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Antioxidant capacity in renal transplant patients

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The aim of the study was to analyse the relation between total antioxidant capacity and immunosuppressive therapies, renal function and hematocrit in kidney transplant patients. The study included 46 adult patients during the maintenance period (>1 year) following renal transplantation, treated with different combinations of immunosuppressive agents – most commonly with cyclosporine (n = 23) or tacrolimus (n = 15). The total antioxidant capacity (TAOC) of plasma was measured using Trolox-equivalent antioxidant capacity (TEAC) assay. Patients treated with cyclosporine had significantly greater TAOC compared with those treated with tacrolimus (1.16 ± 0.46 mmol/L vs. 0.80 ± 0.37 mmol/L, $p = 0.018$, respectively). There was a significantly negative correlation between TAOC and plasma creatinine ($r_s = -0.551$, $p = 0.033$) and a positive correlation between TAOC and creatinine clearance or hematocrit in patients treated with tacrolimus but not with cyclosporine ($r = 0.525$, $p = 0.045$ or $r_s = 0.629$, $p = 0.012$, respectively). Immunosuppressive therapy with cyclosporine was associated with higher TAOC. Anemia can be an independent risk factor for an increase of oxidative stress. Although subject numbers were limited, TAOC was positively associated with renal function in patients treated with tacrolimus.

1. Introduction

Oxidative stress is caused by imbalance between the produced reactive oxygen species (ROS) and the efficiency of the antioxidant system in the disorders of the homeostasis of the organism (Matyska-Piekarska et al. 2006). During recent years, the problem of oxidative stress in renal transplant patients has become more significant. The formation of ROS results from various biochemical reactions (Wang et al. 1994). It has been noted that unfavorable processes that are related to oxidative stress can take place in organs prepared for transplantation. During storage, the blood and oxygen supply is insufficient. Large amounts of ROS that are generated after transplantation and reperfusion result in damaging the cells of the transplant (Kim et al. 2009).

Oxidative stress can also be caused by immunosuppressive therapy. The most common immunosuppressive drugs used for preventing the rejection of transplanted organs are calcineurin inhibitors (CNI) such as cyclosporine (CsA) and tacrolimus (Tac). The side effects that may occur during CNI pharmacotherapy are a significant problem. Nephrotoxicity is particularly important because, among others, it is linked to the increase in ROS formation (Tada et al. 2002; Perrea et al. 2006).

Numerous studies confirm that the nephrotoxicity of CsA is caused by the increased production of ROS in the kidney during immunosuppression. Nevertheless the molecular mechanism behind the induction of ROS formation by CsA has not been fully unraveled. The increase of ROS results in impairing the kidney functions and damaging the cells of the transplanted organ (Parra et al. 2003).

The majority of researchers observed antioxidative properties of Tac. However, it was proved that in some cases Tac can act as a prooxidative agent. This may be linked to the fact that Tac decreases the activity of catalase – one of the antioxidant enzymes (Długosz et al. 2007; Tada et al. 2002).

The mechanism of antioxidation is important for protecting the kidney transplant from reperfusion-caused damage as well as the influence of CNI (Loong et al. 2004). Fending off the oxidative stress is possible by reinforcing the defense against ROS. Nevertheless one should keep in mind that the increase in total antioxidant capacity (TAOC) is not always equal to improving the antioxidative defense system. It can also be a signal of an early phase of oxygen shock or some other pathology, such as kidney insufficiency, that as a consequence result in the increase of antioxidant levels – urea in serum (Sofic et al. 1996). It was reported that supplements containing antioxidants improve renal function (Parra et al. 2003; Blackhall et al. 2005). However, it was shown that in some cases antioxidant supplementation can lead to a prooxidative effect and further damage the kidney as a consequence (Tylicki et al. 2003). In this respect TAOC monitoring is of significant importance in those patients.

The aim of the study was to analyze TAOC in kidney transplant patients depending on the applied CNI as well as on other biochemical indicators reflecting kidney activity.

2. Investigations and results

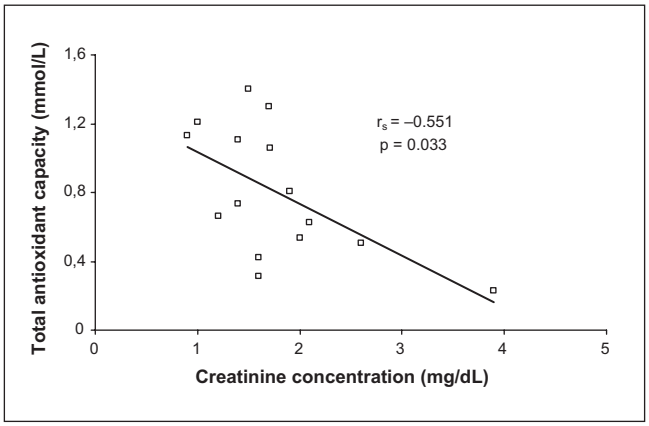
There were no differences in TAOC in terms of gender, age, post-transplant period and body mass index (BMI) (Table 1).

Table 1: TAOC in different groups of patients

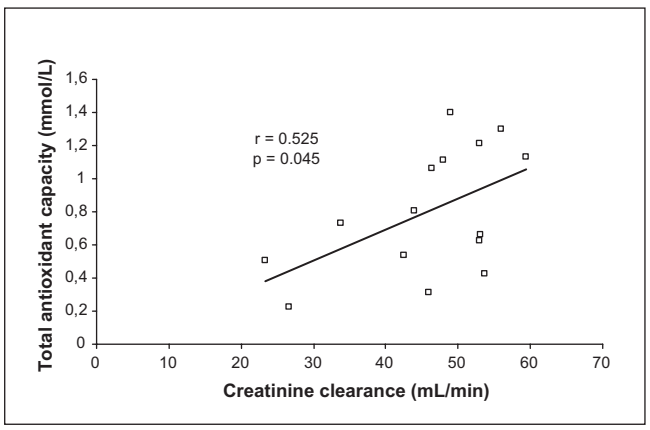
Groups of patients		Number of patients	Mean TAOC ± SD [mmol/L]	p*
Gender	Female	28	0.99 ± 0.44	NS
	Male	17	1.02 ± 0.53	
Age (years)	<50	21	0.90 ± 0.53	NS
	≥50	25	1.09 ± 0.41	
Post-transplant period (years)	<5	15	1.08 ± 0.48	NS
	≥5	31	0.96 ± 0.47	
BMI	≤25	20	0.98 ± 0.50	NS
	>25	26	1.04 ± 0.44	
Diabetes	Yes	9	0.97 ± 0.51	NS
	No	37	1.16 ± 0.25	
Immunosuppressants	CsA	23	1,16 ± 0,46	0.018
	Tac	31	0,80 ± 0,37	

* T-Student test

The lower values of TAOC were associated with higher values of plasma creatinine (Cr) (the Spearman correlation coefficient was −0.551, p = 0.033) and lower values of creatinine clearance (Cl_{cr}) (the Pearson correlation coefficient was 0.525, p = 0.045) or hematocrit (the Spearman correlation coefficient was 0.629, p = 0.012) in patients treated with Tac but not with CsA (Figs. 1 and 2). Patients with renal insufficiency (Cr > 1.5 mg/dL) had significantly lower TAOC than those with normal renal function (Cr < 1.5 mg/dL), but only in the group treated with Tac (0.64 ± 0.35 mmol/L vs. 1.04 ± 0.29 mmol/L, respectively; Fig. 3). In the group of patients treated with sirolimus (n = 12) a positive correlation between TAOC and erythrocytes was observed (the Spearman correlation was 0.592, p = 0.043). In patients who did not receive sirolimus (n = 34) low TAOC values were related to high Cr values (the Spearman correlation coefficient was −0.344, p = 0.046). The investigated correlations between TAOC and Cr, Cl_{cr} and hematocrit were not observed both in the group of renal transplant patients treated with CsA, as well as in the whole group of patients. Additionally, no statistically significant correlation between TAOC and hemoglobin was observed. In the following stage of the research



(A)



(B)

Fig. 1: Correlation between TAOC and Cr (A) or Cl_{cr} (B) in patients treated with Tac (n = 15)

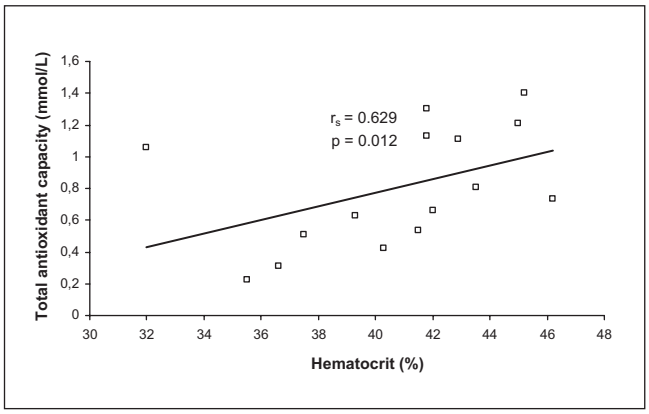


Fig. 2: Correlation between TAOC and hematocrit in patients treated with Tac (n = 15)

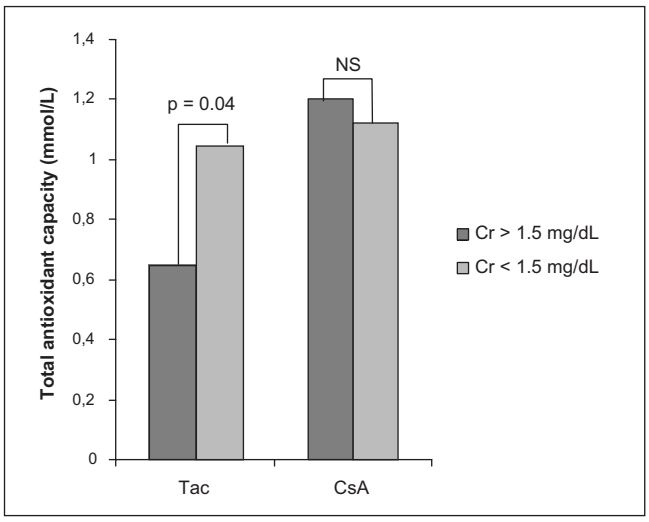


Fig. 3: Relationship between TAOC and Cr in patients treated with different immunosuppressants

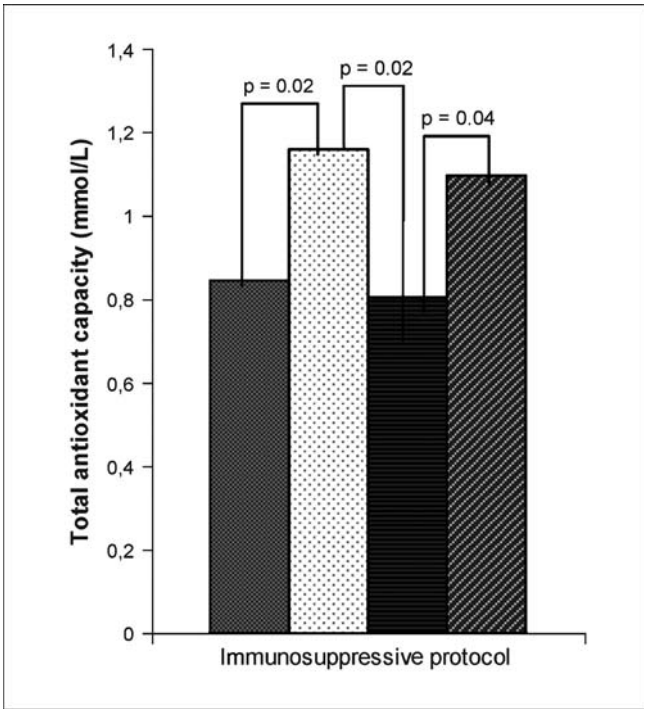


Fig. 4: Relationship between TAOC and immunosuppressive therapy in renal transplant recipients. ■ without CsA (n = 23); □ CsA based (n = 23); ■ Tac based (n = 15); ▨ without Tac (n = 31)

the relation between TAOC and the applied immunosuppressive protocols was investigated. The research revealed that the TAOC of the patients treated with CsA was statistically significantly higher than in the patients, who were not treated with CsA: 1.16 ± 0.46 mmol/L and 0.85 ± 0.43 mmol/L, respectively ($p = 0.02$, Fig. 4). The patients treated with Tac showed statistically significantly lower TAOC when compared to patients not treated with Tac, respectively, 0.80 ± 0.37 mmol/L and 1.10 ± 0.49 mmol/L ($p = 0.04$, Fig. 4). It was observed that TAOC was statistically higher in the patients treated with CsA, compared to the patients treated with Tac: 1.16 ± 0.46 mmol/L and 0.80 ± 0.37 mmol/L, respectively (Table 1, Fig. 4, $p = 0.02$). No statistical relationship in TAOC have been found for azathioprine or sirolimus use.

3. Discussion

Advanced age, diabetes and anemia are usually linked to increased oxidative stress observed in kidney recipients (Vural et al. 2005; Campise et al. 2003). In our research, despite the lack of differences in gender, age, time after transplantation and BMI, it cannot be explicitly stated that TAOC is not related to the listed factors because of the small number of patients in the particular groups.

Kidney plays a central role in the antioxidant system of the human body. Its selenium levels are higher than in other organs and the renal tubules are the main source of human extracellular glutathione peroxidase (Foster and Sumar et al. 1997; Vural et al. 2005). In chronic renal insufficiency incomplete antioxidative defense mechanism and increased prooxidative activity were reported (Descamps-Latscha et al. 2001). Our research investigated the relation between TAOC and renal function based on parameters like Cl_{cr} and Cr. The analysis was performed both in the whole group of 46 patients and in the groups of patients treated with Tac and CsA. The group of patients treated with Tac was the only one where a statistically significant negative correlation between TAOC and Cr and a positive correlation between TAOC Cl_{cr} could be observed. The revealed correlations suggest a relation between the renal functions and TAOC. This relation supports the results of Campise et al. (2003), who proved a positive correlation between Cr and oxidative stress in the group of renal transplantation patients treated with different immunosuppressive protocols. Moreno et al. (2005) and Simic-Ogrizovic et al. (1998) also observed increased oxidative stress in patients with impaired renal function.

Our research considered also the relation between TAOC and hematocrit. In the group of patients treated with Tac, a statistically significant positive correlation between TAOC and hematocrit and in the group of patients treated with sirolimus, a positive correlation between TAOC and erythrocytes were observed.

A similar problem was raised in the study of Campise et al. (2003), who proved the positive correlation between the concentration of the thiol groups occurring in glutathione – one of the most important antioxidants – and hematocrit. Erythrocytes belong to the main antioxidant system. Chronic anemia may result in decreasing the antioxidative properties of glutathione. Therefore, anemia itself can become an independent risk factor for increasing oxidative stress.

The dependency of the oxidative state and the applied immunosuppressive drugs remains unclear. Numerous researchers suggest positive antioxidant properties of Tac, however opinions on prooxidant properties of this drug are also voiced. Moreover, Długosz et al. (2007) proved the increase of lipid peroxidation in renal transplant patients treated with CsA in relation to healthy volunteers. *In vitro* research showed that CsA may increase the level of malondialdehyde (MDA), a marker of oxidative stress, in plasma. However, this is possible only if the concentration of CsA is high. Tac has antioxidant properties in low concentrations, however in high concentration it resulted in an increase of MDA concentration (Długosz et al. 2007). Varghese et al. (1998) reported statistically significantly lower TAOC values ($p < 0.03$) in the group of patients treated with Tac ($n = 10$) if compared to the group treated with CsA ($n = 23$). The authors suggested to apply an antioxidant supplementation in the group of patients treated with Tac. In our research, similarly to Varghese et al. (1998) higher TAOC values were observed in the group of patients treated with CsA. These results show that the way Tac affects the pathways of free radicals in the organism is complex and remains to be further investigated (Długosz et al. 2007).

In the summary of our research it can be stated that renal insufficiency and anemia increase the risk of oxidative stress. The patients treated with CsA showed significantly higher TAOC values than patients treated with Tac. The influence of CNI on renal function and the relation between TAOC and other factors that are clinically significant for renal transplant patients remains to be investigated.

4. Experimental

4.1. Study design and subjects

The study included 46 adult patients aged from 20 to 72 years (average 49 ± 13) during the maintenance period (>1 year) following renal transplantation, treated with different combination of immunosuppressive agents - mostly with CNI:CsA ($n = 23$) or Tac ($n = 15$) and glicocorticosteroids ($n = 43$), some with azathioprine ($n = 17$) and/or sirolimus ($n = 12$). Blood samples were collected in tubes containing EDTA. After separation of the plasma by centrifugation, a portion of plasma sample was stored at $-20\,^{\circ}C$ until analyzed.

Patient characteristics such as age, gender, post-transplant period, Cl_{cr}, Cr and other biochemical parameters are presented in Table 2.

4.2. Methods

The plasma TAOC was measured using a validated Trolox-Equivalent Antioxidant Capacity (TEAC) assay. The TEAC assay is based on

Table 2: Patient characteristics

	CsA (n = 23)	Tac (n = 15)	All (n = 46)
Gender (male/female)	9/14	6/9	18/28
Age (years)	50 ± 11	47 ± 16	49 ± 13
Post-transplant period (years)	7.3 ± 3.8	5.9 ± 2.3	6.6 ± 3.1
BMI	26.0 ± 4.4	23.1 ± 3.6	24.9 ± 4.0
Cr (mg/dL)	1.73 ± 0.80	1.77 ± 0.73	1.76 ± 0.72
Cl_{cr} (mL/min)	55.6 ± 22.2	45.8 ± 10.5	51.8 ± 19.7
Hematocrit (%)	40.0 ± 4.4	40.7 ± 4.0	39.6 ± 6.0
Red blood cells (10^{12}/L)	4.38 ± 0.55	4.44 ± 0.48	4.46 ± 0.54

scavenging of the 2,2′-azinobis(3-ethylbenzthiazoline−6-sulfonate) radical cation ($ABTS^{\bullet}+$) by the antioxidants present in a sample. The $ABTS^{\bullet}+$ is formed by the incubation of ABTS with a peroxidase (myoglobin) and H_2O_2. The $ABTS^{\bullet}+$ has a bluish-green colour with maximum absorbance values at 734 nm. $ABTS^{\bullet}+$ is reducted by antioxidant compounds, which suppresses the absorbance of the test sample corresponding quantitatively to the concentration of antioxidants present. The TEAC assay measures the collective abilities of the antioxidants in the medium concerned to scavenge the free radical in relation to that of 6-hydroxy−2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water-soluble vitamin E analogue (Miller et al. 1993).

The calibration curves were linear in the ranges 0.5–2.5 mmol/L of Trolox. The correlation coefficients varied from 0.987 to 0.998. The interassay coefficients of variation and the accuracy were in the range of 3.8–9.8 % and 96.6–102.7 %, respectively. A calibration curve was prepared for each day and the assay results are expressed as Trolox equivalent (mmol/L).

4.3. Statistical analysis

All statistical tests were performed using Statistica software version 8.0 and p value < 0.05 was considered significant. Normality was determined by Shapiro-Wilk W test. The differences between variables were estimated using Student t and Mann-Whitney test for normal and non-normally distributed data, respectively. The correlation of data was tested by using Pearson or Spearman correlation analysis.

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