Antioxidant Status in Hemodialysis Patients

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Abstarct: The increment of reactive oxygen species (ROS) may be a result of many diseases (cardiovascular, cancer) as well as hypercatabolic states such as sepsis and repeated hemodialysis. The aim of our study was to see the changes of ROS expressed through serum total antioxidant status (S-TAS) in relation to dialysis therapy. S-TAS was performed on patients (pts) on maintenance hemodialysis or acute renal patients treated with hemodialysis. 26 patients were included in the study (12 pts with acute renal failure and 14 pts with chronic renal failure). S-TAS normal range was 0,00 to 1,49mmol/l and it was measured at the beginning of hemodialysis, after first hemodialysis treatment and after 5 treatment with antioxidant drugs. Decrease of the value of S-TAS has been observed in 22 pts in regard of prehemodialysis values, and means percentage of decrease was 25%. It must be emphasized that the prehemodialysis values of S-TAS were above normal values in 23 pts (from slightly increased to a 68% above the normal values). The decreased of S-TAS during the first hemodialysis is partly due to the dialysability of many components of S-TAS (ascorbic acid, uric acid, partially tocoferols). But in 2 pts were not noticed changes in the values. After the third measurement the values of S-TAS were slightly increased in 6 pts and in the other there were not big differences in comparison with the second measurement. When the level of ascorbic acid is reduced, the capacity of vitamins E radicals to bind ROS is diminished. Therefore, the authors consider that a dietary supplementation of antioxidant medications, particularly ascorbic acid, is needed in hemodialysis patients and further follow up of its effect. **Material and methods:**Twenty-six patients (15 male and 11 female) were included in the study in the period of seven months (from February to August). Mean age was 48,8±17.8 (from 16 to 75) years. Twelve of the patients were with ARF and 14 with CRF but all of them were treated with heamodialysis. Total antioxidant status (TAOS) was determined spectrophotometrically using Perkin Elmer, UV/VIS, and (LAMBDA 2S) analyzer. Blood samples were taken before the first treatment with HD, after first HD, and finally after 5 days treatment with amp Multibionta R in infusion (1 amp 10ml is consisting of: Vit. A 3000 IE 1,65 mg, Vit B1 10mg, Nicotinamid 40mg, Dexpanthenol 25mg, Vit. B6 15mg, Vit. C 100mg, Vit. E 5mg).Statistical analysis has been performed by the **statistical package**, Statistic for Windows 5.0. Statistical data processing has been perform with parametric analysis for comparesment of two equivalent groups of patients (before and after the treatment) using Wilkoxon test, for unpaired analysis-Kolmogorof Smirnov test and for correlative analysis Spareman test with significance $p<0.05$.

Index terms:antioxidant status,Hemodialysis

1 INTRODUCTION

Free oxygen radicals are toxic for living cells and they are involved in the pathogenesis of many diseases. This wide spectrum begins with aging, increased risk of atherosclerosis, malignancy, cardiovascular disease and including renal diseases also. Patients with end-stage renal diseases (ESRD) particularly that receiving regular hemodialysis (HD) manifest pronounced oxidative stress (OS). They have a high incidence of premature cardiovascular disease. (1) Cardiovascular complications account for about 50% of deaths in dialysis pts that is a much higher proportion than in the general population. (2)Antioxidants, including vitamin C, Selenium, vitamin E and gluthatione peroxidase in erythrocytes have been shown decreased in patients with chronic renal failure (CRF) (3). Also there is difference in OS and antioxidative system response depends on a type of HD membrane. (4)

Reactive oxygen metabolites (superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid.) are mediators in oxidative injury in patients with acute renal failure (ARF) and are considered to be an important mechanism in patophysiology of this syndrome. Decrease of intracellular and extrace-

llular fluid and presence of endotoxemia may cause impaired antioxidant defense. (5,6)Antioxidants are the substances that protect the tissues from free radical attack by preventing free radical formation, by blocking chain reaction or by repairing the oxidatively damaged bio-molecules. There are a number of antioxidants present in the body and derived from the diet. Based on the location, they can be divided into intracellular and extra cellular antioxidants. Intracellular enzymatic antioxidants are Superoxide Dismutase (SOD), Catalase and Glutathione Main non-enzymatic cellular antioxidant is reduced glutathione (GSH). Glutathione reductase (GR), Antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase maintain a reducing tone within cells. Catalase is a common enzyme found in nearly all living organisms .An enzyme found in the blood and in most living cells that catalyzes the decomposition of hydrogen peroxide into water and oxygen.It is a tetramer of four polypeptide chains, each over 500 amino acids long (7). It contains four porphyrin heme (iron) groups that allow it to react with the hydrogen peroxide. The optimum pH for catalase is approximately 7 (8), while the optimum temperature varies by species (9). Glutathione is found in two forms, a monomer that is a single molecule of the protein, and is the active form of glutathuine; and second a dimmer that is two of the single molecules joined together. The monomer is sometimes called reduced glutathione, while the dimmer is also called oxidized glutathione. The monomer is the active form of glutathione. Oxidized glutathione is broken down to the single molecule by an enzyme called glutathione reductase. Glutathione peroxidase (GPx) is a selenium-containing enzyme whose blood level is a good indicator of the selenium status of the animal; occurs in a plasma form, an enzyme with specificity for phospholipids, and an intracellular form. Glutathione reductase (GR) is a flavin enzyme involved in the defense of the erythrocyte against hemolysis. A partial deficiency occurs relatively frequently but is due to a deficiency of riboflavin. In mammalian tissues, there are at least three district superoxide dismutase isoenzymes, including one managanese from (Mn-SOD) present in the mitochondrial matrix and two copper and zinc forms (Cu, Zn-SOD), one of which is in the cytosol and the other in various extracellular fluids. Superoxide dismutases play a key role in catalyzing the dismutation of O2 .- to O2 and H2O2 . Catalase or GSH-Px must then remove the hydrogen 1468 peroxide formed. In the presence of transition metals, H O can be 2 2 reduced (in the metal-catalyzed Haber-Weiss sreaction) to the extremely reactive ·OH. In many tissues, catalase activity, largely localized to peroxisomes, is very low and frequently not available for decomposition of H O . 2 2 Several extracellular antioxidants such as proteins (Transferrin, lactoferrin, albumin, ceruloplasmin), and urate prevent free radical reaction in the body sequestering transition metal ions by chelation in plasma. Albumin, bilirubin, and urate may also scavenge free radicals directly. Furthermore, plasma has a considerable peroxyl radical scavenging ability, which is mainly determined by its content of ascorbic acid.Some antioxidants are located both intra and extracellularly, such as alpha tocopherol, which is the major lipid soluble antioxidant, present in cellular membrane and plasma lipoproteins. It is an effective chainbreaking antioxidant that protects polyunsaturated lipids from peroxidation by scavenging peroxyl radicals. The effect of free radicals is immediately counteracted by chain breaking antioxidant mainly vitamin E, C and thiols. Reports on plasma vitaminE levels in dialysis patients are highly variable with some reporting low levels (10) and some arguing against them (11-13). Vitamin C is another important antioxidant especially due to its ability to regenerate vitamin E from the vitamin E radical (14-17). Dialysis has been shown to decrease plasma vitamin C levels (18).

2 Materail and methods:

Twenty-six patients (15 male and 11 female) were included in the study in the period of seven months (from February to August). Mean age was 48,8±17.8 (from 16 to 75) years. Twelve of the patients were with ARF and 14 with CRF but all of them were treated with heamodialysis. Total antioxidant status (TAOS) was determined spectrophotometrically using Perkin Elmer, UV/VIS, and (LAMBDA 2S) analyzer. Blood samples were taken before the first treatment with HD, after first HD, and finally after 5 days treatment with amp Multibionta R in infusion (1 amp 10ml is consisting of: Vit. A 3000 IE 1,65 mg, Vit B1 10mg, Nicotinamid 40mg, Dexpanthenol 25mg, Vit. B6 15mg, Vit. C 100mg, Vit. E 5mg).

Statistical processing of material examined. Statistical analysis has been performed by the statistical package, Statistic for Windows 5.0. Statistical data processing has been perform with parametric analysis for comparesment of two equivalent groups of patients (before and after the treatment) using Wilkoxon test, for unpaired analysis-Kolmogorof Smirnov test and for correlative analysis Spareman test with significance $p<0.05$.

3 Results

Pts No	Age	Gen	TAOS I	TAOS II	TAOS III	D	DM	Mgl	HpD	SD
1	65	M	1,68	1,43	1,43	A				
2	58	M	2,40	1,81	1,81	C				
3	62	M	1,41	1,23	1,23	C		Yes		
4	39	M	1,49	1,26	1,26	A			Yes	
5	63	M	1,91	1,32	1,44	A				Yes
6	32	M	2.32	1,83	1,40	C				
7	42	F	2.20	1.65	1.24	C				
8	71	M	1.95	1.40	1.32	A		Yes		
9	18	F	2.39	1.28	1.50	A				
10	59	M	2.50	2.08	2.28	C				
11	55	F	2.22	2.21	2.21	A		Yes	Yes	
12	75	M	2.50	2.13	2.13	A				
13	39	F	1.57	1.77	1.77	C				
14	55	F	1.64	1.25	1.25	A				
15	48	F	1.95	1.51	0.80	C				
16	28	F	1.89	1.06	1.09	C	Yes			
17	68	M	0.99	0.88	0.99	C				
18	72	M	2.02	1.47	1.36	C		Yes		
19	16	M	2.50	2.50	2.50	A				
20	19	F	2.50	2.50	2.50	C				Yes
21	71	F	2.29	2.23	2.23	C			Yes	
22	41	M	2.50	2.26	2.27	A				
23	62	F	2.50	2.40	2.36	C	Yes			
24	38	M	2.00	1.85	1.65	C				Yes
25	35	M	1.85	1.77	1.75	A				
26	40	F	1.75	1.78	1.69	A				

Gen-gender, TAOS-total antioxidant status, Dg-diagnosis, A-Acute renal failure, C-Chronic renal failure, M-male, F-female, Mgl-malignancy, DM-Diabetes Mellitus, HpD-hepatic disease, SD-systemic disease.

4 DISCUSION:

The aim of treatment by dialysis is to replace all the lost functions of the natural kidneys, as far as possible using artificial means. The process of separating solutes using semi permeable membrane in vitro and termed the word “Dialysis”. Dialysis should be instituted when ever early signs of uremia (anorexia, nausea, vomiting and occasionally pericarditis) are present or if fluid overload, electrolyte disorders or acidosis cannot be otherwise controlled. No specific value of creatinine or urea is regarded as critical. People with advanced chronic renal failure (CRF) who have progressed to end stage renal disease (ESRD) usually require dialysis. Hemodialysis requires having a fistula created in the forearm several months before it can be started. The need for dialysis is indicated by various findings in blood analysis, such as a high creatinine level and high levels of blood urea nitrogen, and by a glomerular filtration rate that is, at most, 15 and usually les than 10. Pericarditis (inflammation of the sac that surrounds the heart) is associated with end-stage renal disease (ESRD) and indicates the need for dialysis. The preparation of patients with ESRD for replacement therapy should begin with providing vascular access will be in advance of its need. The Cimino-Brescia arterio-venous fistula in the furred arms is the access of choice for most patients. The standard dialysis schedule is 4 — 5 hours, three times per week depending on residual function, age, body weight and fluid weight status. The initial 4-5 dialysis treatments should be considerably shorter (2-3 h) to prevent the development of the so called dialysis disequilibrium. Dialysis and Antioxidants: Importantly, major antioxidant trials have observed a neutral effect of vitamin E, a lipid-soluble reactive species scavenger, on cardiovascular outcomes (19]. In patients with mild to moderate CKD at high cardiovascular risk, one study showed vitamin E at

dose 400 IU/day has shown no effect on cardiovascular outcomes (20). Exceptionally, the Secondary Prevention with Antioxidants of Cardiovascular disease in ESRD (SPACE) trial reported a benefit of 800 IU/day vitamin E on major cardiovascular outcomes (21). Treatment of HD patients 2.Discussion with the thiol-containing antioxidant N-acetylcysteine significantly decreased cardiovascular events compared with the placebo group (22). However,neither of the studies showed effect of treatment on overall mortality in HD patients. Apparently, there is a need to further elucidate the mechanisms by which reactive species may lead to vascular injury. Effects of Free Radicals on Biological System: Free radicals do not only exert disadvantageous effects, but are also formed deliberately in the body for useful purposes and have important physiological functions. One of the well defined roles of free radicals is when activated phagocytic cells produce superoxide anion radicals and hydrogen peroxide as one mechanism to kill bacteria and fungi and to inactivate viruses. In a biologic system, free radical attack takes place in the presence of an unbalanced ratio between free radicals and antioxidants. Antioxidants Status in Haemodialysis: Ant

The referential values for the TAOS estimated in this way are in the frames of 0.00-1.49mmol/L. The majority of our patients (above 92% or in 24 pts) have the first values above the upper limit. It suggests the existence of oxidative stress, which is due to the primary disease (ARF, CRF). The statistical correlation between the measured values of TAOS (I, II, III) and the primary renal disease (ARF, CRF) does not show any significance in the correlation. Also, there was not any significance in the TAOS values regarding the sex, age, and existence of other chronic disease (DM, hepatic affection or systemic disease).

There has been estimated a significance in the correlation between the values of TAOS I (before I st HD) mean 2.04±0.42 and TAOS II (after I st HD) mean 1.72±0.46 with p=0.00007, than p=0.00007 between TAOS I and TAOS III, (after the therapy with antioxidants) mean 1,67±0,5. There has been an insignificance correlation between oxidative stress for the body, but under the circumstances of the improvement in “milieu” interior with the use of HD, TAOS is improved comparing the firstly measured TAOS. There has been no significance in the correlation of II and III TAOS values although the substitution with antioxidants, but anyway, the significantly decrease of TAOS III versus I implies the improvement of TAOS under the therapy of antioxidants

5 CONCLUSION:

ARF and CRF manifest a great oxidative stress for the body, which can be corrected with everyday low doses of antioxidative therapy that can be used long, enough to avoid adversary effects for the cell damages in oxidative stress.

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