Status of interleukin-6 and hepcidin levels in first-time haemodialysis patients

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Background

The risk of chronic kidney disease stage 5 and its progression depend on the stage and the underlying diagnosis. Haemodialysis has become the long-term maintenance therapy for these patients. The interaction among interleukin-6 (IL-6), hepcidin and the iron exporter ferroportin is a major contributor to the iron-deficiency anaemia of chronic disease. The aim of the present study was to investigate the effects of polysulphone dialyzer membrane on serum IL-6 and hepcidin, including haemoglobin levels, in first-time patients undergoing five cycles of haemodialysis.

Patients and methods

Totally, 11 patients (male: five, female: six) were recruited for the present study after obtaining written informed consent. Their mean age was 57.4±13.4 years, and they were undergoing haemodialysis for the first time. Hepcidin, IL-6 and haemoglobin levels were determined before the first cycle and the fifth cycle and after the fifth cycle of haemodialysis. Pearson's correlation coefficients were also determined.

Results and discussion

No statistically significant differences were observed before the first cycle and after the fifth cycle of haemodialysis in haemoglobin, IL-6 and hepcidin levels nor between pre-fifth and post-fifth cycle values, except for IL-6, which showed a significant mean reduction ($P=0.04$) from the pre-fifth cycle of 67.0 pg/ml to a mean value of 42.9 pg/ml (64.0% reduction). Analysis of variance showed no significant variation in the parameters studied, and no significant correlations between haemoglobin and IL-6, haemoglobin and hepcidin, and IL-6 and hepcidin were found.

Conclusion

In this short study of five-cycle haemodialysis, significant reduction in IL-6 with no significant change in hepcidin levels was found, even though severe anaemia was present. Kidney dysfunction probably results in decreased clearance of inflammatory markers and may not be improved by haemodialysis alone. Moreover, a different approach to reduce these markers is therefore warranted.

Keywords:

haemodialysis, haemoglobin, hepcidin, interleukin-6

Introduction

The risk of chronic kidney disease stage 5 (CKD) and its progression depend on the stage and the underlying diagnosis. Haemodialysis has become the long-term maintenance therapy for CKD. The highest mortality rate is found within the first 6 months of initiating dialysis, and the most common cause of death is cardiovascular disease where mortality is 10–20 times higher [1] and overall life expectancy is shorter than that of the general population with similar demographics [2,3]. Interleukin-6 (IL-6) acts both as a proinflammatory cytokine and an anti-inflammatory myokine. It is secreted by T cells and macrophages to stimulate immune response during infection. Elevated plasma IL-6 has been associated with increased mortality in the elderly population and

in patients with CKD [4,5]. It has been used as a predictor of mortality and nutritional status in prevalent haemodialysis patients [6,7]. The interaction of IL-6, the iron regulatory hormone hepcidin, and the iron exporter ferroportin is a major contributor to the functional iron-deficiency anaemia of chronic disease. Hepcidin is a key regulator of entry of iron into circulation in mammals [8]. It is a peptide hormone synthesised mainly in the liver and was discovered in 2000 [9]. It reduces iron transport across the gut mucosa, reduces

iron exit from macrophages, the main site of iron storage, and reduces iron exit from the liver. Hepcidin better known for its role in iron homeostasis is increased by iron loading and inflammation but iron deficiency and blood loss reduce hepcidin expression [10]. Inflammation and infection increase hepcidin synthesis. In inflammation, such as CKD, high hepcidin production inhibits iron release from macrophages and intestinal absorption of iron, resulting in iron-deficiency anaemia [11]. Discovery of hepcidin and its role in iron metabolism has provided new insight about the management of anaemia in CDK [12]. Human monocytes produce hepcidin in response to adhesion and the proinflammatory cytokine IL-6 [13]. Links between cytokines and hepcidin have shown that IL-6 acts directly on the hepatocytes to stimulate hepcidin production [14]. Kidney function plays an important role in hepcidin clearance, and kidney dysfunction results in decreased clearance [15]. Haemodialysis membranes were used to remove accumulated uraemic toxins, excess ions and water from patients through the dialysate and to supply (deficit) insufficient ions from the dialysate. It has recently been reported that membranes comprising synthetic polymers such as polysulphone or a polyether sulphone show much more improvement in blood compatibility such as suppression of complements compared with regenerated cellulose membranes [16]. An Indonesian report showed that in haemodialysis using polysulphone membrane lower levels of IL-6 were seen compared with cellulose membrane [17].

The aim of the present study was to investigate the effects of using polysulphone dialyzer membrane on serum IL-6 and hepcidin levels, as well as haemoglobin, after five cycles in first-time haemodialysis patients.

Patients and methods

The present study received ethics approval from the Health Research Ethical Committee (No. 136/KOMET/FK/2015), Faculty of Medicine, University of North Sumatera, Indonesia. It was conducted at the Department of Clinical Pathology, Faculty of Medicine, University of North Sumatera/Haj Adam Malik Hospital.

Patients

A total of 11 patients were recruited on the basis of purposive sampling (by purpose), where the purpose was to recruit new patients only before undergoing haemodialysis. These patients fulfilled the criteria of Grade V of KDIGO: Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease 2013 [18]. Patient informed consent was obtained. Inclusion criteria for haemodialysis were as follows: age greater than 18 years, haemoglobin less than 11.0 g/dl, never been transfused or received erythropoietin and Grade V kidney disease. Exclusion criteria included the following: chronic liver disease, evidence of haematological malignancy, gastrointestinal haemorrhage (haematochromasia or evidence of faecal blood), malnutrition (BMI<18.5) and evidence of sepsis. Patients were recruited from Rasyida Haemodialysis Clinic ($n=4$) and Dr Pirngadi Hospital ($n=7$) in Medan.

Blood sampling

About 5 ml of blood by venepuncture was collected into 2.5 ml EDTA vacutainer tubes for haemoglobin estimation, and another 2.5 ml was collected into a plain tube. The blood samples were left to clot for about 2 h at room temperature, and then centrifuged at 2500 g for 15 min. The serum was aliquoted and stored at −70°C for IL-6 and hepcidin analysis. Blood sampling was performed before haemodialysis, which was carried out for 2 weeks (haemodialysis twice per week), and blood sampling was repeated again before the fifth cycle and after the fifth cycle of haemodialysis.

Laboratory analysis

Haemoglobin was determined by the automated Haematology Analyzer Sysmex XN400 (Sysmex Corporation, Kobe, Japan). Enzyme-linked immunoassay was used to determine the levels of hepcidin (Awareness Technologies, Palm City, Florida, USA) and IL-6 (Affymetrix eBioscience, San Diego, California, USA). Normal values as mentioned in the manufacturer's pamphlets were as follows: hepcidin less than 47 ng/ml and IL-6 less than 5 pg/ml.

Statistical analysis

The statistical package for the social sciences (SPSS 22; IBM Corp, Chicago, Illinois, USA) was used to perform all statistical analyses. One-way analysis of variance was performed to test for variations in the parameters studied. The group mean samples paired *t*-test and the Mann–Whitney test for differences between groups were performed. Pearson's cor- relation was calculated. A *P*-value less than 0.05 was considered statistically significant.

Results

Characteristics of first-time haemodialysis patients

A total of 11 patients (males five, females six) with a mean age of 57.4±13.1 years (range: 33–86 years) were

recruited for the present study. The underlying diagnosis was hypertension ($n=7$) and diabetes mellitus ($n=4$). Their clinical presentation before and after the fifth cycle of haemodialysis are shown in Table 1.

Haemoglobin, IL-6 and hepcidin levels in first-time haemodialysis patients before the first cycle of haemodialysis and before the fifth cycle and after the fifth cycle of haemodialysis as well as values before the fifth cycle and after the fifth cycle were compared.

No statistically significant differences were observed before the first cycle compared with pre-fifth and post-fifth cycle haemodialysis values for haemoglobin, IL-6 and hepcidin. No significant differences in IL-6 levels before the first cycle and before the fifth cycle and after the fifth cycle were found, except for a significant mean reduction ($P=0.04$) in IL-6 levels between the pre-fifth cycle (mean: 67.0±53.1 pg/ml) and the post-fifth cycle values (mean: 42.9±42.0 pg/ml) (64.0% reduction). Moreover, the levels remained above the normal reference of less than 5 pg/ml. Mean hepcidin levels showed fluctuation before the first cycle (mean: 39.4±25.5 mg/ml) and after the fifth cycle (mean: 45.2±24.6 mg/ml) (14.7% reduction) and before the fifth cycle (mean: 89.3±125.9 mg/ml) and after the fifth cycle (50.6% reduction), but they did not reach statistical significance probably due to the wide variation in the results seen. The normal range for hepcidin is less than 47 ng/dl. An outlier of hepcidin levels of 454.5 ng/ml was seen at the pre-fifth cycle; when excluded, no statistically significant differences were observed. Haemoglobin levels did not show any significant improvement after five cycles of haemodialysis, even though a mean rise to 7.4±1.7 g/dl from before the first cycle mean of 6.7±1.7 g/dl was seen. Analysis of variance showed no significant variation in the parameters studied (Table 2). The mean levels of haemoglobin, IL-6 and hepcidin after the fifth cycle of haemodialysis are shown in Figure 1.

Correlation studies

Pearson's correlation coefficients were obtained, and no statistically significant correlations were seen between haemoglobin and IL-6 ($r=-0.031$, $P=0.87$), haemoglobin and hepcidin ($r=-0.076$, $P=0.67$), and between IL-6 and hepcidin ($r=-0.075$, $P=0.68$).

Discussion

Haemodialysis has become the long-term maintenance therapy for CKD stage 5. The highest mortality rate is seen within the first 6 months of initiating dialysis, and

**Table 1 Characteristics of patients for first time haemodialysis $n=11$**

Age mean ± SD	Age Range	Sex		Hypertension	Diabetes Mellitus
		Male	Female		
57.4 ± 13.1 years	33–86 years	5	6	7	4

Presentation before haemodialysis: nausea/weakness $n=1$; dizziness/breathless $n=1$; weakness/dizziness $n=1$; nausea/fatigue/dizziness $n=1$; nausea/dizziness/weakness or vomiting $n=6$; weakness/dizziness/breathless $n=1$. Presentation post 5th cycle of haemodialysis: Weakness $n=3$; dizziness $n=1$; nausea $n=1$; dizziness/weakness $n=3$; nausea/weakness $n=2$; nausea/dizziness $n=1$

Table 2 Haemoglobin, interleukin-6 and hepcidin levels in patients on first time haemodialysis compared between before 1st cycle haemodialysis and before 5th and post-5th cycle haemodialysis and between pre 5th and post-5th cycle. Group mean paired samples t-test and Analysis of Variance (ANOVA)

	Hb g/dL	IL-6 pg/mL	Hepcidin ng/mL
$N=11$			
Before 1st cycle Haemodialysis			
Mean (SD)	6.7 (1.7)	71.0 (96.2)	39.4 (25.5)
Range	4.6–10.7	0.12–259.08	15.1–103.50
Pre-5th cycle			
Mean (SD)	7.3 (1.4)	67.0 (53.1)	89.3 (125.9)
Range	5.6–9.0	0.12–160.5	18.8–454.50
P	0.23	0.90	0.23
Post-5th cycle			
Mean (SD)	7.4 (1.7)	42.9 (42.0)	45.2 (24.6)
Range	4.6–10.7	3.21–139.2	21.9–90.0
P	0.16	0.41	0.59
Pre-5th cycle vs post-5th cycle			
P	0.41	**0.04**	0.29
ANOVA P	0.56	0.58	0.25

Figure 1
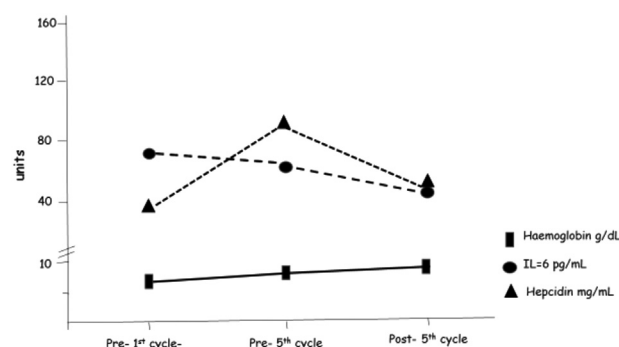
Mean haemoglobin, IL-6 and hepcidin levels at pre-haemodialysis, pre 5th cycle and post 5th cycle haemodialysis.

the most common cause of death is cardiovascular disease where mortality is 10–20 times higher [1]. The overall life expectancy is shorter than that of the general population with similar demographics [2,3]. The use of a synthetic dialysis membrane is recommended over

cellulose-based membranes, as they offer benefits in terms of biocompatibility [18]. Haemodialysis seems to be on the decline, and biocompatibility is probably less significant than long-term survival. Cytokine production is not only related to haemodialysis with cellulose membranes, hemophane or polyamide dialyzers, they have comparable plasma concentration of IL-6 predialysis at the end of first treatment and after 48 treatments have been reported [19]. Elevated IL-6 levels were found to correlate with increased mortality in the general population, in the elderly and in patients with CKD [4,5]. It has been used as a predictor of mortality and nutritional status in prevalent haemodialysis patients [6,7]. High levels of hepcidin found in haemodialysis patients could be related to the underlying chronic inflammation, as has been suggested by Costa *et al.* [20]. In this study, a polysulphone dialyzer membrane used for haemodialysis for five cycles showed a significant reduction in mean IL-6 levels by 64.0% when compared between the pre-fifth and the post-fifth cycles and a 60.4% reduction from the pre-first cycle state. However, the level remained above the normal reference of less than 5 pg/ml. Mean hepcidin levels fluctuated above 14.7% before the first cycle and after the fifth cycle, and 50.6% reduction was found between the pre-fifth and the post-fifth cycle values with the mean level remaining at the upper normal reference of less than 47.0 ng/ml after five cycles of haemodialysis. Mean haemoglobin levels appreciate to 10.4% (mean: 7.4±1.7 g/l) from before first cycle and remained below the normal range but severe anaemia still present after five cycles of haemodialysis. Haemodialysis in this study showed some improvement from the precycle state and after five cycles, especially IL-6 reduction, although the levels remain above normal, whereas hepcidin and haemoglobin did not show significant changes. Anaemia is a common complication in maintenance haemodialysis patients and it contributes towards reduced quality of life [21]. Higher levels of IL-6 have been found in patients undergoing continuous dialysis [22], and in inflammatory states hepcidin production is no longer regulated by iron burden but is rather increased through IL-6 stimulation [23]. Lowest haemoglobin levels were found to correlate with the highest concentration of inflammatory markers, and IL-6 has been found to be the independent factor to define haemoglobin level in untreated advanced epithelial ovarian cancer [24]. We found no significant correlation between haemoglobin and hepcidin, haemoglobin and IL-6, and between IL-6 and hepcidin in patients after five cycles of haemodialysis. These findings are in contrast to patients on long-term maintenance dialysis where there was positive correlation between hepcidin and IL-6 and hepcidin and haemoglobin, except that a correlation between haemoglobin and IL-6 was not reported [23]. No significant correlation between hepcidin and inflammatory markers including IL-6 in haemodialysis patients have been reported by others [25]. Kidney dysfunction was reported to decrease clearance of hepcidin [15] and further long-term dialysis will improve the situation. Elevated inflammatory markers including hepcidin have been reported in patients on long-term dialysis maintenance [19,22]. A different approach to reduce these markers is therefore warranted, especially IL-6 and hepcidin levels to release iron stores out of cells to regenerate red blood cells enhancing haemoglobin levels.

Conclusion

This study reported on five short cycles of haemodialysis; significant reduction in IL-6 with no significant change in hepcidin levels was seen using polysulphone dialyzer membrane, even though severe anaemia was still present. Kidney dysfunction possibly resulted in decreased clearance of the inflammatory markers and this may not be improved by haemodialysis alone. Moreover, a different approach to reduce these markers is therefore warranted.

Conflicts of interest

There are no conflicts of interest.

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