Effect of diet on selected parameters of antioxidative system and inflammatory processes in patients dialyzed due to diabetic kidney disease

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Abstract

The aim of the study was to determine the effect of balanced diet on selected parameters of oxidative stress, immunological response and nutritional status in patients undergoing haemodialysis (HD) due to diabetic kidney disease. Forty HD patients took part in the study. The nutritional status and diets of all the patients were initially assessed and the patients were divided into two groups. The control group (C) got unchanged diet regarded by those patients as optimal. For patients in the P group, a standard diet for a patient undergoing haemodialysis treatment was introduced, considering the fundamental disease – diabetes. Albumin, total protein, leptin and interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) cytokine concentrations were determined in blood plasma, and CAT and SOD enzymes activity was determined in erythrocytes by the spectrophotometric method. On the basis of the results of BMI, albumin and total protein analyses it can be stated that a well-balanced diet can limit the loss of body weight. Performed studies show that the proper diet can reduce the secretion of proinflammatory cytokines and ensure proper activity of antioxidative enzymes in the blood of HD patients.

Key words: diabetic kidney disease, haemodialysis, nutritional state, CAT, SOD, cytokines, leptin.

Introduction

The number of people with type 2 diabetes has been constantly increasing in the world both in civilized and developing countries. It is estimated that by 2025 this number can exceed 300 million. Despite the widespread screening tests and diabetologists’ efforts, this disease is still diagnosed too late when the complications affect the organs, especially the cardiovascular system and kidneys. Diabetes is the most common cause of end-stage renal disease and the need of dialysis, and poses a significant death risk in cardiovascular diseases.

Despite the newest dialysis techniques, new generation medicines and improved medical procedures, the majority of patients show symptoms of malnutrition. This state becomes more serious along with the progression of chronic kidney disease and is a significant factor which increases the morbidity and mortality in this group of patients. Moreover, in chronically dialysed patients there are also determined lipid metabolism disorders, increased oxidative stress and increased production of free radicals, which can stimulate cytokines synthesis and secretion [1, 2].

In people suffering from diabetes, a decreased activity of antioxidative mechanisms is observed, which is confirmed by a reduced concentration of antioxidants in intra-
cellular and body fluids [3]. Moreover, ill people, as compared to healthy people, were reported to have a lower concentration of endogenous antioxidants: glutathione, ascorbic acid and tocopherol, in plasma, erythrocytes and platelets [4]. Also a lower activity of antioxidative enzymes was observed: catalase (CAT) and superoxide dismutase (SOD), which can be a result of an increased level of glycated form of these enzymes or the effect of adaptive processes to increased oxidative stress [5].

In the healthy state, in the organism there is a balance between oxidative and antioxidative processes. It has been proved that the contact of blood with dialysis membrane induces an increased production of reactive oxygen species (ROS) by the neutrophils [6-8]. Thus, there are mechanisms activated to protect the cells against excessive oxidation.

Despite the worldwide and European scientific societies’ recommendations, in Poland the routine nutritional status assessment has not been implemented, which would make it possible to detect malnutrition among the ill people and start proper treatment in later stages. It seems that the regular nutritional care is an important part of the treatment for patients with a renal failure. Moreover, in this group of people the levelling of carbohydrate metabolism is of great importance. Hyperglycaemia intensifies thirst but also increases the synthesis of glycation end products, thus intensifying vascular changes [9]. Moreover, it leads to increased secretion of peroxide anion. In healthy humans, it is neutralized by SOD, activity of which is lowered in people with diabetes. The result of this is oxidative stress, which injures the integrity of vascular membrane, thus decreasing its efficiency as a filtration barrier [10]. Hypoglycaemia, on the other hand, can lead to arrhythmia and myocardial ischaemia. Due to that it is recommended to use dialysis fluids containing 100-200 ml of glucose per dl [8]. Proper implementation of dietetic recommendations can prevent nutritional deficiencies and, in consequence, malnutrition, and can balance patients’ glycaemia, thus improving the quality of life and reduce the risk of premature death.

The aim of performed studies was to determine the effect of a balanced diet on selected parameters of oxidative stress, immunological response and nutritional status in patients treated with haemodialysis (HD) due to diabetic kidney disease.

Material and methods

Characteristics of investigated groups

The studies were carried out for 12 months in 40 HD patients dialysed mainly due to diabetic kidney disease (38), who were divided into two groups. The patients were treated in the Clinical Department of Nephrology, Transplantology and Internal Medicine, Pomeranian Medical University in Szczecin, Private HMO Dialysis Station Hand-Prod in Drawsko Pomorskie and Private HMO Dialysis Centre Fre-

senius Nephrocare branch in Stargard Szczeciński, Dialysis Station No. 7. All the patients were treated with haemodialysis 3 times a week for 4 hours using Fresenius machine (Hamburg, Germany) with polysulphone dialyser, bicarbonate buffer and dialysis fluid containing glucose.

The study was performed on a group of 20 people (10 women and 10 men) (group P) at the age of 71.6 ±3.14. Patients were dialysed regularly for at least 4 months. The average period of dialysis treatment was 8.8 ±2.09 months. The group willingly benefited from nutritional education and gave consent to work with a dietician.

A control group consisted of 20 people (11 women and 9 men) (group C) at the age of 70.8 ±3.63. The average time from the first dialysis was 7.95 ±2.01 months. People in that group decided that their diet was sufficient for their needs and that they did not need the care of a dietician. Blood samples only were collected from the patients in this group for periodical analyses.

The age and sex in both analysed groups of patients were not statistically significantly different. All the people gave their written consent to take part in this study. The study was approved by the Bioethics Commission at the Pomeranian Medical University in Szczecin.

Study design

1. Dietary assessment. In both groups, the initial assessment was made on the basis of the analysis of weekly food records taken on a current basis for days with and without dialysis treatment. The correctness of the diet was assessed, including the coverage of patients’ dietary energy requirements and the intake of proteins, fats, carbohydrates, vitamins and bioelements. Resulting values were compared to current dietary guidelines.

2. Nutritional status measurement on the basis of body mass index (BMI). Calculation was made on the basis of dry mass (after dialysis). Reference values were BMI > 23 (with lower BMI there is a higher risk of malnutrition). Patients were periodically subjected to anthropometric tests (after 3, 6 and 12 months).

3. Patients were divided into groups and given, respectively:
   • C – unchanged diet, regarded by the patients as optimal;
   • P – diet used in patients treated with haemodialysis, taking into consideration the fundamental disease, i.e. diabetes. The following diet guidelines were established:
     – recommended calorie intake 30-35 kcal/kg/day, 50-60% should come from carbohydrates,
     – recommended diet with protein content of 1.0-1.2 g/body mass/day – 50% of consumed protein was of animal origin,
     – the amount of fluids 500-800 ml/day (with those included in the products) plus the amount of excreted urine,
     – the intake of mineral compounds: sodium, due to hypertension limited to 1500 mg/day, potassium to
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2000 mg/day, calcium to 2000 mg/day including calcium supplements, phosphorus to 1000 mg/day [10].

4. Nutritional education in group P. The patients were given proper dietary guidelines and information guide-books containing the list of recommended/not recommended/forbidden products in diets of dialysed people and substitutes of food products rich in proteins. During the dialyses the diets were individually analysed with each patient. During the 12-month study, individual weekly menus were prepared for the patients from this group.

5. Throughout the 12-month study the nutritional status of each patient was assessed and the adherence to given recommendations was checked periodically. Moreover, blood samples were collected from each patient for biochemical and immunological analyses (after 3, 6 and 12 months).

Serum and erythrocytes preparation

Blood for the analyses was collected from arteriovenous fistula at 0, 3, 6 and 12 months from the start of diet application directly before and after dialysis treatment. The samples were centrifuged at 2000 g at 4°C for 15 minutes to separate the plasma from red blood cells (RBC). The plasma was separated into sterile microtubes and saved for the analyses of cytokines [interleukin 6 (IL-6), tumor necrosis factor α (TNF-α)], and leptin. Erythrocytes, after removing theuffy coat, were rinsed three times with 0.9% NaCl solution and centrifuged for 10 min at 2500 g after each rinsing. Red blood cells were separated into microtubes and saved for the analyses of CAT and SOD enzymes activity.

Due to high costs of the kits, the concentrations of leptin, IL-6 and TNF-α were determined in the plasma before and after HD at 0 and 12th month.

Biochemical and immunological analysis

To determine the concentrations of total protein and albumin a clinical chemistry analyser RX-Imola (Randox) was used. The concentration was determined before the HD. The assumed reference values were 62-84 g/l for total protein concentration and 35-50 g/l for albumin concentration. The results of the analyses of total protein and albumin concentrations made it possible to assess the patients’ nutritional status.

All of the analyses based on spectrophotometric measures were carried out using UV/VIS Lambda 20 spectrophotometer (Perkin Elmer).

Catalase (CAT) activity was determined spectrophotometrically using its ability to decompose peroxides [9]. The decrease in extinction of the solution containing RBC hemolysate and hydrogen peroxide was measured at 240 nm. The reaction was carried out in phosphate buffer, pH 7.0.

Superoxide dismutase (SOD) activity was measured spectrophotometrically using its ability to oxidize adrenochrome to adrenochrome [11]. The enzyme was extracted from RBC with ethanol and chloroform mixture. Another carbonate buffer containing adrenalin was added to the extract. The change in adrenochrome concentration was measured at 320 nm at 30°C. One unit of SOD was defined as the amount of enzyme needed for 50% reduction of maximal adrenochrome oxidation.

Concentrations of leptin, and IL-6 and TNF-α cytokines were determined by immunoenzymatic method – ELISA. For those analyses commercial kits of R&D Systems USA were used. Test results were determined by ELISA microplate reader (ELx 808, BIO-TEK Instruments, Inc.). Calibration curves and calculations of concentration values were made using calculation software KC Junior for Windows, BIO-TEK Instruments, Inc. Samples were prepared and tested in duplicate according to the manufacturers’ instructions. The lower limit of sensitivity of the assay for serum samples was 7.8 pg/ml for leptin, 0.016 pg/ml for IL-6 and 0.5 pg/ml for TNF-α.

Statistical analysis

All the results were presented as arithmetic mean ± standard deviation (SD). The results were analysed statistically. The Kolmogorov-Smirnov test with Lilliefors correction showed normality of the distribution of the results, which were subjected to analysis of variance ANOVA using post hoc Tukey’s test for SOD, CAT, IL-6 and TNF-α. In the case of BMI, protein and leptin, a Student’s t-test was used showing significant/insignificant differences in analysed groups for p ≤ 0.05. All the statistical analyses were calculated using STATISTICA v 10 software.

Results and discussion

According to the data of Fresenius Medical Care, in 2011, 2,786,000 people in the world were treated for renal failure, including 1,929,000 patients treated with haemodialysis (HD) and 235,000 with peritoneal dialysis (PD). Despite the progress in dialysis techniques and improving medical care, high mortality is still an important problem. Major causes of chronically dialysed people deaths are cardiovascualr diseases.

It was shown that 20-70% of patients treated with haemodialysis show signs of malnutrition, which lowers the quality of life, worsens prognosis and increases mortality. The most commonly mentioned causes of a lower nutritional value of daily food ratios (DFR) are frequent hospitalisations, laboratory tests demanding fasting, tiredness after dialyses and too restrictive diet, unadjusted to patient’s preferences and unpalatable due to limitations. The diet should meet patient’s dietary energy requirements and supply a proper amount of nutrients. Properly chosen diet should also limit the symptoms of uraemia and metabolic disorders. Therefore, the diet quality and nutritional status of the patients should be monitored. The education of patients (and their families) seems to be of great importance.
The low albumin concentration in the plasma is among the nutritional status indexes that is most commonly regarded as the prognosis of increased mortality among the patients treated with haemodialysis. Also BMI and total protein concentration are taken into consideration.

On the basis of BMI analyses, it can be stated that a well-balanced diet can reduce the loss of body weight. During one-year nutritional therapy, the loss of weight was not observed in patients from the group adhering to the recommendations (Fig. 1). The value of BMI in the control group (C) during the course of the study was decreasing. In this group, statistical differences were observed between BMI values at the beginning of the study (C0) and in the sixth month (C6), and between initial BMI (C0) and the last examination in the 12th month (C12). In the guided group (P), on the other hand, BMI value increased from the initial mean value of 21.6 ± 1.3 kg/m² to 23.7 ± 2.4 kg/m². It indicates a statistically significant difference (p < 0.05) between the initial BMI (P0) and the index value determined after 12 months (P12). BMI values at the beginning of the study were not statistically significantly different between the C and P groups, but after 12 months significant differences were observed. BMI values between the C and P groups were statistically significantly different in the 6th and 12th month of the study (between C6 and P6 and between C12 and P12).

Kalantar-Zadeh [12] stresses that BMI below 25 poses a high risk of increased mortality in this group of patients. It should be noted that for healthy people BMI value between 18.5 and 24.9 is normal. Moreover, the author showed a positive correlation between overweight (BMI 25-30) and obesity (BMI > 30) and increased survival in HD patients.

Literature reports on BMI values in this group of patients are divergent. In studies performed by other authors the observed BMI values in patients were between 20 and 25 [13, 14] and below 20 [15]. Aoyagi et al. [16] in literature review report other studies’ results showing systematic weight loss in patients chronically dialysed for 10-15 years. Different results, but confirming the results of this study, were reported by Louden et al. [17]. They showed that proper intake of energy and protein in the group of dialysed patients can reduce the loss of body weight and even contribute to the increase in BMI value. They reported that BMI value in the examined group of people exceeded 25.

Taking into consideration the total protein concentration (Fig. 2) and albumin concentration (Fig. 3) as nutritional status indexes, the results of this study are promising. Initial average concentrations of both parameters were different between the groups (C0 and P0). Average levels of albumins and total protein in people from the P group in each stage of the study were in the middle of the reference range. In the C group, however, a systematic decrease in levels of both parameters was observed. In the control group, the concentration of total protein decreased from the initial mean value of 68.225 ± 4.35 g/l to mean value of 57.28 ± 2.85 g/l after one year of the study. Statistically significant differences were shown between total protein concentrations in 0 month (C0) and 6 months (C6). Moreover, a significant decrease in total protein levels in plasma after 12 months of the study (C12) were observed, as compared to initial values (C0). In the guided group (P), an increase was observed from the mean value of 68.64 ± 4.13 g/l at the beginning of the study to the mean value of 73.13 ± 2.82 g/l in the 12th month. The differences were statistically significant.

Fig. 1. Changes in BMI value in analysed groups of patients during 12 months of the study

Fig. 2. Changes in total protein levels [g/l] in examined groups of patients during 12 months of implementation of diet recommendations
In group C, the level of albumins was decreasing during 12 months of the study from the initial mean value of 42.24 ± 3.85 g/l to the mean value of 32.39 ± 2.15 g/l after 12 months, showing statistically significant differences between consecutive analyses. Similar results were obtained by Sanlier and Demircioglu [12]. Mayer et al. [18] reported the albumin concentration to be under 35 g/l and the level of total protein below the reference range. Commonly observed low albumin and total protein concentrations in dialysed patients were also confirmed by other researchers [14, 15]. In group P, after the 3rd month a decrease in mean value of albumin concentration from the initial 43.84 ± 2.45 g/l to average value of 43.21 ± 2.4 g/l was observed. Despite the decreased value the difference was statistically insignificant and the increase to 46.28 ± 1.33 g/l was observed after 12 months, showing statistically significant differences between the values in groups P0 and P12. Also statistically significant differences were noted between groups C and P in the 3rd, 6th and 12th month of the study.

It was, therefore, shown that having a well-balanced diet can affect the nutritional status of patients treated with haemodialysis by slightly increasing body mass index, albumins and total protein levels or maintaining the initial values of those parameters.

One of the basic causes of malnutrition is the lack of appetite, which appears in pre-dialysis period and leads to limited calorie and protein intake. Kalantar-Zadeh et al. [19] showed the correlation between anorexia in dialysed patients and worse prognosis, increased frequency of hospitalisation and worsened quality of life. A high role in malnutrition development is attributed to leptin. It is assumed that hyperleptinaemia in HD patients is caused by the renal dysfunction, ongoing inflammatory process or hyperinsulinaemia. In patients treated with haemodialysis, an increased level of leptin is observed. This leads to the lack of appetite, further worsening energy and protein deficiency. The loss of appetite is caused by exogenous toxins, including medicines used in treatment of this group of patients. They have a depressive effect on the hunger centre causing vomiting. Infectious diseases increase eating disorders by the effect of bacterial toxins. The infection itself can lead to increased catabolism [20].

In this work in group C, after 12 months of the study, a significant increase in leptin concentration was observed – from 382.63 ± 83.73 pg/ml to 623.21 ± 120.09 pg/ml. Also in group P the concentration of leptin significantly increased from the initial value of 310.79 ± 57.41 pg/ml to 368.55 ± 65.96 pg/ml (Fig. 4).

It is stressed that proinflammatory cytokines: IL-1β, IL-6 and TNF-α, secreted during haemodialysis, reduce appetite

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**Fig. 3.** Changes in albumin levels [g/l] in examined groups of patients during 12 months of the study

**Fig. 4.** Changes in leptin concentration [pg/ml] in examined groups of patients during 12 months of the study

**Fig. 5.** Changes in IL-6 concentration [pg/ml] in examined groups of patients during 12 months of the study
observed a deteriorated nutritional status in patients treated with haemodialysis. The author suggested that an excessive level of IL-6 is the cause of protein metabolism disorders affecting the nutritional status. He attributed the increased cytokine secretion to bioincompatible membrane [24].

Joannidis et al. [25] assessed the effect of 6-month supplementation with nutrient preparations (Aminomel Nephro, Ello lipid, L-carnitine and vitamin E) on the nutritional status and inflammatory parameters in patients treated with haemodialysis. It turned out that used supplementation caused the increase in BMI but did not significantly affect albumin concentration. Moreover, a decreased TNF-α secretion and slightly increased IL-6 level were observed [25].

Tayyebi-Khosroshahi et al. [26] showed a favourable effect of proper diet (as far as energy and protein are concerned), enriched with n-3 fatty acids at a dose of 3 g/day for 2 months, on the concentration of proinflammatory cytokines. They observed a reduced secretion of cytokines in patients using the supplementation.

Many authors emphasise that increased secretion of proinflammatory cytokines is mainly affected by haemodialysis treatment itself and the type of dialysis membrane used. In this study it was confirmed that haemodialysis causes a significant increase in proinflammatory cytokine levels in plasma of patients from all the groups. Other authors in their studies also showed the increase in cytokine concentrations after the dialysis [6, 27]. The authors suggest that the mechanism responsible for the increase in IL-6 is different from that responsible for overproduction of TNF-α and IL-1β. The type of dialysis membrane used seems insignificant here.

In response to increased production of ROS an organism activates defence mechanisms protecting from oxidative damage. Red blood cells possess a highly effective defence mechanism. One of the key antioxidant enzymes, taking part in hydrogen peroxide decomposition, is catalase. In this study significant differences in CAT activity were obtained between the groups (Fig. 7). The average CAT activity in the control group of patients before HD was statistically significantly different in months 0 and 3 and in months 3 and 12. The decrease was noted from the initial value of 281.8 ±21.3 A/g Hb to the value of 234.0 ±30.2 after 12 months. No significant differences were observed in CAT activity in group P before HD in particular months of the analyses. Dialysis caused a decrease in CAT activity in group C from the mean value of 299.9 ±22.05 A/g Hb to 240.85 ±29.78 A/g Hb, giving statistically significant differences between the consecutive tests. In group P, however, dialysis caused a decrease in average CAT activity from the initial value of 293.5 ±27.35 A/g Hb to 283.5 ±34.56 A/g Hb, and no statistically significant differences were shown between the mean values of the test results in particular months. The lowest values of catalase activity were
observed in both groups before and after HD in the 6th month of the study.

Reduced activity of the antioxidative system in chronic renal failure has been previously reported [28, 29]. On the other hand, Peuchant et al. [30] showed elevated CAT activity in this group of patients, as compared to healthy volunteers. Additionally, the use of very low protein diet (VLPD) in HD patients caused a slight increase in CAT activity. However, the study of Sommerburg et al. [31] shows that erythrocyte CAT activity in adult HD patients and dialysed children can be on a lowered level, as in healthy people.

Own studies show that dialysis caused a little – statistically insignificant – increase in CAT activity in particular groups, regardless of the period of treatment. Other research confirmed obtained results [28].

The main role of superoxide dismutase (SOD) is to catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Reports on SOD activity in HD patients and healthy people are divergent. Some researchers observed significantly higher values of SOD activity in HD patients than in healthy people [32]. Different results were presented by the authors of other studies [28, 33], where SOD activity in HD patients was significantly lower than in healthy people. Decreased SOD activity implicates a defensive role of SOD against superoxide anions. Haemodialysis induces granulocytes to produce superoxide. Its accumulation intensifies SOD consumption in defence against free radicals. Lower SOD activity in HD patients is also attributed to an increased concentration of hydrogen peroxide. Due to decreased activity of GSH-Px in erythrocytes the accumulated hydrogen peroxide may inhibit SOD activity. A high level of hydrogen peroxide is a result of neutrophil activation in the haemodialysis process or overproduction by erythrocytes [33]. Drai et al. [34] and Zachara et al. [35] showed the lack of differences in SOD activity between patients treated with haemodialysis and healthy people.

In the presented study in group C, in patients before HD, the activity of SOD decreased from the value of 1718.86 ±8.72 U/g Hb to 1614.44 ±53.56 U/g Hb, showing a statistically significant difference between the measurement taken at the beginning (C0) and at the end (C12) of the study. Superoxide dismutase activity in group P before HD increased from 1739.87 ±50.18 U/g Hb to 1790.12 ±51.38 U/g Hb, and no statistically significant differences were observed between the months when the measurements were taken (Fig. 8).

In the group of patients after haemodialysis, similarly as in blood plasma collected before HD, in group C the decrease in SOD activity was observed, whereas in group P – an increased SOD activity was noted. Statistically significant differences were determined for group C between the measurements taken in 0 and 6th month and between 0 and 12th month.

Dialysis caused the increase in SOD activity in group P during 12 months of the study. It could be the evidence of organism preparation to defend against free radicals, which form as a result of oxidative stress caused by haemodialysis. Obtained results are in agreement with reports of other authors [28, 36]. Zwolińska et al. [37] observed decreased SOD activity in a group of HD children.

The results of this study indicate the necessity to monitor the nutritional status in order to prevent malnutrition. The results of performed analyses show that a properly selected diet can slow down progressive malnutrition, decrease the secretion of proinflammatory cytokines and assure correct activity of antioxidative enzymes in blood of patients treated with haemodialysis.

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**Fig. 7.** Changes in catalase activity [A/g Hb] in examined groups of patients during 12 months of the study

**Fig. 8.** Changes in SOD activity [U/g Hb] in examined groups of patients during 12 months of the study
The authors declare no conflict of interest.

References
