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C-reactive protein and hemodialysis treatment

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SUMMARY
Objective: To identify if a single hemodialysis session or long-term (one year) hemodialysis treatment influences serum C-reactive protein (CRP) level in patients with chronic renal failure.
Settings: Department of Clinical Biochemistry, St. Anna's Faculty Hospital, Brno, Czech Republic.
Methods: A total of 27 patients on maintenance hemodialysis (bicarbonate hemodialysis with hemophan membrane) were included in the study. Their chronic dialysis protocol consisted of 3-4 hours sessions three times a week. Plasma CRP concentration, blood leukocyte differential counts and albumin were determined by routine methods. Blood samples were taken before and after a single hemodialysis sessions at the start of the study, and after 12 months of regular hemodialysis treatment. Two-factor analysis of variance in parametrically ordered data and the Wilcoxon paired test for non-parametrically ordered data were used.
Results: The mean CRP level after a 4-hour hemodialysis session was significantly higher than that before hemodialysis (12.3 ± 2.22 and 9.9 ± 2.12 mg/l, $p < 0.01$). No significant changes in mean CRP levels were found after 12 months of regular hemodialysis treatment in comparison with CRP levels at the start of the study (8.6 ± 2.83 and 9.9 ± 2.12 mg/l, $p = 0,191$).
Conclusion: Our study corroborates the hypothesis that the hemodialysis procedure itself can affect inflammation markers (CRP), but CRP level does not depend on the length of time of regular hemodialysis treatment.
Key words: atherosclerosis, chronic kidney failure, C-reactive protein, hemodialysis, inflammation.

SOUHRN
Soška V., Sobotová D.: C-reaktivní protein a hemodialyzační terapie
Cíl práce: Posoudit vliv jednorázové hemodialýzy a dlouhodobé pravidelné hemodialyzační léčby (1 rok) na hladinu C-reaktivního proteinu (CRP) u pacientů s chronickým renálním selháním.
Název a sídlo pracoviště: Oddělení klinické biochemie, FN u sv. Anny v Brně.
Materiál a metody: Do sledování bylo zavzato celkem 27 nemocných podstupujících pravidelnou hemodialýzu (bikarbonátová hemodialýza, membrána hemophan). Dialýzy probíhaly po dobu 3–4 hodin 3krát týdně. Plazmatická koncentrace CRP, albuminu a diferenciální rozpočet leukocytů byly stanoveny běžnými rutinními metodami. Krev na vyšetření byla odebrána před hemodialýzou a po hemodialýze na počátku sledování a znovu po 12 měsících pravidelné hemodialyzační léčby. K vyhodnocení byly použity párové přístupy statistické analýzy: dvoucestná analýza rozptylu (ANOVA) u parametricky rozložených dat a Wilcoxonův párový test pro neparametrická data.
Výsledky: Průměrná koncentrace CRP byla po jednorázové hemodialýze signifikantně vyšší, než před hemodialýzou (12,3 ± 2,22 a 9,9 ± 2,12 mg/l, $p < 0,01$). Po 12 měsících pravidelné hemodialýzy nebyl nalezen signifikantní rozdíl v hladině CRP ve srovnání s hodnotou na počátku sledování (8,6 ± 2,83 a 9,9 ± 2,12 mg/l, $p = 0,191$).
Závěr: Výsledky podporují hypotézu, že aktivaci mediátorů zánětu (CRP) může ovlivnit jednorázová hemodialyzační procedura, hladina CRP však nezávisí na délce trvání chronické hemodialyzační léčby.
Klíčová slova: ateroskleróza, chronické selhání ledvin, C-reaktivní protein, hemodialýza, zánět.

Introduction

It has been demonstrated that patients on hemodialysis (HD) are at a high risk of cardiovascular disease [1]. As atherosclerosis is a multifactor disease, this risk may be the result of both classic risk factors (dyslipidaemia, hypertension, smoking, diabetes) and newly established risk factors (oxidative stress, lipoprotein (a), homocysteine, hypercoagulation, low paraoxonase activity etc. [2]. One of the most important factors in the onset and progression of atherosclerotic lesions is the inflammation – and atherosclerosis itself has inflammatory origins [3]. Inflammation can induce endothelial dysfunction leading to vascular damage and to atherosclerotic lesions [4]. As the C-reactive protein (CRP) is an excellent marker of systemic inflammation, its increased values, indicating inflammatory response, are linked to a higher risk of cardiovascular disease [5, 6]. It has been demonstrated that uremia and HD are associated with chronic inflammation, and increased CRP level has been found to be an important predictor of cardiovascular mortality even in patients with end-stage renal disease and HD patients [7, 8]. There is no doubt that the CRP serum level is higher in hemodialysed patients in comparison with healthy subjects, and it has been found that CRP is significantly higher in HD patients than in patients on peritoneal dialysis [9, 10]. But it is not clear yet whether the dialysis procedure itself significantly contributes to the inflammation, and if CRP level depends on the duration of dialysis treatment.

The objective of this study was to compare serum CRP levels before and after HD session in patients on chronic HD treatment, and to compare CRP levels after 1 year of regular HD with the concentration at the start of the study.

Methods

Patients and blood collection

27 patients (9 males, 18 females) undergoing regular maintenance HD were included into the study. Their mean age was 63.5 years (with a range of 30 to 78 years) and they had been on chronic HD for 3.5 ± 2.9 years (with range from 1 to13 years). Their chronic dialysis protocol consisted of 3–4 hours/three times a week depending on residual renal function. Renal failure was caused by glomerulonephritis (n = 9), tubulointerstitial nephritis (n = 7), diabetic nephropathy (n = 5), polycystic liver and kidney disease (n = 4), and pyelonephritis (n = 2). Patients included into this study underwent bicarbonate HD with hemophan membrane (GFS+16, GAMBRO, Lund, Sweden). Standard unfractionated heparin as an anticoagulant in the dose 3000–5000 IU was used. All patients received phosphate binders, recombinant human erythropoetin (4000–8000 IU/week), and low i. v. doses of iron (the iron supplementation was not carried out before blood sampling at the day of the study). The patients were treated with antihypertensive drugs (ACE inhibitors, beta-blockers, calcium channel inhibitors, diuretics). None of the patients showed signs of systemic infection (leukocytosis or fever) 10 days prior to blood sampling for laboratory analysis. None of them underwent surgery in the previous 10 days before examination. Each patient gave his informed consent to the participation in the study.

Analytical procedures: Heparinised blood samples were drawn from an arterio-venous shunt at the beginning and at the end of the HD procedure. The blood leukocyte counts and differential counts were determined using Coulter STKS (Coulter, England). Plasma CRP levels were measured using the nephelometric assay (wide range CRP assay, Scill), albumin concentration using bromcresol green method (Pliva-Lachema) with ADVIA 1650 analyser (Bayer). All parameters were analysed at the start of the study (T_0) and after the 12th month of regular HD treatment (T_1).

Statistical analysis: All values are expressed as the mean ± SD. All variables were tested for Gaussian distribution with the Kolgomorov-Smirnov and Shapiro--Wilk tests. For parametrically ordered data (albumin, blood leukocyte counts) two-factor analysis of variance (paired ANOVA) was used for the evaluation of the HD effect (before and after HD) and for the differences in time (T_0, T_1). For non-parametrically ordered data (CRP) Wilcoxon paired test was used. Differences at p-value < 0.05 were considered statistically significant.

Results

Patients: 1 patient died during the study, 3 underwent renal transplantation, one switched to peritoneal dialysis. One patient was excluded because of obvious inflammatory conditions at the time of the second examination (1 year). 21 patients underwent the second follow-up examination after 1 year of regular HD treatment (T1).

Table 1 compares the results of laboratory data before and after HD session at time T_0 (at the beginning of the study), table 2 shows the results (before and after HD session) at time T_1 (1 year of regular HD treatment). Non-significant changes both in total leukocyte counts and in absolute neutrophil numbers in the blood were found after HD treatment in comparison with the predialysis status both at T_0 and in T_1. The leukocyte and granulocyte counts did not change significantly at times T_0 and T_1 (p = 0.653 and 0.756 respectively). The mean CRP level increased significantly after the 4-hour HD session at T_0 (P = 0.003) and at T_1 (p = 0.004) in comparison with the predialysis level. The predialysis CRP level was not changed at T_1 in comparison with that at T_0 (p = 0,191). The serum albumin concentration after single HD sessions showed significant elevation due to hemoconcentration during HD. When predialysis albumin concentrations at T_0 and T_1 were compared, a significant decline after 1 year of regular HD program was found (39.2 ± 0.50 vs. 37.6 ± 0.78; p = 0.002).

Table 1. Summary of the results of laboratory analysis in hemodialyzed patients before and after hemodialysis session at time T_0 (at the beginning of the study)

	Before hemodialysis	After hemodialysis	P-values
Leukocytes (10^9/l)	7.44 ± 0.41	7.08 ± 0.40	n.s.
Granulocytes (10^9/l)	4.99 ± 0.33	4.99 ± 0.37	n.s.
C-reactive protein (mg/l)	9.9 ± 2.12	12.3 ± 2.22	< 0.01
Albumin (g/l)	39.2 ± 0.50	41.0 ± 0.92	< 0.05

Data are expressed as the mean ± standard deviation.

Table 2. Summary of the results of laboratory analysis in hemodialyzed patients before and after hemodialysis session at time T_1 (after 1 year of regular hemodialysis treatment)

	Before hemodialysis	After hemodialysis	P-values
Leukocytes (10^9/l)	6.97 ± 0.32	6.57 ± 0.47	n.s.
Granulocytes (10^9/l)	4.92 ± 0.31	4.89 ± 0.41	n.s.
C-reactive protein (mg/l)	8.6 ± 2.83	10.8 ± 3.12	< 0.01
Albumin (g/l)	37.6 ± 0.78†	40.1 ± 1.26	< 0.01

Data are expressed as the mean ± standard deviation; † – significant difference of T_0 vs. T_1 at p < 0.01.

Discussion

The present study showed that a 4-hour HD session could induce a significant increase in serum CRP concentration when compared with the predialysis level. The HD procedure itself may contribute to the inflammation, as these patients' blood is in regular contact with the poorly biocompatible materials (HD membranes), and also with the nonsterile dialysis solution [12, 13]. This contact can result in the activation of leukocytes and complement with subsequent release of pro-inflammatory mediators. This theory is corroborated by a decrease in CRP level after switching the water purification system from deionisation to reverse osmosis [14]. Thus the dialysis procedure itself could be responsible for the inflammatory reaction in the vascular bed. Significantly elevated plasma concentration of several inflammatory markers was described by Liang after a single HD session [15]. A significant increase in procalcitonin (as a marker of micro-inflammation) after 4 hours of HD in comparison with that at the start of the session, was also reported [16]. On the other hand, no significant changes in CRP and serum amyloid A levels after a HD session in comparison with predialysis values were reported [9, 16]. Some authors therefore suggested that the contribution of the dialysis procedure itself is not likely major factor initiating the inflammatory state because of the high incidence of predialysis inflammation [17, 18]. Thus the increase of CRP at least in part could be explained by hemoconcentration after dialysis as obvious from albumin level increase.

The existence of a correlation between the CRP level and the HD treatment duration is also not certain yet. In the present study we found no significant changes in CRP concentration after one year of HD treatment in comparison with that at the start of the study. In some studies, the induction of increased CRP concentration was reported only after the very start of chronic HD, and CRP levels were only related to the renal function during predialysis chronic renal failure [19, 20]. On the contrary, a significant correlation between CRP levels and the length of dialysis sessions was described, and no significant differences in CRP concentration between healthy subjects and patients with uncomplicated uremia on conservative therapy were found [21]. Elevated CRP may also be connected with malnutrition and it has been documented that a strong correlation between inflammatory markers and indicators of malnutrition (albumin concentration) exists in HD patients [22, 23]. In our study, the predialysis serum albumin concentration at the start of the study was within reference values but we found a significant decline after 1 year of regular HD.

The results of our study corroborate the hypothesis that HD procedure itself can induce CRP elevation, but CRP level does not depend on the length of time of regular hemodialysis treatment (it returns to the initial level in the period between HD) as no CRP changes were found after 1 year of regular HD treatment.

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Zaujalo nás

Patobiochemie diabetu typu 2 (choroba s dvojím defektem)

Diabetes jako choroba s dvojím defektem je charakterizována *inzulinovou rezistencí* a *poruchou funkce beta-buněk*. Glukóza pocházející ze sacharidů v přijímané potravě a z glukózy uvolňované z jater, je regulována inzulinem. Diabetes typu 2 je důsledek porušené rovnováhy mezi citlivostí na inzulin a sekrecí inzulinu. U diabetu chybí adekvátní regulace tvorby glukózy adekvátní sekrecí inzulinu, což vede k nadprodukci glukózy pocházející z jater, ke sníženému vychytávání kosterním svalstvem; k tomu přistupuje zrychlené vyprazdňování žaludku a nadměrná lipolýza v tukové tkáni. Po určité době pankreatické beta-buňky nedokáží secernovat zvýšené množství inzulinu; dochází ke glukózové intoleranci, inzulinové rezistenci a posléze ke klinickému obrazu diabetu typu 2.

S inzulinovou rezistencí jsou pak sdruženy různé klinické syndromy jako diabetes typu 2, obezita, kardiovaskulární choroby, arteriální hypertenze, nealkoholová steatohepatitida, syndrom polycystických ovarií, určité formy nádorových onemocnění, spánková apnoe.

Patobiochemie diabetické dyslipidémie

Diabetická dyslipidémie zahrnuje:
1. Hypertriacylglycerolémii
2. Zvýšený podíl malých denzních LDL
3. Snížení HDL
4. Postprandiální lipémii

Za většinou těchto abnormalit stojí *kaskáda patologických kroků navozená společně inzulinovou rezistencí a dysfunkcí enzymu lipoproteinové lipázy (LPL)*.

Inzulinová rezistence v adipocytech indukuje nadměrnou lipolýzu aktivací hormon-senzitivní LPL. To vede ke zvýšení hladiny neesterifikovaných mastných kyselin (FFA) v krevní cirkulaci. FFA jsou vychytány v játrech, kde pod vlivem inzulinové rezistence jsou příčinou zvýšeného množství apolipoproteinu B (apo B) tím, že je potlačována jeho degradace. Játra produkují a exportují vyšší množství VLDL částic bohatých na triacylglyceroly (TG) a apo B. Za normálních okolností VLDL podléhají na endotelu cévní stěny tukové a svalové tkáně lipolytickému účinku LPL, uvolněné FFA slouží jako energetický zdroj pro svalovou práci, v adipocytech jsou pak skladovány ve formě TG; VLDL částice se tak přeměňují na LDL částice. Aktivací LPL se zvyšují též HDL částice. U diabetu je však aktivita LPL narušena, což se podílí na zvýšení VLDL částic bohatých na TG (VLDL-TG), dále na snížení HDL a na relativním snížení LDL částic.

Na těchto abnormalitách se účastní též „cholesterol-ester transfer protein“ (CETP). Při zvýšení VLDL-TG účinkem CETP se TG přenášejí na HDL a LDL, a naopak cholesterol-estery putují z HDL a LDL na VLDL-TG. To znamená, že diabetici se vyznačují zvýšenými hodnotami VLDL se současným snížením HDL a přítomností „malých denzních LDL“ vzniklých z VLDL bohatých na TG v játrech. Takto se vysvětluje patogeneze 3 složek diabetické dyslipidémie. Čtvrtou – postprandiální lipémii – charakterizuje přetrvávání chylózní plazmy po požití tuků a je způsobena jednak nedostatečnou aktivitou LPL (pomalé snižování chylomikronémie), jednak poruchou v odstraňování „chylomikronových zbytků“ játry pro jejich nedostatečné zachycování prostřednictvím specifického heparansulfát-proteoglykanu.

Jaroslav Masopust