Sialic acid content in erythrocyte membranes of patients on chronic hemodialysis

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KEY WORDS
erythrocyte membrane, hemo dialysis, sialic acids

ABSTRACT

INTRODUCTION  Sialic acids (SA) located in erythrocyte membranes (EM) play an important role in the survival of circulating red blood cells.

OBJECTIVES  The aim of the present study was to evaluate the SA content in EM obtained from patients on chronic hemodialysis (HD) and to examine the relationships between SA and hematological parameters. Moreover, the effects of HD, treatment with human recombinant erythropoetin (epoetin), and some biochemical and hematological parameters were analyzed.

PATIENTS AND METHODS  The total protein (TP) and total sialic acids (TSA), together with SA bound with proteins (PBSA) and lipids (LBSA), were determined in EM of 72 HD patients and compared with the control group of healthy individuals (CG; n = 25). The adequacy of HD, weekly epoetin doses, mean arterial pressure (MAP), comorbidity score, serum levels of albumin, intact parathyroid hormone (iPTH), low-density lipoprotein cholesterol (LDL-cholesterol) were estimated in patients.

RESULTS  Compared to the CG, HD patients had higher levels of TSA (p <0.001), PBSA (p <0.001), LBSA (p <0.001) and decreased TP levels (p <0.001). The TP (p <0.045) and PBSA (p <0.05) levels were higher in patients with diabetic nephropathy than in non-diabetic HD patients. In HD patients there were correlations between TSA, PBSA in EM and some hematological parameters. There were no relationships between the TSA, PBSA content in EM and variables such as HD, epoetin treatment, MAP comorbidity score, albumin, iPTH, and LDL-cholesterol.

CONCLUSIONS  The results of the current study demonstrated there are significantly higher levels of TSA, PBSA, LBSA and lower TP levels in EM obtained from HD patients compared to healthy subjects. Comorbidity score, epoetin and HD treatment, MAP, iPTH, albumin and LDL cholesterol had no influence on SA levels in EM of patients.
The aim of the study was to evaluate the SA content in EM obtained from HD patients and to examine the relationships between SA and some hematological parameters. Moreover, potential associations of HD, anemia treatment, and other measured parameters with the forms of SA in EM analyzed in HD patients were sought.

**PATIENTS AND METHODS** An observational study was conducted on a cohort of 72 patients (36 men and 36 women) with ESKD on HD treatment. The patients were aged 25–89 years (mean age 63.8 ±14.8), with the mean duration of HD treatment of 30 ±12 months (range, 6–108 months). Twelve patients smoked cigarettes. The causes of ESKD included chronic glomerulonephritis (n = 23), diabetic nephropathy (n = 20), tubulointerstitial nephritis (n = 2), hypertensive nephropathy (n = 8), polycystic kidney disease (n = 4), and nephropathy of unknown origin (n = 5). Fifty-seven percent of the patients had arterial hypertension and were treated with calcium blockers, angiotensin-converting enzyme inhibitors, and α- and β-blockers achieving a satisfactory blood pressure control. Moreover, 29 patients (40%) were treated with acetylsalicylic acid (ASA). All patients received a 4 ±0.3-hour HD treatment 3 times weekly with bicarbonate dialysate; low flux membrane dialyzers were reused. The patient demographics and baseline characteristics are shown in Table 1.

The control group (CG) consisted of 25 healthy volunteers (12 men and 13 women) with a mean age of 56.1 ±7.7 years. The control subjects with normal blood pressure and no clinical signs of diabetes mellitus, renal and cardiovascular diseases were recruited from hospital staff and blood donors who underwent health examination. Several biochemical measurements were made to eliminate from the CG subjects with any diseases which could influence the contents of the examined parameters in EM. Moreover, 14 healthy subjects (56%) smoked cigarettes.

In both groups the following parameters of the EM were measured: total protein (TP), total sialic acid (TSA), SA bound with proteins (PBSA) and with lipids (LBSA). Moreover, hematological parameters were assessed: hemoglobin (Hb), red blood count (RBC), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Likewise, in HD patients serum levels of albumin, LDL-cholesterol, intact parathyroid hormone (iPTH), and mean arterial pressure (MAP), the adequacy of HD and Charlson comorbidity score (CS) were assessed. A dose of erythropoietin β was expressed as the weekly units per body weight after dialysis (IU/kg/week).

**Biochemical methods and kinetic modeling** Blood samples were drawn on a midday of a week, prior to HD in EDTA tubes, and after centrifugation supernatants were stored at −80°C until analysis of all biochemical parameters.

TP was measured by the Lowery method with folate reagent. The TSA content was determined using a colorimetric method. PBSA and LBSA were determined after chloroform methanol extraction, followed by protein precipitation by the Katapodis method. There was no need to repeat the measurement of SA content in EM and a single measurement was performed in this study.

Moreover, systolic and diastolic blood pressure were measured in patients before HD. The MAP was then calculated using the following formula (systolic + 2 × diastolic blood pressure)/3. The adequacy of HD was expressed as eKt/V calculated using the Daugirdas method.

iPTH was measured with an immunochromoluminescence method using the DPC iPTH assay with the IMMULITE 1000 automated immunoassay analyzer. Other biochemical parameters were measured by standard automated techniques.

The Bioethical Committee of Scientific Research of Medical School in Lublin approved the project regarding measurements of SA content in EM in both groups (KE – 0254/132/2007).

**Statistical analysis** Statistical analysis was performed using STATISTICA 7.1 (version for Windows, StatSoft®, Kraków, Poland). The distribution of variables was analyzed using the Kolmogorov-Smirnov and W Shapiro-Wilk tests to confirm normal distribution. All measured parameters were expressed as mean values ± standard deviation (SD) or median with interquartile range. The U-test of Mann-Whitney analysis was used to compare levels of all measured parameters between HD patients and the CG because of non-normal distribution of the parameters tested. The Spearman coefficient of correlation was used to determine the relationships between all studied parameters and the SA composition in EM obtained in both groups.

The ANOVA Kruskal-Wallis test was used to calculate the effect of the parameters such as gender, smoking and comorbidity in the study groups on the content of EM. Differences between both groups were considered significant at p <0.05.
**RESULTS** In HD patients the mean TSA level was 49% higher (p <0.001) than in the CG (TABLE 2). Likewise, the mean PBSA level was 52% higher (p <0.001) and that of LBSA 26% higher (p <0.001) than in the CG. Moreover, the mean TP level was 180% lower (p <0.001) than in the CG. No differences in mean levels of these parameters between men and women were observed in both groups. There were no differences between smokers and nonsmokers in the CG with regard to studied parameters in EM. However, in smoking HD patients the levels of all studied parameters in EM were slightly higher than in non smokers. However, these differences were not statistically significant, i.e. TP (p <0.08), TSA (p <0.06), PBSA (p <0.07), and LBSA (p <0.3).

Compared to diabetic HD patients, the TP and PBSA levels were significantly lower in non-diabetic subjects (301.5±144.8 µg/mg vs. 227.1±89.1 µg/mg, p <0.045, and 84.3±35.98 µg/mg vs. 68.2±24.2 µg/mg, p <0.05, respectively). There were no differences in mean levels of all parameters determined in EM of patients treated and not treated with ASA. In the CG no relationships between the studied parameters in the EM and all hematological parameters were observed. However, it was found that in HD patients RBC positively correlated with TSA (r = 0.25, p <0.001) and PBSA (r = 0.27, p <0.0005). Similarly, Hb positively correlated with TP (r = 0.32, p <0.006), TSA (r = 0.21, p <0.008), and PBSA (r = 0.23, p <0.004).

Moreover, Ht showed positive associations with TP (r = 0.32, p = 0.009), TSA (r = 0.24, p <0.004), and PBSA (r = 0.32 p <0.001). An inverse correlation was observed between MCV and the 3 following variables, i.e. TP (r = −0.24, p <0.03), TSA (r = −0.23, p <0.04), and PBSA (r = −0.26, p <0.02).

Additionally, there were significant inverse associations between MCH and such variables as TP (r = −0.25, p <0.04), TSA (r = −0.23, p <0.04), and PBSA (r = −0.25, p <0.03). There were no associations between the parameters evaluated in EM and the following variables, i.e. eKt/V, epoetin β dose, and the duration of HD treatment. Moreover, LDL-cholesterol, albumin, iPTH, CS, and MAP had no influence on the examined parameters in EM.

**DISCUSSION** The present study showed substantial alterations in EM in chronic HD patients. Compared to the CG, the ESRD patients had significantly higher levels of TSA, PBSA and LBSA. The findings showed that HD treatment and adequacy of HD had no influence on the content of all the 2 variables studied in EM. It was demonstrated only in patients that some studied components of EM show significant positive correlations with hematological parameters. Associations between Hb, RBC, Ht and both TSA and PBSA were observed. It might be speculated that due to extremely high levels of SA, EM are more resistant to uremic toxins and HD treatment. Likewise, Szprynger et al. observed positive relationships between TSA, Hb, Ht in children on continuous ambulatory peritoneal dialysis (CAPD) and HD treatment. Moreover, the authors demonstrated that TSA positively correlated with the duration of renal replacement therapy. They concluded that elevated TSA levels in dialyzed children could be the result of non-specific body response characterized by tissue and organ damage induced by extracorporeal circulation (in the HD patients) or the presence of the dialysate solution in the peritoneal cavity (in the CAPD patients).

The SA content that decreases with age is a physiological process and the measurement of SA in the EM is a tool to assess the age of RBC. Gafter et al. observed that mean SA in membranes were higher in immature RBC, including reticulocytes, in HD patients compared to mature RBC. The high TSA levels in EM of HD patients could result from measurement of SA in immature fraction of RBC, whose production was stimulated by epoetin β. In the present study we did not observe any relationship between anemia treatment and the SA content in EM.

The RBC have the membrane protein skeleton located at the membrane-cytosal surface. This structure participates in several membrane
functions, including the control of shape, viscoelastic properties, surface topology and membrane stability. The findings of the current study indicate that the study patients demonstrated 180% lower TP levels than those found in the CG. In our opinion, such a significant deficiency in proteins was probably compensated by a higher content of TSA and LBSA. Causes of this abnormality were sought. No association between TSA and the serum albumin level was observed. Moreover, we suspected that a low TP level was probably induced by a high iPTH level. Bogin et al. examined the effect of iPTH on erythrocyte osmotic fragility and concluded that iPTH could damage EM and be at least partially responsible for the shortened survival of RBC in uremia and cause protein loss. No influence of iPTH on all the analyzed in EM parameters was observed in the current study.

We examined the effect of CS on the content of EM and we found no relationship between those parameters. However, it could be demonstrated that diabetic HD patients had significantly higher TP levels than non-diabetic patients. Mazzanti et al. studied solely the content of TSA in diabetic and non-diabetic patients with normal renal function and observed that the mean SA level was significantly higher in this group than in the CG.

The activity of Na⁺, K⁺-ATPase in RBC is reduced in the majority of uremic patients. Potential mechanisms underlying the inhibition of Na⁺, K⁺-ATPase in ESKD patients involve not only toxin accumulation, impaired hormone activity, but also altered lipid content. Lipid composition of EM affecting fluidity is in a state of dynamic balance with plasma lipoproteins and reflects the processes occurring in extracellular environment. Broncel et al. evaluated the effect of statins on plasma lipid profile and EM fluidity in patients with hyperlipidemia type II. The authors concluded that statin therapy not only favorably alters plasma lipids, but also causes an increase in EM fluidity. The results of the present study showed that EM were rich in LBSA, but these parameters did not correlate with any blood cell parameters or with serum LDL-cholesterol levels. A relationship between statin therapy and lipid-bound SA in EM was not evaluated.

Raznikova et al. reported increased LBSA levels in EM in patients with primary hypertension. There was no association between MAP and the LBSA content in EM.

Salbas studied the effect of acute smoking on the RBC deformability in healthy young and elderly smokers versus nonsmokers. He concluded that age and smoking are independent factors that affect human RBC deformability. We examined the influence of smoking on the SA content in EM in both groups. The results demonstrated that there were no differences in mean levels of studied parameters between smokers and nonsmokers in the CG. However, it was found that the levels of all studied EM parameters were higher in smokers than in the nonsmoking HD group. However, these differences were not statistically significant.

ASA has been reported to induce disorders in the lipid-protein matrix and membrane protein conformation. We did not observe any differences in mean levels of all studied parameters in EM between patients treated and not treated with ASA.

Megaloblastic macrocytic anemia is a disorder in which the RBC size is increased and the macrocyte is thicker than normal. This abnormality could be caused by vitamin B12 and folate deficiencies with their potential association with altered SA content in EM. We observed negative relationships between TSA and PBSA and MCV and demonstrated no associations between RDW or MCHC and the EM parameters. These findings appear elusive. The design of this study was observational and a size of HD patients’ group was limited. The study was focused on the whole RBC population with no analysis of the SA content in EM for the immature and mature RBC. Because of the cognitive character of the study, we determined potential mechanisms involved in anemia observed in HD patients. Inflammation, which probably affects the SA content in EM in HD patients, has not been examined.

In conclusion, the current study shows markedly higher levels of TSA and PBSA and LBSA, combined with low levels of TP in EM observed in HD patients compared to healthy subjects. The HD and epoetin β treatment, CS, albumin, iPTH, MAP and LDL-cholesterol had no influence on the SA content in EM of HD patients. Due to extremely high levels of SA, RBC are more resistant to uremic toxins and HD treatment.

REFERENCES

Zawartość kwasów sialowych w błonach erytrocytarnych chorych przewlekle hemodializowanych

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SŁOWA KLUCZOWE
błona erytrocytarna, hemodializa, kwas sialowy

STRESZCZENIE

WProwadzenie Kwasy sialowe zlokalizowane w błonach erytrocytarnych (BE) są istotnym czynnikiem determinującym przeżycie erytrocytów w krwiobiegu.

CELE Celem badań wykonanych u chorych leczonych przewlekle hemodializami (hemodialysis – HD) była ocena wpływu terapii na stężenia w BE białka (total protein – TP), kwasów sialowych (total sialic acids – TSA), form związanych z białkami (protein-bound sialic acids – PBSA) oraz z tłuszczami (lipid-bound sialic acids – LBSA). Poszukiwano również zależności między oznaczanymi w BE a leczeniem niedokrwistością za pomocą ludzkiej rekombinowanej erytropoetyny (epoetyna), wybranymi parametrami biochemicznymi oraz morfologicznymi krwi.

PACJENTI I METODY Oznaczenia wykonano u 72 chorych leczonych HD oraz u 25 osób zdrowych (grupa kontrolna [GK], n = 25). Ponadto u chorych oceniono adekwatność HD, tygodniową dawkę epoetyny, średnie wartości ciśnienia tętniczego (mean arterial pressure – MAP), punktację chorobowości, stężenie albuminy, intackt para thyroid hormone (iPTH) oraz cholesterolu frakcji lipoprotein o małej gęstości (low-density lipoprotein – LDL).

WYNIKI W porównaniu z GK w BE chorych stwierdzono istotnie wyższe stężenia: TSA (p <0,001), PBSA (p <0,001), LBSA (p <0,001) oraz niższe stężenie TP (p <0,001). Natomiast w grupie hemodializowanych osób z nefropatią cukrzycową zaobserwowano znamienne wyższe stężenie TP (p <0,045) oraz PBSA (p <0,05) w porównaniu z chorymi hemodializowymi bez cukrzycowej choroby nerek. Tylko u chorych obserwowano zależności między oznaczanymi w BE frakcjami kwasów sialowych: TSA i PBSA oraz wybranymi parametrami morfologicznymi krwi. Nie stwierdzono korelacji między zawartością TSA, PBSA lub LBSA w BE a takimi zmiennymi jak HD, leczenie epoetyną, punktacją chorobowości, MAP oraz stężeniem albuminy, iPTH i cholesterolu LDL.

WNIOSKI Nasze badania wykazały, że BE chorych hemodializowanych zawierają istotnie wyższe stężenia TSA, PBSA, LBSA oraz niższe stężenia TP w porównaniu z osobami zdrowymi. Chorobowość, hemodializoterapia, leczenie epoetyną, MAP, iPTH, albumina ani cholesterol LDL nie miały wpływu na stężenia SA w BE badanych chorych.