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ORIGINAL PAPER

NUTRITIONAL STATUS, IL-6 SERUM CONCENTRATION AND PREDICTION OF CARDIOVASCULAR COMPLICATIONS IN HAEMODIALISED PATIENTS WITH END-STAGE CHRONIC RENAL DISEASE*

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Abstract

There are over 21 thousand haemodialysis patients in Poland. The mortality rate is over 20%. New clinical problems are observed, such as the MIA syndrom (malnutrition, inflammation, atherosclerosis). Aim: The aim of the study was to evaluate the correlation between the nutritional status, IL-6 serum concentration and the frequency of cardiovascular complications in a group of patients with end stage renal kidney insufficiency treated by haemodialysis. The prospective study covered 53 patients divided into two groups: malnourished (group 1) and with the correct state of nutrition (group 2). All patients were haemodialised three times a week in accordance with the established medical procedures. Laboratory tests (IL-6, CRP), and observations were carried out in three consecutive measurements, marked as I, II and III study, covering a period of 6 months. At the same time, anthropometric parameters of the nutritional status were evaluated. Cardiological complications were analysed over a 7-month period. The results of the nutritional study obtained in group 1 were statistically significantly lower than in group 2, but the incidence of cardiovascular complications was higher. A significant correlation between the serum IL-6 concentration and the risk of cardiovascular complications (OR 1.53; $p = 0.04$) was observed. The probability of cardiovascular complications depending on the thickness of the triceps skin-fat fold in the entire study group was statistically significant (OR 0.34; $p = 0.04$). Results of the study suggest that the monitoring of selected anthropometric, biochemical measurement not only gives an opportunity to assess the nutritional status of haemodialysed patients, but may be also crucial in early diagnosis of cardiovascular complications.

Keywords: anthropometric measurement, nutritional status, cytokines, complications, end-stage renal disease.

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INTRODUCTION

Owing to the progress in medicine, including nephrology, possibilities of renal replacement therapy are constantly increasing, leading to an increase in the number of patients and, on the other hand, the emergence of new clinical problems, such as MIA syndrome (malnutrition, inflammation, atherosclerosis) and a greater number of complications. Currently, there are over 21 000 haemodialysed patients in Poland. Despite the constant improvement of treatment methods, the mortality rate of haemodialysed patients reaches 15-20%, which is 4 to 7 times higher than in the general population. The most common complications observed in this group of patients are cardiovascular complications, which cause more than 50% of deaths (Załuska et al. 2015). A relationship is observed between mediators of inflammation (concentration of proinflammatory cytokines) and the severity of atherosclerosis and the development of cardiovascular complications, as well as the development of protein-caloric malnutrition, also correlated with atherosclerosis (MIA syndrome). The presence of MIA in dialysis patients is a cause of the reverse epidemiology of cardiovascular disease. Factors that reduce the risk of cardiovascular diseases in the general population become important contributors to an increased likelihood of the occurrence and development of these diseases and death in patients who are dialysed.

Malnutrition

Despite the fact that malnutrition of chronically dialysed patients has long been recognised as a serious clinical problem, inadequate nutrition still occurs in about one third of the patients, i.e. according to various sources in 20-76% of hemodialysed patients (Bergström 1995, Essadik et al. 2017). Two types of malnutrition are distinguished in patients with kidney disease (Stenvinkel et al. 2000). One is associated with an insufficient supply of protein, energy and protein absorption disorders, while the other one is due to chronic inflammation, which may lead to accelerated development of the MIA syndrome. The most common causes of malnutrition in haemodialysed patients are anorexia and gastrointestinal complaints. Failure to comply with dietary recommendations is another reason for eating disorders. Protein supply recommendations are often difficult to achieve by patients; in practice, there is a shortage of protein (less than 10^{-3} kg BW / day) and energy $< 117.3 \cdot 10^{-3}$ J kg^{-1} (<28 kcal kg^{-1} BW / d). A large proportion of patients, despite the initiation of renal replacement therapy, continue to apply the low-protein diet recommended in the pre-dialysis period, and all of them consume significantly less meals on dialysis treatment days. Dietary counseling for patients with kidney disease is practically non-existent (Kistler et al. 2018, Stevenson et al. 2018).

Another important cause of malnutrition are inflammatory states manifested by elevated CRP and a higher level of proinflammatory cytokines in

the serum. IL-6 plays a significant role in the development of malnutrition through the catabolism of muscle proteins, and the anorectic impact of cytokines (increased leptin production, lipolysis). It was found that an increased level of cytokines is characteristic for hypoalbuminemic patients, and is associated with their shorter survival (Qureshi et al. 2002, Stenvinkel et al. 2002).

Cardiological complications

The prevalence and worse prognosis of cardiovascular (CV) diseases associated with kidney diseases are an important social problem, and therefore their prevention and treatment ought to be a priority. Cardiological complications in the group of HD patients are: left ventricular hypertrophy, pericardial disease, ischemic heart disease, vascular diseases, heart failure, bacterial endocarditis, arrhythmias.

The role of inflammation in the pathogenesis of atherosclerosis is underlined, in which the key importance is attributed to the adhesion of leukocytes to the vascular endothelium, and consequently to vascular damage by inflammation products, such as free radicals and cytokines. In patients undergoing haemodialysis, there was a correlation between the inflammation markers (concentration of proinflammatory cytokines) and the severity of atherosclerosis and the development of cardiovascular complications, as well as the development of protein-caloric malnutrition, also correlated with atherosclerosis (the MIA syndrome). Cardiovascular diseases are the most common complication in HD patients and account for over 50% of deaths in patients. Mortality resulting from the causes of the CV, after correction in relation to age and comorbidities, is 3-20 times more frequent in the dialysed group than in the general population. Chronic inflammation is found in about 35-65% of hemodialysed patients (Kalantar-Zadeh et al. 2003, Dai et al. 2017).

Already in the early stages of CRF, patients with no heart disease have an increased level of proinflammatory cytokines (IL-1, TNF alpha, IL-6), acute phase proteins (CRP, fibrinogen), adhesion molecules (selectins) and some coagulation blood factors (factors VII, VIII) as well as reduced expression of anti-inflammatory cytokines. The level of IL-6 increases with the decrease of GFR, and is a strong predictor of poor prognosis. IL-6 levels correlate with atherosclerotic lesions in the vessels, contribute to the development of atherosclerosis by interfering with the endothelial function and coagulation processes, and enhance smooth muscle proliferation. In people without kidney disease, it was found that increased serum IL-6 and CRP levels predispose to the development of CV disease as well as increased mortality. A high concentration of IL-6 in patients initiating renal replacement therapy is associated with a worse long-term prognosis (Wanner et al. 2002, Tosic Dragovic et al. 2016).

MATERIALS AND METHODS

Based on a retrospective analysis of the results of examinations of patients with end-stage renal disease treated with repeated hemodialysis in the Department of Dialysis of the Medical University of Warsaw, 53 patients (20 women and 33 men) aged from 25 to 83 (mean 58.5 ± 5.6) years were qualified for further prospective studies. They were divided into two groups depending on the state of nutrition. The criterion of malnutrition was: weight loss (5% - 10% in the period of 3 or 6 months, respectively), serum albumin below $35 \cdot 10^{-3}$ kg m^{-3}, total lymphocyte count below $1.5 \cdot 10^{-9}$ m^{-3}.

Group 1 consisted of 27 malnourished individuals. The second group included 26 individuals without malnutrition symptoms. Co-morbid chronic infections, decompensated heart failure and cancerous diseases were adopted as clinical exclusion criteria. All patients were haemodialised three times a week in accordance with the established medical procedures. Laboratory tests and observations were carried out in three consecutive measurements marked as I, II and III study, covering a period of 6 months. During that time, the patients were not included in the nutritional treatment program and did not receive any nutritional supplements. Blood was collected immediately before dialysis from veins of the opposite arm than the shoulder with a fistula. Determination of the CRP concentration was performed by immunoturbidimetry. The biochemical determinations were performed on a Cobas Integra 800 analyzer. The total number of peripheral blood lymphocytes was determined by flow cytometry with a semiconductor laser using a Cytmex XT 2000I. IL-6 cytokine assays were performed with a Fluorokine MAP kit cytokine multiplex kit (company R'n'D) and a Luminex 100™ instrument. The reference group for cytokine concentrations were 10 healthy blood donors (3 women, 7 men), aged 20-33 (mean 27 ± 4.4).

At the same time, the anthropometric parameters of the nutritional status (body weight, BMI, triceps skinfold thickness and skinfold thickness over the shoulder, arm circumference, arm muscle circumference, waist and hips circumference) were evaluated using aTanita BF-662W weight scale, sewing tape measure, and a caliper Harpender BATY. In both groups, selected clinical parameters were monitored over a period of 7 months, taking into account the frequency and type of complications. All patients signed an informed consent form approved by the Bioethics Committee of the Medical University of Warsaw.

Cardiological complications that were analysed include myocardial infarction and stroke.

Statistical analysis

SPSS for Windows 12.0 and R 2.7.0 were used in the analysis. Comparisons between groups were made using *t*-Student or the Mann-Whitney tests

and chi-square tests or the Fisher's test. Potential factors influencing a greater risk of complications were examined using logistic regression analysis. The results are presented as odds ratio (OR) with its 95% confidence interval and the corresponding *p*-value. $P < 0.05$ was assumed as the level of significance.

RESULTS

The compared groups 1 and 2 did not differ significantly in terms of sex and age. The causes of end-stage renal disease and coexistence of diabetes were similar in both groups. In group 1, compared to 2, significantly lower values of anthropometric indicators of the nutritional status were observed throughout the observation period; body weight ($p = 0.02$), BMI ($p = 0.001$), skinfold thickness over the shoulder ($p = 0.01$), arm circumference ($p = 0.01$), arm muscle circumference ($p = 0.01$), arm muscle surface ($p = 0.01$), waist circumference ($p = 0.001$), hip circumference ($p = 0.01$).

The results of the skin-fat fold measurement over the triceps muscles of the arm did not undergo significant changes during the observation period, and were on average in group 1: $16.6 \cdot 10^{-3}$ m ± $6 \cdot 10^{-3}$ m in the first and second tests and $15.5 \cdot 10^{-3}$ m ± $5 \cdot 10^{-3}$ m in the third study, while in group 2, they were $19 \cdot 10^{-3}$ m ± $8 \cdot 10^{-3}$ m on average in the three subsequent studies.

The concentration of C-reactive protein (C-reactive protein, CRP) in subsequent studies was on average: $14.7 \cdot 10^{-6}$ kg m^{-3} ± $19.4 \cdot 10^{-6}$ kg m^{-3}, $20.11 \cdot 10^{-6}$ kg m^{-3} ± $28 \cdot 10^{-6}$ kg m^{-3}, $14.57 \cdot 10^{-6}$ kg m^{-3} ± $23.8 \cdot 10^{-6}$ kg m^{-3} in group 1, and $8.1 \cdot 10^{-6}$ kg m^{-3} ± $8.7 \cdot 10^{-6}$ kg m^{-3}, $10 \cdot 10^{-6}$ kg m^{-3} ± $13.3 \cdot 10^{-6}$ kg m^{-3}, $16.1 \cdot 10^{-6}$ kg m^{-3} ± $34.5 \cdot 10^{-6}$ kg m^{-3} in group 2. Results of CRP protein concentrations did not differ significantly between group 1 and group 2 ($p = 0.1$).

The results of IL-6 concentrations obtained in both groups are presented in Table 1. The results of the tests in patients were statistically significantly higher than in the control group ($p = 0.001$), but they did not differ significantly in comparison with the results obtained between the groups ($p = 0.96$).

Table 1

Results of IL-6 concentration in group 1 and group 2

Result	Group 1						Group 2						*P*
	n	mean	SD	median	I quartile	III quartile	*n*	mean	SD	median	I quartile	III quartile	
1	27	2.6	1.7	1.8	1,.65	2.81	26	2.3	1.8	1.94	1.65	2.23	0.96
2	26	1.6	0.9	1.65	0.96	1.94	26	62.8	287.4	1.65	0.96	1.94	0.99
3	26	1.6	1.7	0.96	0.96	0.96	26	1.4	1.2	0.96	0.96	0.96	0.92

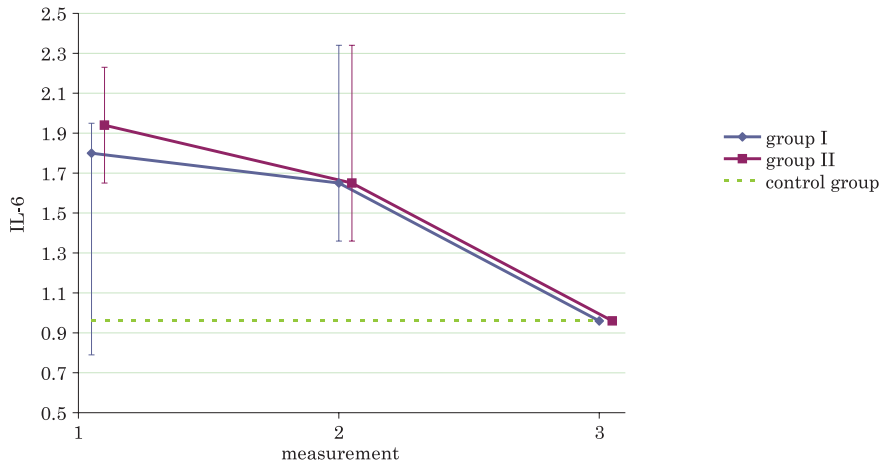

Fig. 1. Median IL – 6 concentration in groups 1, 2 and control group in three studies. Differences statistically significant between group 1 and control group ($p = 0.001$) and group 2 and control group ($p = 0.001$)

There were more cardiovascular complications in the group of patients with malnutrition ($n = 18$) than in the group without malnutrition ($n = 13$), although these differences were not statistically significant (Figure 1).

The probability of cardiovascular complications in the subsequent quarter of the year depending on the interleukin-6 concentration level was similar in both groups of patients (OR 1.53; $p = 0.04$) – Table 2. The odds ratio

Table 2

Odds Ratio for cardiovascular complications in groups 1 and 2 depending on the concentration of IL-6

Group	OR	95% CI		*P*
1	1.49	0.82	2.69	0.19
2	1.56	0.92	2.65	0.10
ALL	1.53	1.03	2.28	0.04

for occurrence of a complication is above 1 and is comparable in groups 1 and 2. The lack of statistical significance results from the small size of individual groups.

The probability of cardiovascular complications depending on thickness of the triceps skin-fat fold in the entire study group was statistically significant (OR 0.34; $p = 0.04$) – Figure 2. The results obtained for group 1 ($p = 0.21$) and 2 ($p = 0.14$) did not constitute a statistically significant correlation.

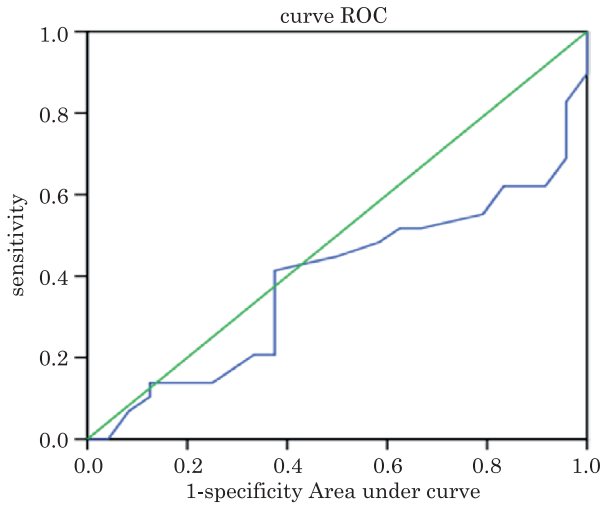

Fig. 2. The relationship between the thickness of the triceps skin-fat fold and the occurrence of cardiovascular complications in both groups

Area under the curve
Test variable: triceps skin-fat fold

Area	Standard error (a)	Asymptotic significance (b)	Asymptotic 95% CI	
			lower limit value	upper limit value
.386	.078	.158	.234	.539

DISCUSION

Disorders of the nutritional status and persistence of chronic inflammation are among the most important factors affecting the mortality of patients with end stage renal disease treated by haemodialysis. An increase in markers of inflammation like IL-6 and CRP protein is absolutely related to the arteriosclerosis process and may have a prognostic value. Studies on changes in cytokine concentrations and the incidence of complications in haemodialysed patients may allow us to gain better understanding of the mechanisms regulating the immune response, and to focus on patients with an increased risk of complications. What seems to be particularly valuable is the early recognition of nutritional and immune disorder, even before the onset of clinical symptoms, as this may be helpful in early diagnosis and therapy (Wanner et al. 2002, Pupim et al. 2004).

Anthropometric parameters are a valuable element in every patient's examination. Among the numerous anthropometric studies conducted in the

examined groups of patients, strong correlation between triceps skinfolds (OR 0.29; $p < 0.05$) and mid-arm circumference (OR 0.81; $p = 0.04$) only were potential predictors of cardiovascular complications and greater mortality risk. These correlations did not occur in patients from group 1 or group 2, but were only evidenced for the whole group of haemodialysed patients. The probability of cardiovascular complications depending on the triceps skinfold in the initial evaluation group was statistically significant (OR 0.34; $p = 0.04$). Other anthropometric measurements show a limited prognostic capacity to foresee complications, and it was only the estimated death risk of dialysed patients that was dependent on the body composition, arm circumference, and arm muscles (Stosovic et al. 2011, Castellano et al. 2016).

In the dialysed population, the relationship between elevated levels of CRP or IL-6 and mortality due to cardiovascular complications, both in the general population (Honda et al. 2006) and patients undergoing dialysis (Stenvinkel 2001, Snaedal et al. 2009, Tosik et al. 2016), has been repeatedly pointed out. The results of the present study showed increased levels of CRP and IL-6, compared to the control group. They indicate, same as the results of the other tests, the presence of chronic inflammation in haemodialysed patients (Liakopoulos et al. 2017).

In our study, there was no significant correlation between the state of nutrition and the IL-6 concentration. This could be due to the small dynamics of changes in the adopted parameters of the nutritional status and the adopted research schedule.

Our research indicates a statistically significant correlation between the IL-6 concentration and cardiovascular complications in haemodialysed patients. Similar observations, repeatedly described in the literature, indicate a relationship between elevated levels of CRP or interleukin-6 and mortality due to cardiovascular complications (Honda et al. 2006, Dai et al. 2017, Taheri et al. 2017). The results reported in available refereces do not prove the relationship between the level of interleukin-6 and increased mortality of dialysed patients, nor do they draw attention to this possibility (Pecoits et al. 2002, Tosik et al. 2016). However, this factor is considered to be an indicator of cardiovascular complications. In research involving dialysed patients, attention is paid to the high incidence of complications. For instance, exacerbation of ischaemic heart disease was reported in 38% of the patients included in the HEMO study, while 24% presented exacerbation of peripheral vascular disease, and 19% had central nervous system vascular disease are (Beddhu et al. 2002). The results of our study showed that the total number of cardiovascular complications in the compared groups of patients did not differ significantly. Among numerous anthropometric and biochemical parameters obtained from both groups, it was only the results of the skinfold thickness and the concentration of IL-6 that corresponded to the risk of these complications. The results of anthropometric studies in hemodialysis patients indicate limited chances of early identifica-

tion of patients at a high risk of clinical complications by means of simple tests, meaning that immunological techniques are necessary. It should be emphasised that determined IL-6 levels are likely to allow us to distingish patients who are at risk of developing cardiovascular complications. This can enable doctors to detect, reduce or – in some cases – even eliminate this threat through early diagnosis, appropriate treatment or early feeding interventions.

CONCLUSIONS

1. The results of the study indicate that the monitoring of anthropometric, biochemical and immunological indicators allows us not only to assess the nutritional status of hemodialysis patients, but also to assess the risk of future cardiovascular complications.

2. Among the anthropometric indicators examined in the study, only the thickness of the triceps skin-fat fold was an important risk factor for cardiovascular complications. The results should be confirmed in studies on a large population of hemodialysis patients.

3. An elevated IL-6 concentration in blood serum correlating with the CRP level indicates the presence of chronic inflammation in the group of hemodialysed patients, suggesting a higher risk of cardiovascular complications.

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