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COMPARATIVE ANALYSIS OF SOME MARKERS OF OXIDATIVE STRESS IN PATIENTS WITH DIABETES NEPHROPATHY ON CHRONIOHEMODIALYSIS – FOUR YEARS STUDY

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ABSTRACT
Balance between generation and elimination of ROS is vital for the survival of aerobic organisms and their normal existence. Imbalance between levels of ROS and antioxidants is marked with the term "oxidative stress" (OS). It has been found that OS underlies a number of diseases and pathological processes, resulting in the occurrence of inflammation, hypersensitivity, autoimmune reactions, carcinogenesis etc. The nature of haemodialysis-procedure itself is a source of ROS on account of bio-incongruent dialysis membranes. This study is designed to determine to examine the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) and the changes in the amount of malondialdehyde (MDA) as a manifestation of oxidative stress in patients with diabetic nephropathy (DNP) immediately before and after chronic hemodialysis (CHD) and compare those to a control group of healthy persons, and also to trace the changes of these indicators for oxidative stress (2008 vs 2012). We found enhanced oxidative stress in all patient groups due to an increase in lipid peroxidation and changed activists of antioxidant enzymes. In conclusion, it has been noticed that the OS persists in IDDM patients whit chronic renal failure undergoing haemodialysis.

Key words: diabetes mellitus, diabetic nephropathy, oxidative stress, antioxidant enzymes

INTRODUCTION

Reactive oxygen species (ROS) are formed by oxy-reduction biochemical processes in aerobic organisms as part of their normal metabolism [1, 13, 24]. They are highly reactive and often have harmful effects on humans. Mechanisms for their elimination belong to some antioxidant enzymes. Balance between generation and elimination of ROS is vital for the survival of aerobic organisms and their normal existence. Imbalance between levels of ROS and antioxidants is marked with the term "oxidative stress" (OS):

- increased formation of pro-oxidants
- reduction of the activity of antioxidant defense mechanisms
- a combination of the two elements

The degree of OS can be measured by changes in the activity of antioxidant enzymes or by the amount of lipid peroxidation in the resultant malondialdehyde (MDA) [9].

It has been found that OS underlies a number of diseases and pathological processes, resulting in the occurrence of inflammation, hypersensitivity, autoimmune reactions, carcinogenesis etc. For years, discusses his role in diabetes, kidney disease and others. [2, 16].

Today, diabetes is increasingly being seen as an example of chronic oxidative damage [12, 37]. It is known that hyperglycemia leads to the formation of ROS caused and thus oxidative

stress is involved in the pathogenesis of chronic vascular complications of diabetes [13, 24].

Clinically, one of the worst manifestations of microangiopathy is diabetic nephropathy (DN) [5, 6, 17]. In this case, under discussion is not only the role of hyperglycemia, but also increaseing the concentration of urea as a source of ROS and causing inefficient operation of the antioxidant defense systems [5, 11, 14, 15, 21, 22]. These changes are more pronounced in patients on chronic haemodialysis treatment, because the procedure itself is an additional incentive for the development of the OS. There is evidence of adverse effects, due to biouncompatibility of dialysis membranes and activation of macrophages [23, 33]. It is possible to fall potentially toxic substances in the dialysis water flow to provoke hemolysis and passage of free iron in the circulation [29, 39]. Furthermore, the serum levels of certain trace elements, for example selenium [26] and zinc can be reduced. Of importance is also the duration of chronic hemodialysis treatment, and other factors .

AIM OF THE STUDY

To examine the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) and the changes in the amount of malondialdehyde (MDA) as a manifestation of oxidative stress in patients with diabetic nephropathy (DNP) immediately before and after chronic haemodialysis (CHD) and compare those to a control group of healthy persons. Also to be traced the changes of these indicators for oxidative stress (2008 vs 2012).

MATERIAL AND METHODS

10 patients from the hemodialysis ward of "Prof. Stoyan Kirkovich" Hospital, Stara Zagora, (5 women and 5 men) with IDDM and nephropathy aged from 40 to 66 years and diagnosed with diabetes 8 to 41 years prior were studied in 2008 and of these 6 were followed-up in 2012 (3 women and 3 men).

The patients received four hours of bicarbonate dialysis three times per week for 2 to 12 years. The type of dialysis membrane used – a cellulose diacetate membrane from the Bulgarian company "Etropal", sterilized with ethilenoxide. The membranes and the dialysis solution were not treated with antioxidants.

The control group comprised of 17 healthy subjects - eight women and nine men - aged from 18 to 66.

Venous blood was taken on the day of the study immediately before and after haemodialysis, a standard procedure. Traced was routine clinical laboratory parameters, characterizing the state of diabetes – blood sugar profile (BSP), glycosylated hemoglobin (HbA1C), lipid profile - and the degree of renal insufficiency (creatinine, urea, blood count, plasma proteins). Laboratory tests were conducted using a closed system COBAS - Integra 400 Roche, CCL at "Stara Zagora" Hospital EAD.

The activity of SOD and CAT were assessed spectrophotometrically in erythrocyte lysate in the Laboratory for antioxidant enzymes, Institute for Chemistry and Biochemistry of the Medical Faculty, TU Stara Zagora.

- CAT (KU/g Hb) - at wavelength 240 nm [7]
- SOD (KU/g Hb) - at wavelength 560 nm [34]
- MDA (mol/l) - at wavelength 532 nm [30]

The concentration of hemoglobin in the research lysate was determined by the method of cyanmethemoglobine Mahoney et al., 1993 [19].

Results are presented using the software "R: A language and environment for statistical computing" as mean ± standard error of mean.

Statistically significant differences between the results obtained in the groups were determined by Student's t test after application of the test of Shapiro for normal distribution.

Values of $p < 0.05$ are considered statistically significant

RESULTS

Was traced the activities of antioxidant enzymes SOD and CAT, and the concentration of MDA Controls and patients immediately before and after dialysis in 2008 and in 2012. The results obtained are presented in **Table 1**.

Table 1. *Activity of SOD and CAT and amount of MDA in patients with diabetic nephropathy (DNP) before and after CHD vs controls – 2008 and 2012 Values are shown as arithmetic mean ± standard error of mean / n - number of patients*

	controls n = 17	IDDM patients			
		before CHD n = 10 2008 г.	after CHD n = 10 2008 г.	before CHD n = 6 2012 г.	after CHD n = 6 2012 г.
SOD kU/gHb	2.52 ± 0.22	3.74 ± 0.62*	2.58 ± 0.51	1.44 ± 0.31*^	0.58 ± 0.15*#^
CAT kU/gHb	20.29 ± 1.64	36.22 ± 3.37*	40.33 ± 3.18*	84.29 ± 12.67*^	72.59 ± 13.48*^
MDA umol/l	1.73 ± 0.07	3.62 ± 0.18*	2.62 ± 0.11*#	3.24 ± 0.27*	3.79 ± 0.39*^

* - significant difference in the diabetic group compared to controls, p <0.05
\# - significant difference in the diabetic group before and after the CHD
^ - significant difference in the diabetic group between 2008 and 2012

In 2008 We found a significantly higher SOD activity in patients before dialysis (3.74 ± 0.62 kU/gHb) compared to controls (2.52 ± 0.22 kU/gHb), ($p < 0.05$).

In 2012 there is significant reduction of SOD in patients compared to controls (2.52 ± 0.22 kU/gHb) both before (1.44 ± 0.31 kU/gHb) and after (0.58 ± 0.15 kU/gHb) CHD, ($p < 0.05$).
Juxtaposition 2012 vs 2008: the activity of SOD in the diabetic group (before/after CHD) decreased significantly over time (before CHD 2008: 3.74 ± 0.62 kU/gHb vs 2012: 1.44 ± 0.31 kU/gHb), (after CHD 2008: 2.58 ± 0.51 kU/gHb vs 2012: 0,58 ± 0.15 kU/gHb), ($p < 0.05$).

Significant reduction in the activity of SOD in the diabetic group after CHD only for 2012 (before CHD: 1.44 ± 0.31 kU/gHb vs after CHD: 0.58 ± 0.15 kU/gHb), ($p < 0.05$). **(Figure 1)**

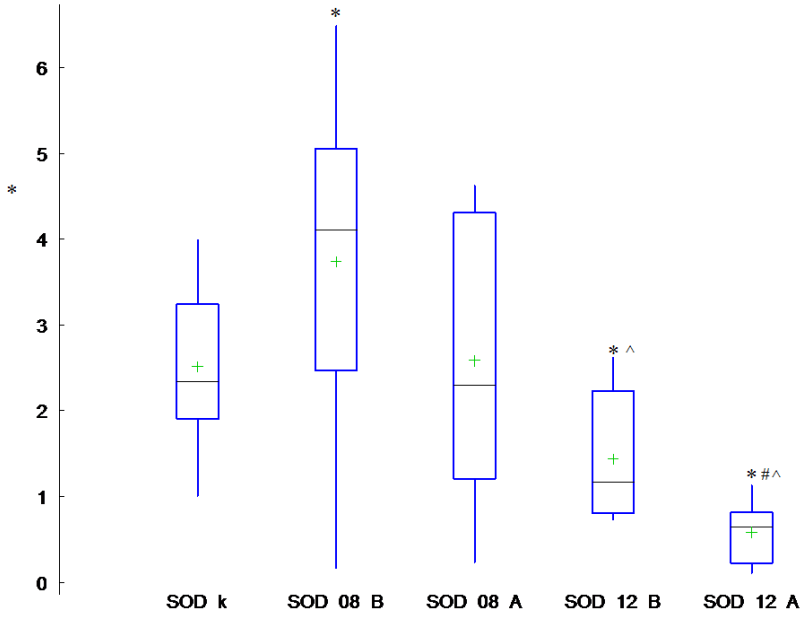

Figure 1. Comparison of SOD results.

* - significant difference in the diabetic group compared to controls, p<0.05
\# - significant difference in the diabetic group before and after the CHD
^ - significant difference in the diabetic group between 2008 and 2012

The results point that there is significant increase of CAT activity in patients compared to controls: before and after the CHD, in both study years. Also there is significant increase of CAT activity over time in both diabetic groups in both study years, before (2008: 36.22 ± 3.37 kU/gHb vs 2012: 84.29 ± 2.67 kU/gHb) and after (2008: 40.33 ± 3.18 kU/gHb vs 2012: 72.59 ± 3.48 kU/gHb) the CHD, ($p < 0.05$). **(Figure 2)**

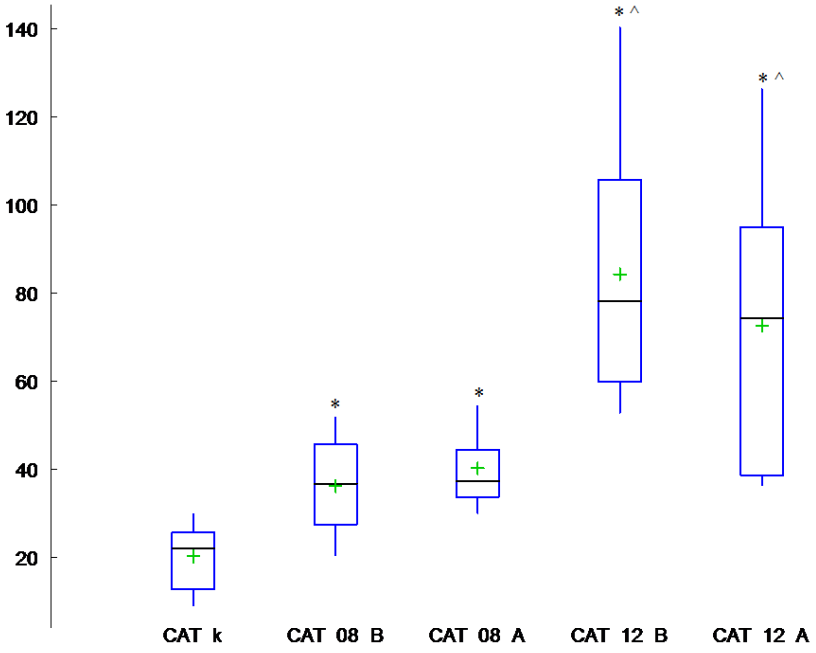

Figure 2. Comparison of CAT results.
* - significant difference in the diabetic group compared to controls, p <0.05
\# - significant difference in the diabetic group before and after the CHD
^ - significant difference in the diabetic group between 2008 and 2012

Study show statistically significant ($p < 0.05$) higher MDA levels in patients compared to controls (1.73 ± 0.07 μmol/l).

Although MDA levels decrease significantly following the procedure in 2008, they remain significantly higher compared to controls (2.62 ± 0.11 μmol/l), ($p < 0.05$). In 2012 is significant increasing of MDA levels in patients after haemodialysis compared to 2008 (2008: 2.62 ± 0.11 μmol/l vs 2012: 3.79 ± 0.39 μmol/l), ($p < 0.05$). **(Figure 3)**

CONCLUSIONS

It is well known that diabetes is accompanied by pronounced oxidative stress. Oxidative damage can cause an imbalance in the activity of antioxidant enzymes. Of particular interest is the role of OS in patients with CKD and undergoing hroniohaemodialysis. There are lots of accumulated data about changes in the antioxidant status of the body and the degree of lipid peroxidation associated with the process of hemodialysis , and the type of dialysis membranes , which have been described different biocompatibility [21,23,3,28]. Most of these data are mixed.

In this study, using selected indicators of antioxidant activity and lipid peroxidation was studied OS in patients with insulin-dependent DM and nephropathy on chronic hemodialysis treatment . We tried to trace change their OS , respectively, over time.

In our study, the levels of MDA in patients immediately before and after CHD were found to be significantly higher compared with the controls for both study years. We reported a statistically significant decrease in 2008 after the procedure. This was consistent with literature [4, 7, 8, 9, 18, 25, 27, 31, 35, 36].

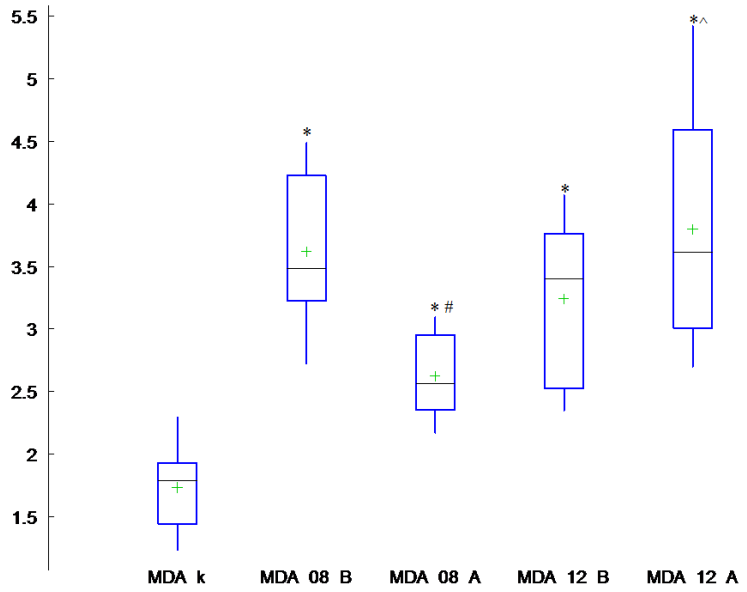

Figure 3. Comparison of MDA results.
* - significant difference in the diabetic group compared to controls, p <0.05
\# - significant difference in the diabetic group before and after the CHD
^ - significant difference in the diabetic group between 2008 and 2012

However a significant increase of MDA was found in the diabetic groups over time (2012 vs 2008), as well as a significant increase of MDA in 2012 after CHD; Hence, in IDDM patients undergoing continuous hemodialysis intense lipid peroxidation takes place and OS is observed. The established trend of decrease in SOD activity over time (2012 vs 2008), can be considered a marker for cell damage; [20, 32, 38]. The reason for increased CAT activity can be linked to a secondary compensatory activation of the enzyme. Catalase activity remained significantly high after dialysis relative to controls in both study years, with values similar to the high levels before CHD. CAT activity increased also significantly over time. This increased activity indirectly proves the presence of OS in patients with IDDM and CKD. The fact that MDA levels (respectively lipid peroxidation) is high in IDDM patients with CKD on CHD, is enough to warrant uremia as a secondary source of reactive carbonyl derivatives (carboxymethyllyzin, pentosidine, MDA, etc.) [1, 10, 13, 14, 16, 21, 22].

OS in patients with IDDM and CKD on dialysis persisted after dialysis treatment. Great importance is given to the biocompatibility of dialysis membranes [3,23,33]. For these reasons, the study of dialysis membranes, hemodialysis techniques and use of various exogenous antioxidants, scavenging of reactive oxygen species, is critical for ensuring the quality of life of patients undergoing chroniohaemodialysis.

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