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Effect of Correction of Metabolic Acidosis on Serum Interleukin 10 Levels in Chronic Hemodialysis Patients

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ABSTRACT
Background: Metabolic acidosis is a common complication in chronic hemodialysis (HD) patients and can cause loss of lean body mass. Interleukin 10 (IL-10) also is increased in chronic HD patients and has a prognostic value.
Objectives: The aim of this study was to evaluate the effect of correction of metabolic acidosis on serum IL-10 levels in chronic hemodialysis patients.
Patients and Methods: Forty chronic hemodialysis patients with predialysis bicarbonate levels <22 mEq/l were treated by increasing dialysate bicarbonate concentration till achieving serum bicarbonate ≥ 22 mEq/l. Serum IL-10 levels were measured at baseline and one month after correction of metabolic acidosis.
Results: Bicarbonate levels significantly increased at end of study (22.83±0.71 vs 18.85±1.12 mEq/l, p<0.001). Serum IL-10 levels significantly decreased after correction of metabolic acidosis (19.91±6.68 vs 25.89±10.64 pg/ml, p<0.001) and the change of IL-10 was significantly correlated with the change in bicarbonate (r = - 0.436, p = 0.005).
Conclusions: Correction of metabolic acidosis in chronic hemodialysis patients was associated with decrease in serum IL-10.
Keywords: Hemodialysis (HD), Interleukin 10 (IL-10), Metabolic acidosis.

INTRODUCTION
Metabolic acidosis is a common complication in hemodialysis patients, which can cause loss of lean body mass by impairing the activation of adaptive responses that maintain protein stores [(1)]. Some effects of acidosis include negative nitrogen balance, protein catabolism, muscle wasting, increased corticosteroid, and parathyroid hormone production [(2)]. It is recommended to maintain predialysis serum bicarbonate at ≥22 mEq/L [(3)].

In patients with renal failure, the systemic concentrations of both pro-inflammatory and anti-inflammatory cytokines are high compared to healthy individuals due to both decreased renal clearance and increased production [(4)]. Interleukin 10 (IL-10) is a cytokine with potent anti-inflammatory properties. It counter-regulates the cascade of proinflammatory cytokines, including IL-6, as part of the acute-phase reaction.

The importance of IL-10 as an anti-inflammatory cytokine is emphasized by a study that showed a higher cardiovascular morbidity and mortality in maintenance HD patients who had a low producing IL-10 gene polymorphism [(5)].

HD induces production of cytokines, which can induce protein catabolism and promote apoptosis [(6)]. High levels of interleukins and presence of metabolic acidosis are described as independent risk factors for morbidity and mortality in HD patients. Although regular hemodialysis causes decreased levels of mortality, it is considered a condition associated with inflammation [(7)]. To that end, we hypothesized that serum IL10, anti-inflammatory cytokine, will decrease after metabolic acidosis correction.

The aim of this study was to evaluate the effect of correction of metabolic acidosis on serum IL-10 levels in chronic hemodialysis patients.

PATIENTS AND METHODS
The study was conducted at Ain Shams University Hospital Dialysis Center. It included forty adult patients aged ≥18 years with end-stage renal disease (ESRD) treated with regular hemodialysis thrice weekly for at least 3 months and having a mean predialysis serum bicarbonate of <22 mEq/L on monthly laboratory analyses during the 3 months before initiation of the study. Exclusion criteria were catabolic state (infection, surgery, steroid therapy) for 1 month before the study, patients with hemodialysis catheter as access for dialysis and patients on chemotherapy or other immunosuppressive drugs.

A group of 20 healthy subjects served as the control group only for measurement of serum IL-10 for comparison with study population. We collected demographic data including age, gender. Medical history including cause of ESRD, hypertension, diabetes mellitus (DM), smoking, cardiovascular disease (CVD). The interdialytic weight gain (IWG) was calculated as the mean of body weight gain before the mid-week HD session.

Ethical consent:
An approval of the study was obtained from Ain Shams University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association

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(Declaration of Helsinki) for studies involving humans.

Hemodialysis data:

HD was performed three times weekly, 4 hours by session using a low-flux polysulfone membrane dialyzer (POLYFLUX 17L, Baxter Renal Care) using bicarbonate-buffered dialysate delivered by a proportionate system (GAMBRO AK-96). The blood flow rate was 300 ml/min, and dialysate flow rate was 500 ml/min. The dialysate fluid composition was sodium 138 mEq/l, potassium 2 mEq/l, calcium 3.5 mg/dl, magnesium 0.5 mEq/l, chloride 109.5 mEq/l, and acetate 3 mmol/l. The dose of HD was measured by single-pool Kt/V (k = dialyzer clearance of urea, t = dialysis time, V = volume of distribution of urea) according to the Daugirdas formula [(8)].

Study design:

This was prospective cohort study. Before study onset, dialysate bicarbonate was fixed at 32 mEq/l. It was increased gradually in the selected patients in 2-4 mEq/l increments and plasma bicarbonate levels were measured before the next session till being ≥ 22 mEq/l then patients were dialysed against these dialysate bicarbonate concentrations for one month and were advised not to change their usual diets. Other dialysate content was not changed during study period.

Laboratory parameters:

Blood samples were drawn from dialysis access before onset of the mid-week HD session. The following laboratory parameters were measured at onset of study and was repeated at the end for comparison: hemoglobin, calcium, phosphorus, intact PTH, CRP, ferritin, iron, total iron binding capacity (TIBC), albumin, and pre- and post-dialysis BUN. Blood gases were analyzed for blood pH, bicarbonate and PCO2. Serum IL-10 was measured by commercially available ELISA kits with lower limit of detection 1.56 pg/ml.

Blood samples were centrifuged and serum was separated and stored at -80°C till use. The syringes for blood gas were transported to the laboratory for analysis in a container with ice within 30 minutes.

Statistical Analysis

Data analysis was performed using SPSS for Windows v.25. Continuous variables were presented as mean ± standard deviation (SD) or median (range) as appropriate. Categorical data were presented as frequency and percentages. Pearson correlation test was used to assess the correlation between baseline IL-10 levels and other variables and between the IL-10 change and changes in other parameters. Independent t-test was used to compare IL-10 between both groups. Paired samples T-test was used to compare between baseline and laboratory parameters at end of study in patients' group. P-value of <0.05 was considered statistically significant.

RESULTS

Patients' characteristics

The study was conducted on 40 patients. Most of them were men with mean age 51.78 ± 9.26 years and median hemodialysis vintage 17 months (range; 3 – 84 months). The etiology of ESRD was mainly hypertensive nephropathy 25 (62.5%), followed by diabetic kidney disease in 12 (30%) (Table 1).

Table (1): Baseline characteristics of the studied population

Parameter	Value
Age (years), mean±SD	51.78± 9.26 (35 – 68)
Gender (male), (n, %)	36 (90.0%)
Dry weight (kg), mean±SD	81.01 ± 9.86 (64 – 101)
Cause of ESRD, (n, %)	
Hypertensive nephropathy	25 (62.5%)
Diabetic kidney disease	12 (30%)
Obstructive uropathy	1 (2.5%)
Analgesic nephropathy	1 (2.5%)
Unknown	1 (2.5%)
Hemodialysis vintage (months), median (range)	17 (3 – 84)
DM (n, %)	12 (30.0%)
HTN (n, %)	39 (97.5%)
CVD (n, %)	14 (35.0%)
Smoking (n, %)	29 (72.5%)

Clinical and laboratory parameters:

Compared to healthy subjects, hemodialysis patients had significantly higher levels of baseline IL-10 (25.89±10.64 vs 5.72 ± 1.77 pg/ml, $p < 0.001$).

Changes of laboratory parameters after correction of metabolic acidosis:

Before the study, dialysate bicarbonate concentration was 32 mEq/l for all patients. To achieve plasma bicarbonate ≥ 22 mEq/l, dialysate bicarbonate concentration increased to 34 mEq/l in 14 patients, 36 mEq/l in 24 patients and 38 mEq/l in 2 patients.

Mean baseline CRP, and IL-10 levels were significantly higher compared to the values at the end of the study, while blood pH and HCO_3^- were significantly higher at the end of the study compared to the basal values (Table 2).

Table (2): Baseline clinical and laboratory parameters of study group

Parameter	Baseline value	End of study value	P value
IWG, kg	2.60±0.28	2.66±0.2	0.175
Systolic BP, mmHg	131.50±18.33	128.25±16.46	0.368
Diastolic BP, mmHg	79±11.72	76.25±7.74	0.202
Sodium, mEq/l	136.4±2.62	137.50±4.48	0.200
spKT/V	1.11±0.10	1.14±0.14	0.257
Hemoglobin, g/dl	11±1.16	10.73±1.22	0.122
Calcium, mg/dl	8.74±0.76	8.62±1	0.403
Phosphorus, mg/dl	5.4±1.38	5.35±1.80	0.709
iPTH, pg/ml	357.92±57.95	389.98±26.65	0.051
Iron, ug/dl	71.37±3.92	73.82±7.74	0.573
Ferritin, ug/l	519.26±96.35	520.25±93.11	0.932
TIBC, ug/dl	240.30±36.61	224.97±46.45	0.029
CRP, mg/l	7.02±1.42	5.98±1.13	0.004
Albumin, g/dl	3.82±0.43	3.91±0.38	0.106
Blood pH	7.32±0.04	7.41±0.02	<0.001
PCO2, mmHg	36.75±3.03	37.14±2.61	0.538
HCO_3^-, mEq/l	18.85±1.12	22.83±0.71	<0.001
IL-10, pg/ml	25.89±1.64	19.91±3.68	<0.001
Values are presented as mean±SD			

Correlation between baseline serum IL-10 and other parameters:

Baseline serum IL-10 has significant positive correlation with baseline serum CRP and significant negative correlation with blood pH and bicarbonate (Table 3).

Table (3): Correlations between serum IL-10 levels and other parameters at baseline

Parameter	Correlation coefficient	p-value
Age	-0.292	0.067
Dry weight	0.021	0.900
IDWG	-0.210	0.194
Systolic BP	-0.122	0.453
Diastolic BP	0.243	0.131
Hemodialysis vintage	-0.165	0.308
Sodium	0.059	0.718
spKT/V	-0.143	0.377
Hemoglobin	0.304	0.057
Calcium	0.019	0.909
Phosphorus	-0.128	0.430
iPTH	-0.065	0.692
Albumin	-0.227	0.159
CRP	0.607	<0.001
Iron	0.247	0.125
Ferritin	0.050	0.761
TIBC	0.055	0.737
pH	-0.411	0.008
PCO2	0.101	0.534
$HCO3^-$	-0.525	0.001

Correlation between change in IL-10 levels and change in other parameters:

The change in IL-10 levels were significantly correlated with the change in bicarbonate levels as shown in table 4.

Table (4): Correlations between change in IL-10 and change of other parameters

Parameter	Correlation coefficient	P value
Δ CRP, mg/l	-0.039	0.810
Δ $HCO3^-$, mEq/l	-0.436	0.005
Δ TIBC, ug/dl	0.138	0.395
Δ is the difference between baseline value and value at end of study		

DISCUSSION

This prospective cohort study was designed to determine the effect, if any, of correction of metabolic acidosis on serum IL-10 levels in chronic HD patients. Our results showed that correction of metabolic acidosis in HD patients leads to decrease of serum IL-10 levels. It should be mentioned that the lack of a control group did not allow interpretation of the findings exclusively as a result of metabolic acidosis correction. As reported previously [(9)], baseline serum IL-10 in study population was significantly higher compared with healthy individuals. This may be due to reduced clearance of cytokines or the HD procedure itself, which increases proinflammatory cytokines, mostly due to bio-incompatible dialysis membranes [(10)].

We demonstrated that blood pH and plasma bicarbonate increased significantly with increasing dialysate bicarbonate concentration and the target level has been achieved and maintained till the end of the study. We did not show increase in serum sodium concentration by increasing the dialysate bicarbonate as described before [(11)]. Also, there was no significant change in blood pressure control or interdialytic weight gain as a result of this intervention.

Correction of metabolic acidosis was associated with a significant decrease in serum IL-10 levels. This result is in agreement with previous study [(12)] that showed that correction of metabolic acidosis in non-dialysis CKD patients by oral alkali therapy resulted in a significant decline in IL-10 secretion by peripheral blood mononuclear cells. Contrary to our results, the same group of investigators studied IL-10 production in two groups of hemodialysis patients with and without metabolic acidosis and found that IL-10 levels were lower in metabolic acidosis group compared with group without metabolic acidosis [(7)]. An explanation to this contradictory result is that our study measured the changes that occurred in the same study population before and after correction of metabolic acidosis and levels of IL-10 measured in serum sample while **Ori *et al.*** [(7)]**,** compared between IL-10 levels in two different groups with different bicarbonate levels and also they measured secreted IL-10 after incubation of peripheral blood mononuclear cells in a culture media and not in a serum sample.

The decrease in serum IL-10 levels after correction of metabolic acidosis can be explained as follow; metabolic acidosis is a contributing factor in pathogenesis of the malnutrition-inflammation-atherosclerosis (MIA) syndrome in hemodialysis patients [(13)], and its correction might decrease the inflammation with subsequent decrease in serum IL-10, which is considered as anti-inflammatory cytokine produced to counter-regulate the inflammation. A supporting evidence for this speculation is that serum CRP also significantly decreased after correction of metabolic acidosis.

There are some limitations to this study. First, blood gas analysis was done once at end of study after the target bicarbonate level achieved and not before every dialysis session and it is possible that variability of serum bicarbonate levels had occurred. However, bicarbonate levels were at the target level at the end of study and accordingly this limitation may not be important. Second, there was no control group of chronic HD patients so we cannot establish the causal relation between correction of metabolic acidosis and reduction of IL-10 levels. Third, our cohort is small in number and derived from one center so we cannot extrapolate the results to all HD patients.

CONCLUSION

The results of this study suggest a decrease in IL-10 levels following correction of metabolic acidosis in chronic HD patients and this effect may be attributed to decreased inflammation after correction of metabolic acidosis.

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