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NEPHROLOGY - ORIGINAL PAPER

Regenerative potential of platelets in patients with chronic kidney disease

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Abstract

Introduction Chronic kidney disease (CKD) is a systemic disease affecting many organs. Progression of renal failure aggravates ongoing inflammation and increases oxidative stress. In the final stage of CKD, it is necessary to use renal replacement therapy. A side effect of dialysis therapy is the synthesis of proinflammatory factors and increased oxidative stress, which activates platelets and immune cells.

Aim of the study To determine the regenerative potential of platelets in patients with CKD based on the analysis of the relationships between substances with potential regenerative action, as well as analysis of the influence of the type of renal replacement therapy used on regeneration of platelets.

Materials and methods The study group consisted of 117 patients. Based on the type of therapy used, patients were divided into four groups: hemodialysis, peritoneal dialysis, kidney transplant patients, and conservative treatment (30, 30, 27, and 30 patients). The control group consisted of 30 healthy volunteers. The concentrations of IGF-1, TGF-β, and PDGF-B in the blood serum were measured by ELISA methods.

Results It was shown that renal replacement therapy significantly influences the concentration of platelet growth factors (IGF-1: $p = 0.025$ and PDGF-B: $p = 0.012$). There was a relationship between the type of renal replacement therapy and the duration of dialysis, and the concentration of IGF-1, PDGF-B ($p < 0.00001$, $p < 0.001$).

Conclusions The type of renal replacement therapy has a different effect on the concentration of platelet-derived growth factors IGF-1 and PDGF-B. PD patients had the highest concentrations of all growth factors, and this may be due to the presence of inflammation induced by dialysis-related advanced end-products of glycosylation (AGE).

Keywords Platelet growth factors · IGF-1 · PDGF-B · TGF-β · Chronic kidney disease

Introduction

Platelets are the first molecules that reach the site of tissue damage and actively participate in the first stages of the inflammatory process and tissue healing. At the site of tissue damage, platelets secrete a broad spectrum of platelet-derived growth factors (PDGF) and other molecules such as chemokines, arachidonic acid metabolites, extracellular matrix (ECM) proteins, nucleotides, or ascorbic acid [1, 2].

Platelet growth factors are released from the granules contained in plaques after their adhesion and subsequent activation by agonists [1, 2]. Platelet growth factors have been shown to play an important role in all phases of tissue healing. The active secretion of these proteins by platelets begins within 10 min after thrombus formation, with more than 95% of the pre-synthesized growth factor (GF) being secreted within 1 h. Then, within 5–10 days, to maintain balance and survival, the platelets produce and secrete additional GF [1, 3].

GFs secreted by platelets include PDGF, epidermal growth factor (EGF), insulin-like growth factor one (IGF-1), TGF-β (transforming growth factor-beta), vascular

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endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (bFGF) [4].

These growth factors are the main components of platelet rich plasma (PRP), and play a very important role in several processes, including increasing the recruitment, proliferation, and differentiation of cells involved in tissue regeneration and bone remodeling, vascular remodeling, angiogenesis, inflammatory processes, and coagulation.

PDGF stimulates the proliferation of many cells, including connective tissue cells. After its release, PDGF is chemotactic to monocytes, neutrophils, and fibroblasts [5]. PDGF also affects cell growth, cell migration, metabolic effects, and modulates cell-membrane receptors [6]. It occurs in four isoforms; A, B, C, and D, and contains two receptor chains, PDGF receptor-alpha (PDGF-Rα) and PDGF receptor-beta (PDGF-Rβ). All isoforms increase bone cell proliferation [7]. PDGF plays an important role in wound healing, atherosclerosis, fibrosis, and neoplasia.

Numerous studies on genes encoding PDGF show that their expression is altered in most kidney diseases. PDGF-C has been shown to mediate renal interstitial fibrosis and PDGF-B and D are key factors associated with mesangioproliferative disease and interstitial fibrosis [8–10]. Although many GFs participate in the wound healing process, PDGF and TGF-β1 seem to be the most involved modulators [11]. TGF-β belongs to the family of recently discovered secretory proteins. It has three isoforms (TGF-β1, β2, and β3). It is mainly produced in platelets and in macrophages. TGF-β causes chemotaxis and activation of monocytes, macrophages, and fibroblasts. Activated fibroblasts increase the formation of extracellular matrix and collagen and stimulate cells to form a temporary matrix on the wound [12].

In the literature, there are reports on the relationship between plasma concentrations of TGF-β and the progression of kidney disease. The kidneys respond to injury by the release of proinflammatory cytokines and growth factors, such as transforming growth factor. Long-term overexpression of TGF-β occurs in patients undergoing dialysis as a result of the need to maintain vascular access, and causes ECM to accumulate in the damaged kidney [13, 14] and ultimately leads to glomerular and interstitial fibrosis [15–18].

A characteristic feature of IGF-1 is its structural similarity to insulin, which explains its ability to bind to insulin receptors and induce insulin-like effects. IGF-1 promotes osteoblast proliferation and stimulates osteocalcin synthesis. In addition, it stimulates the proliferation and differentiation of mesenchymal stem cells in the processes of chondrogenesis, adipogenesis, and myogenesis, promotes neuronal differentiation, induces vascular endothelial cell chemotaxis, and is an important modulator of cell apoptosis [7].

Chronic kidney disease (CKD) leads to a decrease in the bioavailability of IGF-1, despite normal or even elevated levels of this protein in the blood [19–21]. In the literature, there are reports on the relationship between the concentration of IGF-1 in the blood, and increased mortality in patients suffering from CKD. This may be due to an increased resistance to growth hormone (GH) and IGF-1 in this group of patients, which in turn leads to numerous metabolic disorders [22, 23].

CKD is a systemic disease, caused most often by diabetic or hypertensive nephropathy, glomerulonephritis, acute kidney injury, and polycystic kidney degenerations. Progression of renal failure aggravates ongoing inflammation and increased oxidative stress. In the early stages of CKD, conservative treatment is used. In the final stage, it is necessary to use dialysis or kidney transplantation. A side effect of dialysis therapy is the synthesis of proinflammatory factors and increased oxidative stress, resulting from the creation of vascular access and repeated blood contact with artificial dialysis materials. This leads to the activation of platelets and transient leukopenia, followed by the activation of cells of the immune system and complement system, as well as an increase in interleukin concentration. In addition, tissues and blood vessels are also damaged during dialysis and organ transplantation.

The aim of the study was to determine the regenerative potential of platelets in patients with chronic kidney disease, based on the analysis of relationships between substances with potential regenerative action, as well as analysis of the influence of the type of renal replacement therapy used on the regeneration of platelets.

Materials and methods

Ethical approval and consent

The Bioethical Commission at the Pomeranian Medical University in Szczecin approved the research carried out (no. KB-0012/36/11). All participants, including the healthy volunteers in the control group, were informed about the purpose and scope of the study and gave their consent to donate samples and for the resulting data to be published.

Study group

There were 147 participants: a control group of 30 healthy volunteers (NK) and 117 patients with CKD attending the Nephrology, Transplantology and Internal Diseases Clinic of the Pomeranian Medical University in Szczecin. The patients were divided into four groups based on the treatment they received: 30 patients before (HD A) and after (HD B) hemodialysis (biological material was collected from patients immediately before and after dialysis); 30 patients who received peritoneal dialysis (PD); 27 patients before (TE) and after (TE A) kidney transplantations (5–7 days

Table 1 General characteristics of hemodialyzed patients (HD), peritoneal dialysis (PD)-treated conservatively (CT), kidney transplantation (TE), and control group (NK) participating in the study (mean ± OS)

Parameters	HD	PD	CT	TE	NK	*P**	*P***
Gender							
M—male	M-18	M-16	M-17	M-14	M-18	NS	NS
F—female	F-12	F-14	F-13	F-13	F-12		
Age (years)	63 ± 16	55 ± 15	66 ± 15	57 ± 11	50 ± 8	< 0.001	0.029
Dialysis duration (months)	25 ± 16	26 ± 22	–	54 ± 34	–	–	0.003
Causes of CKD							
1—DM	5 (17%)	5 (17%)	4 (13%)	1 (4%)	–	–	NS
2—HA	15 (50%)	3 (10%)	6 (20%)	0 (0%)	–	–	NS
3—KZN	2 (7%)	9 (30%)	6 (20%)	3 (11%)	–	–	NS
4—ADPKD	0 (0%)	0 (0%)	4 (13%)	2 (7%)	–	–	NS
5—other	5 (17%)	10 (33%)	4 (13%)	6 (22%)	–	–	NS
6—unknown	3 (10%)	3 (10%)	6 (20%)	15 (56%)	–	–	NS

**P* statistical significance for differences between HD, PD and CT groups, TE and NK exact Fisher test for qualitative variables, and for quantitative variables—one-way ANOVA and

***P* statistical significance for differences between HD, PD and CT groups and TE exact Fisher test for qualitative variables for quantitative variables—one-way ANOVA or *DM* diabetic nephropathy, *HA* hypertension, *KZN* glomerular inflammation kidney, *ADPKD* polycystic kidney disease inherited autosomal dominant, *NS* no statistically significant differences

Table 2 General characteristics of hemodialyzed patients (A—before, B—after HD), peritoneal dialysis (PD)-treated conservatively (CT) before and after kidney transplantation (TE and TE A) and control group (NK) taking partin the study (mean ± OS)

Parameters	HD A	HD B	PD	CT	TE	TE A	NK	*P**	*P***
Kt/V	1.28 ± 0.21	–	2.77 ± 1.06	–	–	–	–	–	< 0.001
Creatinine concentration (mg/dl)	7.9 ± 2.4	3.5 ± 1.3	4.4 ± 2.2	2.5 ± 1.1	7.4 ± 3.3	3.4 ± 2.9	0.8 ± 0.1	< 0.001	< 0.001
Stage of CKD									
1	0 (0%)	–	0 (0%)	0 (0%)	0 (0%)	–	29 (97%)	–	–
2	0 (0%)		0 (0%)	3(10%)	0 (0%)		1 (3%)	NS	NS
3	0 (0%)	–	0 (0%)	10 (33%)	0 (0%)	–	0 (0%)	NS	–
4	0 (0) %)	–	0 (0%)	12 (40%)	0 (0%)	–	0 (0%)	–	–
5	30 (100%)	–	31 (100%)	5 (17%)	27 (100%)	–	0 (0%)	–	NS

Kt/V dialysis index (volume fraction *V* purified by clearance *K* at time *t*), *NS* no statistically significant relationships were found

**P* statistical significance for differences between HD, PD, and CT groups, TE and NK for quantitative variables—Kruskal–Wallis ANOVA, ANOVA one-way ANOVA, or Student's *t* test

***P* statistical significance for differences between HD, PD, and CT and TE groups for Kruskal–Wallis's ANOVA quantitative variables or ANOVA one-way analysis

after surgery); and 30 patients who received conservative treatment (CT) (CKD stages 2–5). The gender, age, duration of dialysis, cause and stage of CKD, and creatinine concentration in the test and control groups are given in Tables 1 and 2.

Samples

Blood samples [K_2EDTA (8 ml), 3.8% trisodium citrate (9:1; v/v), and serum (8 ml)] were drawn from all study participants. Blood was drawn from hemodialyzed patients via their arteriovenous fistula, and peripheral venipuncture was used for all other participants. Samples were taken from hemodialysis patients before (HD A) and after the procedure (HD B). Transplant patient blood was collected before transplantation (TE) and 5–7 days after surgery (TE A). K_2EDTA and clotted blood samples were centrifuged at 2600 rpm for 10 min at 20 °C to obtain plasma and serum, respectively.

Concentrations of platelet growth factors

The concentrations of IGF-1, PDGF-B, and TGF-β were each determined by an ELISA (Quantikine® Colorimetric Sandwich ELISAs, R&D Systems, USA).

Table 3 Concentration of platelet-derived growth factors of patients with chronic renal hemodialyzed disease (before and after HD A, HD B), peritoneal dialysis (PD), conservative treatment (CT), before and after kidney transplantation (TE, TE A) and in the control group (NK) (mean ± OS, median–lower and upper quartile)

Concentration of platelet-derived growth factors Groups	IGF-1 (ng/ml)	TGF-B (pg/ml)	PDGF-B
HD A	94.6 ± 91.7 34.2 (64.6; 121.2)	19,712.4 ± 12,284.1 16,280.0 (11,560.0; 23,293.0)	2737.1 ± 1347.1 2475.0 (1795.0; 3776.0)
HD B	95.8 ± 37.2 91.33 (66.3; 126.0)	20,964.1 ± 9916.8 18,760.0 (13,360.0; 24,160.0)	2188.1 ± 1342.0 1756.8 (1059.4; 3522.0)
PD	128.2 ± 66.3 124.4 (75.3; 180.4)	56,828.7 ± 73,770.7 15,026.7 (9320; 113,200)	3297.7 ± 1649.1 2882.0 (2098.0; 4270.0)
CT	113.4 ± 39.04 115.3 (81.4; 132.4)	24,163.4 ± 11,741.4 23,293.4 (16,093.3; 30,226.6)	3011.3 ± 1458.0 2926.0 (1169.8; 3580.0)
TE	115.6 ± 47.7 105.6 (78.9; 156.2)	33,709.1 ± 40,069.5 16,640.0 (11,640; 33,360)	2109.0 ± 1230.8 1584.2 (11,640; 3135.0)
TE A	86.7 ± 34.7 80.0 (63.2; 108)	29,125.5 ± 36,241.6 15,560.0 (8760.0; 27,826.7)	2403.6 ± 1393.1 2142.0 (1302.4; 3118.0)
NK	107.6 ± 38.7 104 (75.3; 119.0)	42,679.2 ± 48,566.2 20,720.0 (17,040.0; 33,480.0)	3211.1 ± 2879.0 2103 (1000.0; 5350.0)
p	0.025	NS	0.012

Statistical analysis

To assess data distributions, the Kolmogorov–Smirnov (K–S) test was used, which in the case of some variables (IGF-1 concentrations) showed a non-parametric distribution. Exact Fisher and Chi-square tests were used to analyze quantitative data. A Student's *t* test and ANOVA were used for univariate systems, and the differences between associated (paired) and unrelated (unpaired) variables were evaluated in the case of variables with a parametric distribution. For variables with non-parametric distributions, a Kruskal–Wallis ANOVA was used to evaluate differences, as well as the Mann–Whitney *U* non-parametric test for unpaired data or the Wilcoxon test for paired data. A linear multiple regression model was used to determine the multifactor evaluation of relationships between the parameters studied. Statistical analysis of the results was carried out using Statistica PL 12 Trial (StatSoft).

Results

The concentrations of individual growth factors involved in the regenerative processes in the body are presented in Table 3. The concentration of IGF-1 differed significantly between groups ($p = 0.025$) (Fig. 1). The highest concentration of IGF-1 was observed in patients on peritoneal dialysis and before transplantation, while the lowest was in patients after kidney transplantation. Other differences between the different groups are shown in Table 4.

There were no significant differences in TGF-β concentration between the study groups (Table 3). The highest concentration of TGF-β was found in patients with peritoneal dialysis and in the control group, and the lowest was in patients before and after hemodialysis. However, there is a statistically significant relationship in the concentration of TGF-β between patients before hemodialysis and the control group ($p = 0.045$).

The concentration of PDGF-B was significantly different between the groups ($p = 0.012$) (Fig. 2). The concentration of PDGF-B was highest in peritoneal dialysis patients (DO) and lowest in patients before kidney transplantation and after hemodialysis. Other differences between the different groups are shown in Table 4.

Concentration of growth factors is influenced by sex, duration of dialysis, and the cause and severity of CKD

There was no statistically significant difference in the concentration of PDGFs depending on sex (Table 5). A relationship between IGF-1 and PDGF-B concentrations and the type of renal replacement therapy and duration of dialysis was observed ($p < 0.00001$); ($p < 0.001$) (Table 5). There were also statistically significant differences between the concentration and type of renal replacement therapy and the age of patients (IGF-1 $p < 0.0001$, PDGF-B and TGF-B $p < 0.001$) (Table 5). A statistically significant relationship between the cause of CKD and IGF-1 concentration was also observed ($p = 0.05$) (Table 5).

Discussion

In the last decade, there has been a lot of progress in our knowledge of the axis of growth hormone (GH), IGF-1, and high-affinity IGF-binding proteins (IGFBP) in the context

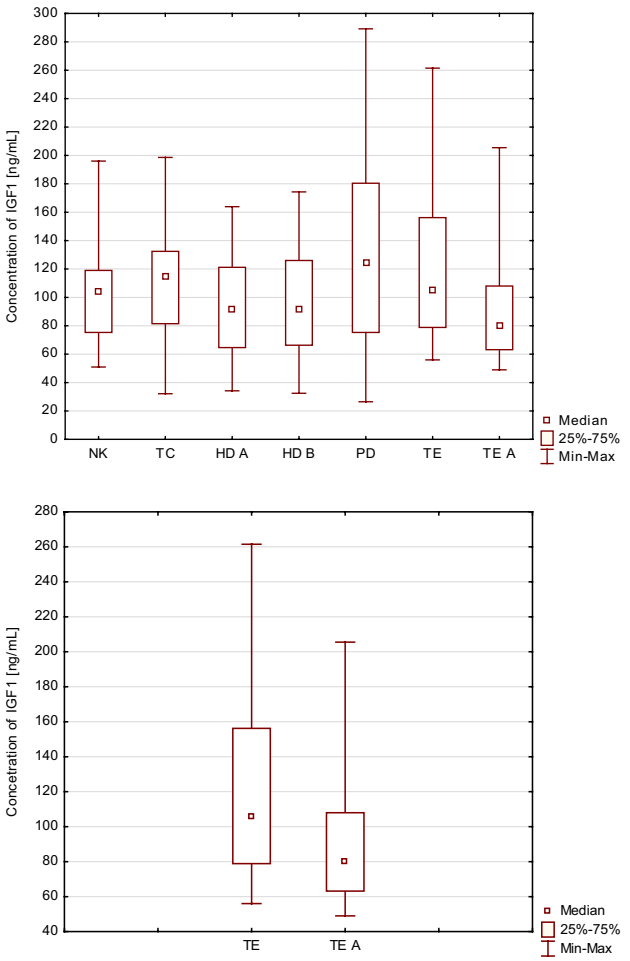

Fig. 1 Kruskal–Wallis's ANOVA analysis of the relationship between the type of group and the concentration of IGF1. IGF1 concentration—differences between PNN, HD A, HD B, DO, TE, TE A, NK groups ($p=0.025$). NK—control group TC—treated conservatively; HD A—before hemodialysis; HD B—after hemodialysis; DO—peritoneal dialysis; TE—before kidney transplantation: TE A—after kidney transplantation. **a** Comparison of IGF1 serum concentration in patients before (TE) and after kidney transplantation (TE A) ($p=0.034$). IGF1-insulin-like growth factor

of normal renal function and pathogenesis and progression of CKD [24].

However, the relationship between IGF-1 level and the type of renal replacement therapy used is unknown [24]. The level of IGF-1 can be used to assess the survival of patients after organ transplantation. It has been proven that a high concentration of this factor may indicate a short survival of the patient after liver transplantation and may be a marker indicating the recovery of the synthetic liver function [25]. It was also shown that the faster the IGF-1 concentration decreases after liver transplantation, the higher the chance of 3-year survival after organ transplantation [26].

In our study, there was a significant difference between IGF-1 concentration and the studied groups ($p=0.025$). The highest concentration of IGF-1 was observed in patients on peritoneal dialysis and before transplantation, and the lowest was observed in patients after kidney transplantation. The IGF-1 concentration before transplantation was significantly higher than after renal transplantation ($p=0.034$). These data suggest that transplantation may inhibit platelet activation by two potential pathways. First, the improvement in kidney function following transplantation results in low-grade inflammation. Second, immunosuppressive agents may decrease inflammation and activation of platelets and, consequently, the concentration of platelet growth factors. The decrease in IGF-1 after transplantation may indicate a higher chance for several years of survival in this group of patients, suggesting that IGF-1 levels should be monitored annually in transplant patients.

In the literature, there are no reports on the relationship between serum IGF-1 concentration and the type of applied nodular therapy. High mortality in patients suffering from CKD may be due to a resistance to growth hormone (GH) and IGF-1 which in turn leads to numerous metabolic disorders, including loss of energy contained in protein energy wasting (PEW) proteins (creatinine, albumin) [22, 23]. Nilsson et al. showed that low levels of IGF-1 are associated with increased mortality of hemodialyzed patients regardless of the levels of markers of inflammation (high-sensitivity C-reactive protein, hs-CRP) and PEW [27]. Reinhard et al. explained that low IGF-1 is associated with an increased concentration of IGFBPs in chronically hemodialyzed patients [27]. However, CKD patients do not usually have low levels of IGF-1. Instead, CKD patients suffer from a reduction in the bioavailability of IGF-1, due to increased production and reduced clearance of IGFBP, which in turn is caused by impaired renal function [28]. Our study found a low concentration of IGF-1 in hemodialyzed patients, which may indicate a high risk of death in this group of patients. The highest concentration of IGF-1 in PD patients was also higher than in the control group. This may confirm the thesis that in patients with CKD, the level of IGF-1 in the blood serum is not being underestimated, but instead, the bioavailability is actually lower than normal.

In some studies, it is suggested that IGF-1 administration to patients suffering from CKD can delay the need for dialysis, as a result of improved kidney function. Administration of IGF-1 to patients after acute renal failure was also associated with an improvement in kidney function. In the case of resistance to IGF-1, it is suggested to administer analogs of an insulin-like growth factor that have an affinity for IGFBP, but which have no affinity to the IGF receptor. This may restore the bioactivity of IGF-1 in CKD patients [28–32].

The relationship between the duration of dialysis and the concentration of PDGFs is a very interesting issue, but not yet well described in the literature. Jia et al. studied the IGF-1 concentration in dialysis-dependent ESRD patients.

Table 4 Statistical differences in the concentration of platelet-derived growth factors, between the studied groups (*p* value)

Groups	HD A	HD B	PD	CT	TE	TE A	NK
IGF-1							
HD A	–	NS	< 0.001	0.048	NS	NS	NS
HD B	NS	–	0.022	NS	NS	NS	NS
PD	0.001	0.022	–	NS	NS	0.008	NS
CT	0.048	NS	NS	–	NS	0.005	NS
TE	NS	NS	NS	NS	–	0.034	NS
TE A	NS	NS	0.008	0.005	0.034	–	0.025
NK	NS	NS	NS	NS		0.025	–
TGF-B							
HD A	–	NS	NS	NS	NS	NS	0.045
HD B	NS	–	NS	NS	NS	NS	NS
PD	NS	NS	–	NS	NS	NS	NS
CT	NS	NS	NS	–	NS	NS	NS
TE	NS	NS	NS	NS	–	NS	NS
TE A	NS	NS	NS	NS	NS	–	NS
NK	0.045	NS	NS	NS	NS	NS	–
PDGF-B							
HD A	–	0.003	NS	NS	NS	NS	NS
HD B	0.003	–	0.006	0.025	NS	NS	NS
PD	NS	0.006	–	NS	0.003	0.031	NS
CT	NS	0.025	NS	–	0.014	NS	NS
TE	NS	NS	0.003	0.014	–	NS	NS
TE A	NS	NS	0.031	NS	NS	–	NS
NK	NS	NS	NS	NS	NS	NS	–

They showed that patients who had an increase in IGF-1 concentration after a year of dialysis had a good prognosis. However, a steady decline in IGF-1 concentration during 1-year follow-up was a poor prognosis for these patients. This indicates the importance of IGF-1 as a marker of mortality in patients undergoing long-term dialysis [22]. This is also confirmed by Nilsson et al., who observed hemodialysis patients for 3 years and determined the concentration of IGF-1 [33]. In another study, Kocyigit et al. determined mean platelet volume (MPV) to demonstrate the importance of platelet activation for predicting the progression of nephrotic syndrome. The increase in MPV correlated positively with proteinuria and hs-CRP and correlated negatively with albuminuria during the 1-year follow-up of patients. This underlines the important role of platelet activation in predicting disease progression [34].

In our study, the relationship between the duration of dialysis, the type of renal replacement therapy, and the concentration of IGF-1 and PDGF-B was discovered. It was observed that in patients who had longer dialysis (dialyzed peritoneal, mean 26 months; patients before kidney transplantation, mean 54 months), IGF-1 concentration was higher than in hemodialyzed patients. This is a very good marker for patients in groups DO and TE and may be associated with a longer survival time compared to hemodialyzed patients. Conversely, the concentration of PDGF was the lowest in the group before renal transplantation. There are no reports on the relationship between the duration of dialysis and the concentration of PDGF-B in the literature. In our study, there was no correlation between the duration of dialysis and the concentration of platelet growth factors. It can, therefore, be argued that the concentration of PDGF-B is more dependent on the type of therapy used than the duration of dialysis.

There are increasing reports in the literature on the relationship between the concentration of PDGFs and conditions that may be the cause of CKD. According to the latest studies, the concentration of IGF-1 and TNF-α is significantly higher in patients with diabetic nephropathy than in the control group. Increased levels of IGF-1 may also exacerbate type II diabetes [33]. Insulin-like growth factor contributes to the development of cystic changes in autosomal dominant polycystic kidney disease (ADPKD), because its expression increases, as the disease progresses. It is already elevated in the early stages of ADPKD [35]. Study in the Chinese population has shown that IGF-1 may contribute to the development of hypertension in patients with elevated body mass index (BMI) and hypertension occurring in other family members [36]. The hypertension caused by IGF-1 deficiency has also been shown to cause

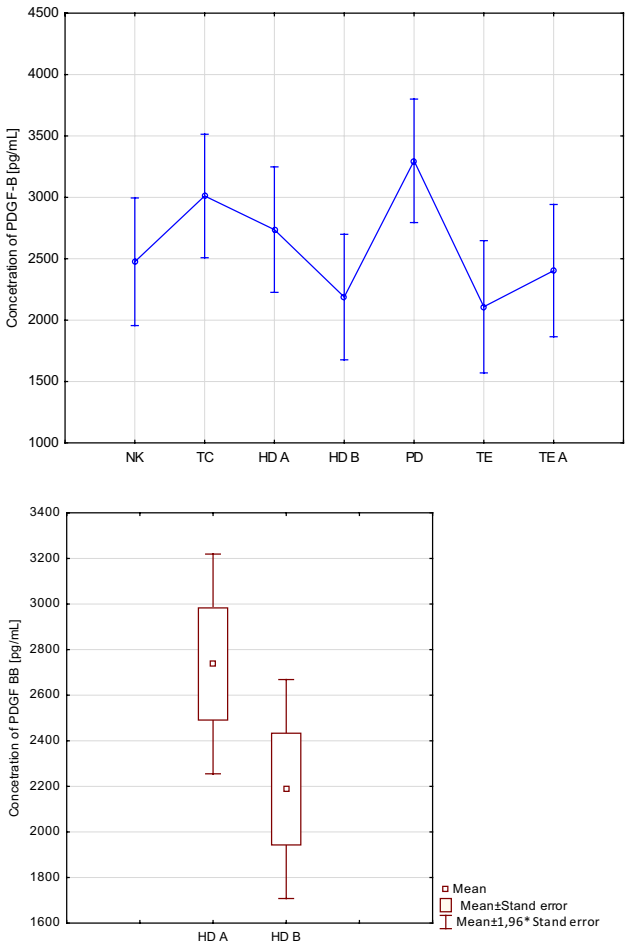

Fig. 2 ANOVA analysis of the relationship between the type of group and the concentration of PDGF-BB. PDGF-B concentration—differences between NK groups, PNN, HD A, HD B, DO, TE, TE A ($p=0.012$). NK—control group PNN—treated conservatively; HD A—before hemodialysis; HD B—after hemodialysis; DO—peritoneal dialysis; TE—before kidney transplantation: TE A—after kidney transplantation. **a** Comparison of PDGF-B concentration in patients' serum before (HD A and after hemodialysis (HD B) ($p=0.003$). PDGF-B—low-cost growth factor

Table 5 Influence of particular parameters on concentrations of platelet-derived growth factors

Parameters	IGF-1	TGF-B	PDGF-B
Gender	NS	NS	NS
Renal replacement therapy and duration of dialysis	< 0.00001	NS	< 0.001
Renal replacement therapy and age	< 0.0001	< 0.001	< 0.001
Stage of CKD	NS	NS	NS
Causes of CKD	0.05	NS	NS

The table presents p values defining statistical significance. The relationship between gender, duration of dialysis, age, stage of chronic kidney disease and the causes of chronic kidney disease and the concentrations of platelet-derived growth factors was assessed using one-way ANOVA

HD and duration of dialysis—the relationship between the type of therapy (hemodialysis, peritoneal dialysis, and patients before kidney transplantation), duration of dialysis, and the concentrations of platelet-derived growth factors

HD and age dependence between the studied groups (hemodialysis, peritoneal dialysis, conservative treatment, patients before kidney transplantation, and control group), patients' age, and concentrations of platelet-derived growth factors

The stage of chronic kidney disease—the relationship between the severity of chronic kidney disease based on eGFR and the activity of concentrations of platelet-derived growth factors

The causes of chronic kidney disease—the relationship between selected causes of chronic disease and the concentrations of platelet-derived growth factors

NS no statistically significant relationship was found

brain damage and can worsen the cognitive function of patients [37].

Our study suggests that the cause of CKD has an effect on IGF-1 concentration. The highest concentration of IGF-1 was observed in patients with glomerulonephritis and the lowest was in patients with diabetic nephropathy.

There are no reports in the literature on the relationship between IGF-1 concentrations and glomerular inflammation disease (GID). Slightly lower but also high levels of IGF-1 have been demonstrated in patients with ADPKD, this is confirmed by the results obtained by other scientists saying that IGF-1 can be increased even in the initial stages of this disease. In the study group, ADPKD was the cause of CKD in only six people; CKD is more frequently caused by glomerulonephritis. It would be necessary to conduct study in a wider group of patients to make more reliable conclusions on the relationship between IGF-1 and ADPKD. The lowest level of insulin-like growth factor in patients suffering from diabetic nephropathy is a good marker for this group of patients, because high levels of IGF-1 may be indicative of exacerbation of diabetes mellitus.

TGF-β belongs to a family of secreted proteins. It is mainly produced in blood platelets and in macrophages [12]. There are reports in the literature on the relationship between plasma TGF-β concentration and the progression of kidney disease. The kidneys respond to injuries by the release of proinflammatory cytokines and growth factors, such as transforming growth factor. Long-term overexpression of TGF-β, which occurs in patients undergoing dialysis, as a result of the need to maintain vascular access, and progressive renal failure, causes the extracellular matrix to accumulate in the damaged kidney [13, 14], and ultimately leads to glomerular fibrosis and interstitial fibrosis [13, 16, 17, 19].

The role of TGF-β in the progression of kidney disease is additionally confirmed by studies in which anti-TGF-β antibodies were administered in various animal models of renal damage. This resulted in the alleviation of fibrosis,

which indicates the important role of TGF-β in the fibrotic process [18, 38, 39]. In addition, several clinical trials have demonstrated an increased expression of TGF-β in the kidneys of patients with glomerulosclerosis, including diabetic nephropathy [13, 40, 41] and other inflammatory kidney diseases [42, 43].

Mehta et al. showed that higher plasma levels of TGF-β are independently associated with a lower estimated glomerular filtration rate (eGFR) and a higher incidence of CKD in older people. They also demonstrated the need for further observation of this group of patients to confirm whether serum TGF-β is associated with a faster loss of renal function in the elderly and whether therapies that lower TGF-β and reduce fibrosis may play a role in the prevention or treatment of CKD and its co-morbidities [44]. Despite numerous studies on the relationship between TGF-β concentration and the progression of CKD, both in animal models and in humans, it is not possible to conclude whether the increase in the concentration of transforming growth factor is unfavorable for patients suffering from CKD.

Chimenz et al. found that TGF-β may be a test marker with an appropriate level of transport across the peritoneal membrane and can be used to select the right peritoneal dialysis technique. In their study, they showed that a high level of transforming growth factor indicates low permeability of the peritoneal membrane [45]. In another study, Zhou et al. demonstrated that TGF-β can be used as a biomarker for fibrosis of the peritoneal membrane in patients on peritoneal dialysis [46].

In our study, there was no correlation between the serum TGF-β concentration and the type of renal replacement therapy used. The highest concentration of TGF-β was observed in patients with peritoneal dialysis and in the control group, and the lowest concentration was found in patients before and after hemodialysis. However, a statistically significant relationship was found between the concentration of transforming growth factor in patients prior to hemodialysis and the concentrations in the control group.

Considering the literature data, the significantly lower concentration of TGF-β in hemodialyzed patients compared to the control group may be a sign of slower renal fibrosis and, consequently, a slower progression of renal failure. High levels of TGF-β in patients with peritoneal dialysis may suggest low permeability of the peritoneal membrane; however, due to the lack of significance of differences between the studied groups, this thesis cannot be unequivocally stated.

PDGF is released as a result of granular degranulation in the platelets during wound healing. It also stimulates the proliferation of many cells, including connective tissue cells [47].

In our study, the concentration of PDGF-B was significantly different between the groups ($p = 0.012$). The concentration of PDGF-B was the highest in peritoneal dialysis patients (DO) and the lowest in patients before kidney transplantation and after hemodialysis. There was also a significant difference between PDGF-B concentration before and after hemodialysis. After hemodialysis, the concentration of the studied growth factor dropped significantly. An increase in the concentration of platelet-derived growth factor after renal transplantation was also observed, as compared to the pre-transplant concentration, but this was not statistically significant.

There are reports in the literature about the relationship between PDGF-B and fibrosis of the peritoneal membrane in peritoneal dialysis patients. Cina et al. showed that overexpression of PDGF in the peritoneal membrane of the rat led to significant angiogenesis, cell proliferation, and endothelial thickening. PDGF also induced the expression of TGF-β, but no activation of this factor was observed, as no increased production of collagen was observed. PDGF-B induces angiogenesis, but does not induce fibrosis of the peritoneal membrane. The lack of significant ultrafiltration dysfunction and mesenchymal epithelial passage, as observed in patients on peritoneal dialysis, suggests that PDGF-B may play a role, but is not an integral component in response to peritoneal damage [47].

In our study, the highest concentration of PDGF-B was observed in patients on peritoneal dialysis. This may confirm the hypothesis that PDGF plays an important role in response to peritoneal damage. PD patients may have higher levels of PDGF-B than other patients due to peritoneal membrane dysfunction which causes AGE accumulation, and subsequently leads to systemic inflammation. On the basis of the obtained results, we cannot unequivocally state whether there is fibrosis of the peritoneal membrane and ultraviolet disorders in this group of patients. To this end, the scope of research should be extended. However, based on the high level of PDGF-B, we can say that this is a probable thesis.

Yamada et al. studied the effect of activated T lymphocytes from patients after kidney transplantation with chronic rejection, PDGF, and vascular smooth muscle cell (VSMC) proliferation. In this study, activated T cells associated with MHC class II expression were shown to promote PDGF-induced VSMC proliferation in renal transplant patients with chronic rejection, and are likely to be involved in the pathogenesis of transplanted kidney disease [48]. Our study showed an increase in PDGF-B concentration after renal transplantation, in comparison with the values obtained before transplantation. However, this was not a statistically significant difference. To observe the above-described relationship, it would be necessary to observe patients after kidney transplantation for transplant rejection and the function of a transplanted kidney. Monroy et al. showed, however, that overexpression of PDGF in hemodialyzed patients causes VSMC proliferation in conditions with high urea

concentration, consequently, causing phenotypic changes associated with vascular remodeling and vascular access dysfunction in this group of patients. This was particularly true for patients with end-stage renal disease (ESRD) who had significantly earlier changes in neointimal vein hypertrophy compared to people with renal normal function. This study even suggested the necessity of VSMC dysfunction before vascular access to hemodialysis patients [49]. In our study, a statistically significant difference was found between PDGF-B concentration before and after hemodialysis, with the concentration of growth factor decreasing after hemodialysis. These results confirm the thesis put forward by Monroy et al. After hemodialysis, the level of urea decreases, which may cause a decrease in PDGF-B concentration, whereas the re-accumulation of urea, as a result of kidney dysfunction, causes an increase in PDGF-B concentration. Monroy et al. suggested that PDGF-B overexpression occurs under uremic conditions, but this is only true in vivo. In vitro, high levels of urea do not increase the concentration of PDGF [48]. The overexpression of PDGF in hemodialyzed patients may result from vascular access dysfunction, which is commonly observed in this group of patients.

Conclusions

1. The type of renal replacement therapy used has an effect on the concentration of platelet-derived growth factors: IGF-1 and PDGF-B.
2. The factors significantly affecting the release of platelet-derived growth factors are the duration of dialysis and the cause of CKD.
3. The concentration of PDGFs in the blood of CKD patients may provide us with information on the selection of appropriate renal replacement therapies, patient prognosis, or mechanisms that occur in the body of a CKD patient on renal replacement therapy.
4. PD patients had the highest concentrations of all growth factors, and this may be due to the presence of inflammation induced by dialysis-related advanced end-products of glycosylation (AGE).
5. The decrease in IGF-1 after transplantation may indicate a higher chance for several years of survival of this group of patients what would be caused by the reduction of platelet activation, as a result of taking immunosuppressive drugs and good functioning of the transplanted kidney.

Limitations

The study determined the concentration of platelet-derived growth factors such as IGF-1, PDGF-B, and TGF-B in patients before and after different types of renal replacement therapy. PDGF-B and TGF-B are mainly produced by platelets after their activation. IGF-1 can be produced by various tissues and organs, and both IGF-1 and TGF-B are markers of inflammation. It is not possible to check whether the platelet-derived growth factors in the study are derived mainly from platelets. The literature states that MPV could be a marker of platelet activation. However, the study group consisted of patients with chronic kidney disease undergoing dialysis or kidney transplantation, and this is often associated with many other diseases. As MPV may indicate an increase in inflammation, trauma, or iron fever, we decided not to use it as a marker of platelet activation in these patients. However, it has previously been suggested that increased platelet activation occurs in patients following surgery or the production of vascular access in hemodialyzed patients, which is supported by the data presented here.

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