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*Evaluation of immunoglobulins, proinflammatory
cytokines and lipid concentrations in patients with
diabetic nephropathy treated with continual ambulatory
peritoneal dialysis (CAPD)*

Ocena stężeń immunoglobulin, cytokin prozapalnych i lipidów u pacjentów
z nefropatią cukrzycową, leczonych ciągłą ambulatoryjną dializą
otrzewnową (CADO)

In the course of diabetes, glucose autooxidation takes place during which free radicals are generated. They intensify lipid peroxidation and protein glycation, which depends on the degree of diabetes balance and diabetes duration time. Nonenzymatic glycation involves structural and enzymatic proteins, apolipoproteins as well as immunoglobulines and other immunological proteins (4,12).

The patients with chronic renal failure on continual ambulatory peritoneal dialysis (CAPD) treatment also have impaired lipid metabolism as well as increased oxidative stress, which contributes to protein glycation, even at the normal glycaemia level (15).

Final protein glycation products due to their changed structure become immunologically reactive and induce release of cytokines, growth factors, increase in endothelial permeability in addition to their being monocyte chemotactic factors (12,13).

The investigations of immunological parameters found disordered immunocompetent cell functions, increased concentrations of proinflammatory cytokines IL-1b, TNF-a, IL-6 and T helper IL-2 and interferon-g produced by lymphocytes, adhesive molecules expression and others (2,5)

Previous investigations of immunoglobulin concentrations in patients with diabetes concerned the aspect of response to cytokine production and reactions to bacterial and mycotic infections, chlamydia and food antigen immunisations (8,14). The studies found the patients demonstrated increased incidence of infections and developed autoimmunological processes. Detected disorders in IgG, IgA and IgM concentrations resulted, among others, from changed immunoglobulin molecule structure due to nonenzymatic glycation over chronic hyperglycaemia (7,11).

In diabetes there are changed lipid parameters concentrations, lipoprotein oxidative modifications, which are found to be connected with disordered immunological processes (2, 9).

Lipoprotein and immunoglobulin glycooxidation in patients with diabetes (15) with simultaneous cytokine system activation made us investigate concentrations of lipid parameters, immunoglobulins and proinflammatory cytokines in patients with diabetes on CAPD treatment for chronic renal failure (13).

The aim of the research was to determine the concentrations of total cholesterol, cholesterol HDL, cholesterol LDL and triglycerides with reference to proinflammatory cytokines IL-1 β , TNF- α , chemokine RANTES and immunoglobulins in patients with complicated nephropathy on CAPD treatment.

MATERIAL AND METHODS

The study covered 20 patients with chronic renal failure on CAPD treatment: of them 10 patients with type 2 diabetes who developed diabetic nephropathy, 10 with renal failure due to other causes (6 patients with glomerulonephritis, 2 with urethro-interstitial nephritis, 1 with renal cyst and 1 with renal failure of unknown origin). The control group consisted of 10 healthy adults. In both groups mean age of dialysed patients was 57.6 years, the duration of CAPD treatment – 27 months, mean length of type 2 diabetes was 13.6 years.

The blood for the examination was collected during patients' routine hospital check-ups. The patients had no signs of infections detected. The evaluation of total cholesterol, triglycerides and HDL cholesterol, was done by the direct enzymatic method, while LDL cholesterol was counted by the Friedewald formula. Cytokines IL-1b, TNF α and chemotactic factor RANTES were determined by the ELISA method with the use of commercial kits by Genzyme and R&D. Immunoglobulins were determined by the Mancini method.

The obtained results were analysed statistically by t-Student's test to find out significant differences between the examined groups and Pearson's correlation test to compare the relation among the parameters. The results were presented as mean arithmetic

standard deviation, $p < 0.05$ assumed as statistically significant. Statistical analysis was done by Statistica 5.0 programme.

RESULTS

The investigation of cytokine concentrations in the group with diabetes on CAPD demonstrated the following mean values: IL-1 β , 3.5 ± 5.7 pg/ml, TNF- α , 3.4 ± 2.2 pg/ml, RANTES 63.4 ± 60 pg/ml (Table 1). Different values of TNF- α , and RANTES in relation to controls were of statistic significance. Cytokine values found in patients with diabetes were not different in relation to the values obtained in patients without diabetes on CAPD treatment. Immunoglobulin concentrations in patients with diabetes on CAPD treatment were not statistically significantly different in relation to the groups of patients

Table 1. Serum concentration values of cytokines, lipids and immunoglobulins in patients with diabetes on CAPD, patients without diabetes on CAPD and healthy controls

Tested parameters	CAPD diabetic patients n=10	CAPD non-diabetic patients n=10	Healthy controls n=10
IL-1 β pg/ml	3.5 ± 5.7	3.4 ± 2.3	3.2 ± 2.3
TNF- α pg/ml	$3.4 \pm 2.2^*$	$3.1 \pm 2.1^*$	1.7 ± 1.2
RANTES ng/ml	$63.4 \pm 60^*$	$71.4 \pm 41^*$	41.2 ± 11.2
Total cholesterol mg/dl	215 ± 62	210.1 ± 58	201.5 ± 25.4
Cholesterol LDL mg/dl	134.3 ± 43	152.8 ± 44	125.0 ± 27.6
Cholesterol HDL mg/dl	$32.6 \pm 12.6^*$	$41.2 \pm 14^*$	61.5 ± 9.7
Triglycerides mg/dl	$239.5 \pm 142.6^*$	176.6 ± 59	74.5 ± 22.1
Immunoglobulin G IgG mg/dl	1006 ± 198	1145 ± 301	1050 ± 245
Immunoglobulin A IgA mg/dl	254 ± 77	218 ± 90	245 ± 82
Immunoglobulin M IgM mg/dl	154 ± 72	165 ± 75	130 ± 37

* statistic significance $p < 0.05$ investigated group in comparison to controls

Table 2. Correlation ratio (r) between immunoglobulin concentrations and concentrations of cytokines and lipids in patients with diabetes (dial - diabetes) and without diabetes on CAPD (dial - contr)

Immunoglobulins		IL-1 β	TNF α	RANTES	T-Chol	Chol-LDL	Chol-HDL	Tg
IgG	Diabetes	X	X	X	-0.71*	X	-0.38	-0.66*
	dial-contr	0.43	X	X	0.21	-0.19	-0.53	0.41
IgA	Diabetes	-0.40	X	X	-0.70*	X	-0.68*	-0.80*
	dial-contr	0.27	0.42	0.54	-0.24	0.53	-0.45	X
IgM	Diabetes	X	0.38	X	X	X	X	X
	dial-contr	0.54	X	0.33	0.86*	-0.44	X	0.75*

X - correlation does not exist

* statistic significance $p < 0.05$ investigated group in comparison to controls

with renal failure without diabetes and controls. Slightly decreased immunoglobulin IgG concentrations were noted in the group with diabetes on CAPD treatment in comparison mainly to the group on dialysis without diabetes ($1,006 \pm 198$ vs. $1,145 \pm 301$) as well as to the healthy controls ($1,006 \pm 198$ vs. $1,050 \pm 245$). The lowest immunoglobulin IgA concentrations (218 ± 90) were noted in the group of dialysed patients without diabetes. However, immunoglobulin IgM concentrations were the highest in both groups of dialysed patients. The study of relation (Table 2) between concentrations of immunoglobulin and cytokine demonstrated positive correlation in patients without diabetes on CAPD (IgG: IL-1 β $r=0.43$, IgA: RANTES $r=0.54$ IgM: IL-1 β $r=0.54$); such relations were not found in the group with diabetes. The study of lipid parameters found no statistically significant differences in concentrations of total cholesterol and cholesterol LDL between the investigated groups. However, lowered cholesterol HDL concentrations were found to be statistically significant in the group of dialysed patients with and without diabetes in relation to healthy controls. Triglyceride testing demonstrated the highest concentrations in the group of patients with diabetes, mean 239.5 ± 142.6 mg/dl, a bit higher values 176.6 ± 59 mg/dl were obtained in the group on CAPD without diabetes and the lowest values in the control group 74.5 ± 22 mg/dl. Triglyceride values obtained in both groups demonstrated statistic significance in relation to the control group. The studies of relations between IgG and lipid concentrations demonstrated negative correlation with total cholesterol, cholesterol LDL and triglycerides ($r=-0.66$, $r=-0.71$ and -0.38 respectively). Similar trends expressed as statistically significant correlation ratio r were noted in the relation between IgA concentrations and above mentioned lipids in the group of patients with

diabetes ($r=-0.80$, -0.70 and -0.68 respectively). However, in the group of patients without diabetes on dialyses positive correlation was found between IgM concentrations and total cholesterol and cholesterol LDL ($r=0.75$ and 0.86 respectively). The evaluation of relations found significant correlation between concentrations of IL-1 β and RANTES $r=0.83$ and between concentrations of TNF α and RANTES $r=0.60$ in the group with diabetes. The studies of correlation between concentrations of cytokines and lipids demonstrated positive relation only between IL-1 β , RANTES and triglycerides in the group of patients with diabetes on CAPD.

DISCUSSION

Immunological activation expressed by increased cytokine concentrations is reflected in immunoglobulin production resulting from antibody synthesis following antigenic stimulation. Relations like these are found in healthy people. Our investigations found positive correlation between increased concentrations of TNF α and RANTES and immunoglobulins IgA and IgM also in the group of patients with chronic renal failure on CAPD without diabetes. However, in the group of patients with diabetes on CAPD treatment no correlation with immunoglobulin concentrations was found despite increased concentrations of cytokines TNF α and RANTES pointing to chronic stimulation. Epidemiological studies performed in the group of patients with diabetes type 1 (IDDM) and type 2 (NIDDM) demonstrated increased IgA and IgM concentrations with simultaneously lowered IgG concentrations (3,11). We also found the lowest immunoglobulin G concentrations in patients with diabetes on CAPD treatment. These patients had increased IgM values in relations to healthy controls and increased IgA in comparison to patients with chronic renal failure without diabetes on CAPD treatment.

Changes in immunoglobulin concentrations in patients with diabetes presented in other numerous reports, also confirmed by our investigation, may result from nonenzymatic immunoglobulin molecule glycation (7,11) which may relate to clinical complications such as bacterial and mycotic infections in diabetes. Increased incidence of infections in patients with diabetes and peritonitis in patients on CAPD treatment may result from lowered IgG concentration and changed IgG, IgA and IgM properties due to glycation. The degree of nonenzymatic glycation depends on the molecule size and is most pronounced in immunoglobulin class M, and subsequently in immunoglobulin A. Glycation may change immunoglobulin properties and their binding by specific antigens and receptors, just as glycation-modified lipoprotein LDL fraction, which may account for the increase in their blood serum concentrations.

Increased concentrations of total cholesterol, cholesterol LDL fraction and triglycerides with simultaneously decreased cholesterol HDL concentration found in our investigations in the group of patients with diabetic nephropathy on CAPD comply with the results generally found in diabetes. Dyslipidemia and glycoxidating lipoprotein modi-

fications in diabetes in connection with immunological activation processes contribute to accelerated vasculopathies due to, among others, autoantibodies of class IgG, IgA and IgM directed against oxidated LDL fractions (1,6).

On the basis of relation between lipid concentrations and concentrations of immunoglobulin class IgG and IgA and concentrations of triglycerides and chemotactic factor RANTES found in our investigation it is difficult to define the mechanism leading to these changes, because of the complexity of influencing factors. Considering possible impact of these changed parameters in accelerated vasculopathies (10,12) they are intensively researched for biochemical, immunological and genetic changes in the clinical models of diabetes type 1 and 2 and in animal studies.

Patients with diabetic nephropathy on CAPD treatment demonstrate the relation between changes in lipid parameters and detected changes in concentrations of proinflammatory cytokines and immunoglobulins.

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STRESZCZENIE

Zarówno u chorych na cukrzycę, jak i u pacjentów z przewlekłą niewydolnością nerek leczonych ciągłą ambulatoryjną dializą otrzewnową stwierdza się zaburzenia metabolizmu lipidów, zwiększoną produkcję wolnych rodników, które mogą stymulować syntezę i sekrecję cytokin, jak też nasilają nieenzymatyczną glikację białek, nawet przy prawidłowych stężeniach glukozy we krwi. Nieenzymatyczna glikacja dotyczy białek strukturalnych enzymatycznych, apolipoprotein a także immunoglobulin i innych białek odpornościowych.

Celem przeprowadzonych badań było określenie stężeń parametrów lipidowych, cytokin prozapalnych (TNF α i IL 1 β), chemokiny RANTES i immunoglobulin u chorych z nefropatią

Cukrzycową, leczonych ciągłą ambulatoryjną dializą otrzewnową. Grupy kontrolne stanowili chorzy z przewlekłą niewydolnością nerek bez cukrzycy leczenia CADO i ludzie zdrowi. W wyniku przeprowadzonych badań stwierdzono, że zarówno u chorych z nefropatią cukrzycową jak i bez cukrzycy leczonych CADO występują istotnie statystycznie wyższe stężenia TNF α , czynnika chemotaktycznego RANTES, triglicerydów i obniżone stężenia cholesterolu frakcji HDL. Co do stężeń immunoglobulin klas IgG, IgA i IgM, aczkolwiek nie wykazywały one znamiennych statystycznie różnic w poszczególnych grupach, to na uwagę zasługuje istotna statystycznie ujemna korelacja pomiędzy stężeniami immunoglobulin IgG i IgA a stężeniami cholesterolu całkowitego i cholesterolu LDL a także pomiędzy stężeniami IgA a triglicerydami w grupie pacjentów z cukrzycą leczonych CADO. Natomiast u chorych bez cukrzycy leczonych CADO stwierdzono dodatnie zależności pomiędzy stężeniami immunoglobulin IgM a cholesterolu całkowitym i cholesterolu LDL oraz czynnikiem chemotaktycznym RANTES. Stężenie RANTES wykazywało również dość wysoką korelację ze stężeniami Ig A. Zaś stężenie TNF α korelowało dodatkowo z IgM u pacjentów z nefropatią cukrzycową i z IgA u pacjentów bez cukrzycy leczonych CADO. Podsumowując, można stwierdzić, że u chorych z nefropatią cukrzycową leczonych CADO występują zależności pomiędzy stężeniami parametrów lipidowych, stężeniem cytokin prozapalnych i immunoglobulin.

