Cancer Epidemiol. Biomarkers* 8:2923-2928.

El-Bayoumy K. (2001).The protective role of selenium on genetic damage and on cancer. *Mutat. Res*. 475:123-129.

Fantel A.G. (1996). Reactive oxygen species in developmental toxicity: Review and hypothesis. *Teratology* 53:196–217.

Fisinin V.I., Papazyan T.T. & Surai P.F. (2009). Producing selenium-enriched eggs and meat to improve the selenium status of the general population. *Crit. Rev. Biotechnol.* 29:18-28.

Flohe L., Günzler W.A. & Schock H.H. (1973). Glutathione peroxidase: a selenoenzyme. *FEBS Lett.* 32:132-134.

Francesconi K. (2006). Urinary excretion of selenium. In: *8th International Symposium on Selenium in Biology and Medicine,* University of Madison, Madison, WI, USA, July, 26-30; Conference book, p. 21.

Francesconi K.A. & Pannier F. (2004). Selenium metabolites in urine: a critical overview of past work and current status. *Clin. Chem*. 50:2240-2253.

Ge K., Xue A., Bai J. & Wang S. (1983). Keshan disease-an endemic cardiomyopathy in China. *Virchows Arch. A Pathol. Anat. Histopathol*. 401:1-15.

Gladyshev V.N., Factor V.M., Housseau F. & Hatfield D.L. (1998). Contrasting patterns of regulation of the antioxidant selenoproteins, thioredoxin reductase, and glutathione peroxidase, in cancer cells. *Biochem Biophys Res Commun*. 251:488-493.

Granata S., Zaza G., Simone S., Villani G., Latorre D., Pontrelli P., Carella M., Schena F.P., Grandaliano G. & Pertosa G. (2009). Mitochondrial dysregulation and oxidative stress in patients with chronic kidney disease. *B.M.C. Genomics* Aug 21; 10:388. 388; doi: 10.1186/1471-2164-10-388.

Guo C.-H., Ko W.-S., Chen P.-C., Hsu G.-S.W., Lin C.-Y. & Wang C.-L. (2009). Alterations in trace elements and oxidative stress in uremic patients with dementia. *Biol. Trace Elem. Res*. 131:13-24.

Hartmann A., Agurell E., Beevers C., Brendler-Schwaab S. & Burlinson B. (2003) Recommendations for conducting the in vivo alkaline Comet assay. 4th International Comet Assay Workshop. *Mutagenesis* 8:45-51

Himmelfarb J. & Hakim R.M. (2003). Oxidative stress in uremia. *Curr. Opin. Nerphrol.Hypertens. 12:593-598.*

Iseki K., Osawa A. & Fukiyama K. (1993) Evidence for increased cancer death in chronic dialysis patients. *Am. J. Kidney Dis*. 22:308-313.

Iwanier K. & Zachara B.A. (1995). Selenium supplementation enhances the element concentration in blood and seminal fluid but does not change the spermatozoal quality characteristics in subfertile men. *J. Androl*. 16:441-447.

Jałoszyński P., Jaruga P., Oliński R., Biczysko W., Szyfter W., Nagy E., Möller L. & Szyfter K. (2003). Oxidative DNA base modifications and polycyclic aromatic hydrocarbon DNA adducts in squamous cell carcinoma of larynx. *Free Radic Res*. 37:231-240.

Joseph M. (1995). The generation of free radicals by blood platelets. In: Joseph M. (ed.), *Immunopharmacology of Platelets*; Academic Press, London – Toronto; pp. 209-225.

Juskowa J., Paczek L., Laskowska-Klita T., Rancewicz Z., Gajewska J. & Jedynak-Ołdakowska U. (2001). Selected parameters of antioxidant capacity in renal allograft recipients. *Pol. Arch. Med. Wewn*. 105:19-27.

Klein M., Ouerdane L., Bueno M. & Pannier F. (2011). Identification in human urine and blood of a novel selenium metabolite, Se-methylselenoneine, a potential biomarker of metabolization in mammals of the naturally occurring selenoneine, by HPLC coupled to electrospray hybrid linear ion trap-orbital ion trap MS. *Metallomics*. - in press.

Koyama H., Omura K., Ejiama A., Kasanuma Y., Watanabe C. & Satoh H. (1999). Separation of selenium-containing proteins in human and mouse plasma using tandem high-performance liquid chromatography columns coupled with inductively coupled plasma-mass spectrometry. *Anal. Biochem*. 267:84-91.

Krol E. & Rutkowski B. (2008). Przewlekła choroba nerek – klasyfikacja, epidemiologia i diagnostyka. *Forum Nefrol*. 1:1-6.

Kuroda M., Imura T., Morikawa K. & Hasegawa T. (1988). Decreased selenium levels of selenium and glutathione peroxidase activity associated with aging, malignancy and chronic hemodialysis. *Trace Elem. Med.* 5:97-103.

L'Abbe M.R. & Friel J.K. (1992). Copper status of very low birth weight infants during the first 12 months of infancy. *Pediatr. Re*s. 32:183-188.

Laffon B., Valdiglesias V., Pasaro E. & Mendez J. (2010). The organic selenium compound selenomethionine modulates bleomycin-induced DNA damage and repair in human leukocytes. *Biol. Trace. Elem. Res*. 133:12-19.

Lai C.-C., Huang W.H., Klevay L.M., Gunning W.T. 3rd, & Chiu T.H. (1996). Antioxidant enzyme gene transcription in copper-deficient rat liver. *Free Radic. Biol. Med.* 21:233-240.

Letavayova L., Vlckova V. & Brozmanova J. (2006). Selenium: from cancer prevention to DNA damage. Review. *Toxicology* 227:1-14.

Lim P.S., Cheng Y.M. & Wei Y.H. (2000). Large-scale mitochondrial DNA deletions in skeletal muscle of patients with end-stage renal disease. F*ree Radic. Biol. Med*. 29:454-463.

Lockitch G. (1989). Selenium: clinical significance and analytical concepts. *Crit. Rev. Clin.Lab. Sci*. 27:483-541.

Loughrey C.M., Young I.S., Lightbody J.H., McMaster D., McNamee P.T. & Trimble E.R. (1994). Oxidative stress in haemodialysis. *Q. J. Med.* 87:679-683.

Luque Galvez M.P., Peri Cusi L. & Corral Molina J.M (2005). Living donor kidney transplantation in pediatric recipients. *Arch. Esp. Urol*. 58:553-652.

MacMillan-Crow L.A., Crow J.P., Kerby J.D., Beckman J.S. & Thompson J.A. (1996). Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. *Proc. Natl. Acad. Sci. USA*. 93:11853-11858.

Maddipati K.R. & Metrnett L.J. (1987). Characterization of the major hydroperoxide-reducing activity of human plasma. *J. Biol. Chem*. 262:17398-17403.

Mandayam S. & Shahinian V.B. (2008). Are chronic dialysis patients at increased risk for ancer? *J. Nephrol*. 21:166-174.

Marchante-Gayón J.M., Sánchez-Uría J.E. & Sanz-Medel A. (1996). Serum and tissue selenium contents related to renal disease and colon cancer as determined by electrothermal atomic absorption spectrometry. J. Trace Elem. Med. Biol. 10:229-236.

Marklund S.L. (1984). Extracellular superoxide dismutase in human tissues and human cell lines. *Clin. Invest*. 74:1398-1403.

Martin-Mateo M.C., Sanchez-Portugal M., Iglesias S., de Paula A. & Bustamante J. (1999). Oxidative stress in chronic renal failure. *Renal Fail*. 21:155-167.

Mekki K., Taleb W., Bouzidi N., Kaddous A. & Bouchenak M. (2010). Effect of hemodialysis and peritoneal dialysis on redox status in chronic renal failure patients: a comparative study. *Lipids Health Dis*. Sep 3;9:93, (in press).

Meydani M. (2002). Antioxidants in the prevention of chronic diseases. *Nutr. Clin. Care* 5:47-49.

Mills G.C. (1957). Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J. Biol. Chem. 229:189-197.

Milly K., Wit L., Diskin C. & Tulley R. (1992). Selenium in renal failure patients. Nephron 61:139-144.

Mimic-Oka J., SimicT., Djukanovic L.J., Stefanowski J. & Ramic Z. (1992). Glutathione and its associated enzymes in peripheral blood cells in different stages of chronic renal insufficiency. *Amino Acids* 2:215-225.

Morris-Stiff G.J., Oleesky D.A., Smith S.C. & Jurewicz W.A. (2005). Sequential changes in plasma selenium concentration after cadaveric renal transplantation. *Br. J. Surg*. 91:339-343.

Navarro-Alarcon M. & Cabrera-Vique C. (2008). Selenium in food and the human body: a review. Sci. Total Environ. 400:115-141.

Olson O.E. & Palmer I.S. (1999). Selenium in foods purchased or produced in South Dakota. *Food Sci*. 49:446-452.

Palmer I.S., Gunsalus R.P., Halverson A.W. & Olson O.E. (1970). Trimethylselenonium ion asgeneral excretory product from selenium metabolism in the rat urine. *Biochim. Biophys. Acta* 208:260-266.

Palus J., Dziubaltowska E. & Rydzynski K. (1999). DNA damage detected by the comet assay in the white blood cells of workers in a wooden furniture plant. *Mutat. Res*. 444:61-74.

Papp L.V., Lu J., Holmgren A. & Khanna K.K. (2007). From selenium to selenoproteins: Synthesis, identity, and their role in human health. *Antioxid. Redox. Signal*. 9:775-806.

Pasaoglu H., Muhtaroglu S., Gunes M. & Utas C. (1996). The role of the oxidative state of glutathione and glutathione-related enzymes in anemia of hemodialysis patients. *Clin. Biochem*. 29:567-572.

Paul J.L., Sall N.D., Soni T., Poignet J.L., Lindenbaum A., Man N.K., Moatti N. & Raichvarg D. (1993). Lipid peroxidation abnormalities in hemodialyzed patients. *Nephron* 64:106-109.

Persson-Moschos M., Huang W., Srikumar T.S., Akesson B. & Lindeberg S. (1995). Selenoprotein P in serum as a biochemical marker of selenium status. *Analyst* 120:833-836.

Reid M.E., Duffield-Lillico A.J., Slate E., Natarajan N., Turnbull B., Jacobs E., Combs G.F. Jr., Alberts D.S., Clark L.C. &

Marshall J.R. (2008). The nutritional prevention of cancer: 400 mcg per day selenium treatment. *Nutr. Cancer* 60:155-163.

Richard M.J., Arnaud J., Jurkovitz C., Hachache T., Meftahi H., Laporte F., Foret M., Favier A. & Cordonnier D. (1991). Trace elements and lipid peroxidation abnormalities in patients with chronic renal failure. *Nephron* 57:10-15.

Richard M.J., Ducros V., Forêt M., Arnaud J., Coudray C., Fusselier M. & Favier A. (1993). Reversal of selenium and zinc deficiencies in chronic hemodialysis patients by intravenous sodium selenite and zinc gluconate supplementation. Time-course of glutathione peroxidase repletion and lipid peroxidation decrease. *Biol. Trace Elem. Res.* 39:149-159.

Rohun K., Kuliś M., Pawłowska A., Kierzkowska I., Kwella N., Kwella B., Iłowska Bierawska A., Napora M., Całka A., Wiatr-Bykowska D., Bandurska-Stankiewicz E. & Stompór T. (2011). Identifying chronic kidney disease in an emergency department: a chance for an early diagnosis. *Pol. Arch. Med. Wewn.* 121:23-28.

Roozbeh J., Shahriyari B., Akmali M., Vessal G., Pakfetrat M., Raees Jalali G.A., Afshariani R., Hasheminasab M. & Ghahramani N. (2011). Comparative effects of silymarin

and vitamin e supplementation on oxidative stress markers, and hemoglobin levels among patients on hemodialysis. *Ren. Fail.* 33:118-123.

Rotruck J.T., Pope A.L., Ganther H.E., Swanson A.B., Hafeman D.G. & Hoekstra W.G. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179:588-590, 1973.

Roxborough H.E., Mercer C., McMaster D., Maxwell A.P. & Young I.S. (1999). Plasma glutathione peroxidase activity is reduced in haemodialysis patients. *Nephron* 81:278-283.

Saint-Georges M.D., Bonnefont D.J., Bourely B.A., Jaudon M.C., Cereze P., Chaumeil P., Gard C. & D'Auzac C.L. (1989) Correction of selenium deficiency in hemodialyzed patients. *Kidney Int.* Suppl. 27:S274-S277.

Sanz Alaejos M. & Diaz Romero C. (1993). Urinary selenium concentrations. *Clin. Chem.*39:2040-2052.

Schiavon R., Biasioli S., De Fanti E., Petrosino L., Cavallini L., Cavalcanti G., Zambello A. & Guidi G. (1994a). The plasma glutathione peroxidase enzyme in hemodialyzed subjects. *ASAIO J.* 40:968-971.

Schiavon R., Guidi G.C., Biasioli S., De Fanti E. & Targa L. (1994b). Plasma glutathione peroxidase activity as an index of renal function. *Eur. J. Clin. Biochem.* 32:759–765.

Schrauzer G.N. (2001). Nutritional selenium supplements: product types, quality, and safety. *J. Am. Coll. Nutr.* 20:1-4.

Schrauzer G.N. (2003). The nutritional significance, metabolism and toxicology of selenomethionine. *Adv. Food Nutr. Res.* 47:73-112.

Schrauzer GN. (2009). Lessons from the selenium and vitamin E cancer prevention trial (SELECT). *Crit. Rev. Biotechnol.* 29:81.

Seo Y.R., Sweeney C. & Smith M.L. (2002). Selenomethionine induction of DNA repair response in human fibroblasts. *Oncogene* 21:3663-3669.

Shigenaga M.K., Hagen T.M. & Ames B.N. (1994). Oxidative damage and mitochondrial decay in aging. *Proc. Natl. Acad. Sci. USA.* 91:10771-10778.

Sies H. (1991). Oxidative stress: from basic research to clinical implication. *Am. J. Med.* 91:31S-38S.

Simic-Ogrizovic S., Simic T., Reljic Z., Markovic S., Blagojevic R., Radivojevic D., Lezaic V., Djukanovic L. & Mimic-Oka J. (1998). Markers of oxidative stress after renal transplantation. *Transpl. Int.*11 Suppl. 1:S125-S129.

Singh N.P., McCoy M.T., Tice R.R. & Schneider E.L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.* 175:184-191.

Suzuki K.T. (2005). Metabolomics of selenium: Se metabolites based on speciation studies. *J. Health Sci.* 51:107-114.

Suzuki K.T., Kurasaki K., Okazaki N. & Ogra Y. (2005). Selenosugar and trimethylselenonium among urinary Se metabolites: dose- and age-related changes. *Toxicol Appl Pharmacol.* 206:1-8.

Suzuki Y., Hashiura Y., Matsumura K., Matsukawa T., Shinohara A. & Furuta N. (2010). Dynamic pathways of selenium metabolism and excretion in mice under different selenium nutritional statuses. *Metallomics* 2:126-132, 2010.

Temple K.A., Smith A.M. & Cockram D.B. (2000). Selenate-supplemented nutritional formula increases plasma selenium in hemodialysis patients. *J. Ren. Nutr.* 10:16-23, 2000.

Thomson C.D. & Robinson M.F. (1980). Selenium in human health and disease with emphasis on those aspects peculiar to New Zealand. *Am. J. Clin. Nutr*. 33:303-323.

Tonelli M., Wiebe N., Hemmelgarn B., Klarenbach S., Field C., Manns B., Thadhani R. & Gill J. (2009). Trace elements in hemodialysis patients: a systematic review and meta-analysis. *BMC Med*. 2009, 7:25; doi:1186/1741-7015-7-25.

Trafikowska U., Sobkowiak E., Butler J.A., Whanger P.D. & Zachara B.A. (1998). Organic and inorganic selenium supplementation to lactating mothers increase the blood and milk Se concentrations and Se intake by breast-fed infants. *J. Trace Elem. Med. Biol*. 12:77-85.

Turan B., Delilbaşi E., Dalay N., Sert S., Afrasyap L. & Sayal A. (1992). Serum selenium and glutathione-peroxidase activities and their interaction with toxic metals in dialysis and renal transplantation patients. *Biol. Trace Elem. Res.* 33:95-102.

Turi S., Nemeth I., Vargha I., Matkovics B. & Dobos E. (1991). Erythrocyte defense mechanisms against free oxygen radicals in haemodialysed uraemic children. *Pediatr. Nephrol.* 5:179-183.

Valavanidis A., Vlachogianni A. & Fiotakis N. (2009). 8-hydroxy-2' -deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis. *J. Environ. Sci. Health, part C*, 27:120-139.

Valee B.L. (2001). Thionein/metallothionein – a metabolic link. *J. Trace Elem. Exp. Med*. 14:368-369.

Valko M., Leibfritz D., Moncol J., Cronin M.T.D., Mazur M. & Telser J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. Internat. *J. Biochem. Cell Biol*. 39:44-84,.

Vamvakas S., Brüning T., Thomasson B., Lammert M., Baumüller A., Bolt H.M., Dekant W., Birner G., Henschler D. & Ulm K. (1998). Renal cell cancer correlated with occupational exposure to trichloroethene. *J. Cancer Res. Clin. Oncol*. 124:374-382.

Vendrely B., Chauveau P., Barthe N., El Haggan W., Castaing F., de Précigout V., Combe C. & Aparicio M. (2003). Nutrition in hemodialysis patients previously on a supplemented very low protein diet. *Kidney Int.* 63:1491-1498.

Wasowicz W. & Zachara B. (1987). Selenium concentration in the blood and in urine of a healthy Polish sub-population. *J. Clin. Chem. Clin. Biochem*. 25:409-412.

Whitin J.C., Tham D.M., Bhamre S., Ornt D.B., Scandling J.D., Tune B.M., Salvatierra O.,Avissar N. & Cohen H.J. (1998).

Plasma glutathione peroxidase and its relationship to renal proximal tubule function. *Mol. Genet. Metab*. 65:238-245.

Wiseman H. & Halliwell B. (1996). Damage to DNA by reactive oxygen and nitrogen species: Role in inflammatory disease and progression to cancer. *Biochem. J.* 313:17-29.

Wlodarczyk Z, Zachara B.A., Masztalerz M., Wasowicz W. & Gromadzinska J. (2003). Selenium, glutathione peroxidases and some other parameters in blood of patients after kidney transplantation. In: *4th International Symposium on Trace Elements in Human*: New Perspectives. Proceedings Book, part I. Athens, Grece, pp. 75-90.

Yang G., Zhou R., Yin S., Gu L., Yan B., Liu Y., Liu Y. & Li X. (1989). Studies of safe maximal daily dietary selenium intake in a seleniferous area in China. I. Selenium intake and tissue selenium levels of the inhabitants. *Trace Elem Electrolytes Health Dis.* 3:77-87.

Yoshimura S., Suemizu H., Nomoto Y., Sakai H., Katsuoka Y., Kawamura N. & Moriuchi T. (1996). Plasma glutathione peroxidase deficiency caused by renal dysfunction. *Nephron* 73:207-211.

Young I.S. & Woodside J.V. (2001). Antioxidants in health and disease. *J. Clin. Pathol*. 54:176-186.

Yu B.P. (1994) Cellular defenses against damage from reactive oxygen species. *Physiol. Rev*. 74:139-162.

Zachara BA. (1992). Mammalian selenoproteins. *J. Trace Elem. Electrolytes Health Dis*. 6:137-151.

Zachara B.A., Mikolajczak J. & Trafikowska U. (1993). Effect of various dietary selenium (Se) intakes on tissue Se levels and glutathione peroxidase activities in lambs. *J. Vet. Med. A*., 40:310-318.

Zachara B.A., Adamowicz A., Trafikowska U., Pilecki A. & Manitius J. (2000). Decreased plasma glutathione peroxidase activity in uraemic patients. *Nephron* 84:278-279.

Zachara B.A., Adamowicz A., T rafikowska U., A. Trafikowska, Manitius J. & Nartowicz E. (2001). Selenium and glutathione levels, and glutathione peroxidase activities in blood components of uremic patients on hemodialysis supplemented with selenium and treated with erythropoietin. *J. Trace Elem. Med. Biol*. 15:201-208.

Zachara B.A., Adamowicz A., Trafikowska U., Trafikowska A., Manitius J. & Nartowicz E. (2001a). Selenium and glutathione levels, and glutathione peroxidase activities in blood components of uremic patients on hemodialysis supplemented with selenium and treated with erythropoietin. *J. Trace Elem. Med. Biol*. 15:201–208.

Zachara B.A., Trafikowska U., Adamowicz A., Nartowicz E. & Manitius J. (2001b). Selenium, glutathione peroxidases, and some other parameters in blood of patients with chronic renal failure. *J. Trace Elem. Med. Biol*. 15:161–166.

Zachara B.A., Pawluk H., Bloch-Boguslawska E., Sliwka K.M., Kolenkiewicz J., Skok Z. & Ryc K. (2001c). Tissue level, distribution, and total body selenium content in healthy and deceased humans in Poland. *Arch. Environ. Health* 56:461-466.

Zachara B.A., Salak A., Koterska D., Manitius J. & Wasowicz W. (2004a). Selenium and glutathione peroxidases in blood of patients with different stages of chronic renal failure. *J. Trace Elem. Med. Biol*. 17:291-299.

Zachara B.A., Koterska D., Manitius J., Sadowski L., Dziedziczko A., Salak A. & Wasowicz W. (2004b). Selenium supplementation on plasma glutathione peroxidase activity in patients with end-stage chronic renal failure. *Biol. Trace Elem. Res*. 97:15-30.

Zachara B.A., Wlodarczyk Z., Masztalerz M., Adamowicz A., Gromadzinska J. & Wasowicz W. (2004c). Selenium concentrations and glutathione peroxidase activities in blood of patients before and after allogenic kidney transplantation. *Biol. Trace Elem. Res*. 97:1-13.

Zachara B.A., Wlodarczyk Z., Andruszkiewicz J., Gromadzinska J. & Wasowicz W. (2005). Glutathione and glutathione peroxifdase activities In blond of patients in early stages following kidney transplantation. *Ren. Fail*. 27:751-755.

Zachara B.A., Gromadzinska J., Wasowicz W. & Zbrog Z. (2006). Red blond cell and plasma glutathione peroxidase activities and selenium concentration in patients with chronic kidney disease: A review. *Acta Biochim. Polon*. 53:663-677.

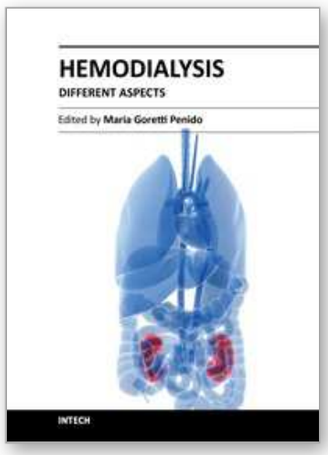
Zachara B.A., Gromadzinska J., Zbrog Z., Swiech R., Wasowicz W., Twardowska E., Jablonska E. & Sobala W. (2009). Selenium supplementation to chronic kidney disease patients on hemodialysis does not induce the synthesis of plasma glutathione peroxidase. *Acta Biochim. Polon.* 56:183-187.

Zachara B.A., Gromadzinska J., Palus J., Zbrog Z., Swiech R., Twardowska E. & Wasowicz W. (2011). The effect of selenium supplementation in the prevention of DNA damage in white blood cells of hemodialyzed patients: A pilot study. *Biol. Trace Elem. Res.*; in press.

Zagrodzki P., Barton H., Walas S., Folta M., Stompor T., Janusz-Grzybowska E., Drozdz M. & Sulowicz W. (2007). Selenium status indices, laboratory data, and selected biochemical parameters in end-stage renal disease patients. *Biol. Trace Elem. Res.* 116:29-41.

Zima T., Mestek O., Nemecek K., Bartova V., Fialova J., Tesar V. & Suchanek M. (1998). Trace elements in hemodialysis and continuous ambulatory peritoneal dialysis patients. *Blood Purif.* 16:253-260.

Zwolinska D., Grzeszczak W., Szczepanska M., Kilis-Pstrusinska K. & Szprynger K. (2006). Lipid peroxidation and antioxidant enzymes in children on maintenance dialysis. *Pediatr. Nephrol*. 21:705-710.



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The book provides practical and accessible information on all aspects of hemodialysis, with emphasis on day-to-day management of patients. It is quite comprehensive as it covers almost all the aspects of hemodialysis. In short it is a valuable book and an essential aid in the dialysis room.

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