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Original Article

Plasma and intracellular (platelet) zinc levels in chronic renal failure (CRF) patients under different treatment modalities

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Abstract: The causes and degree of zinc (Zn) deficiency in uraemia are still controversal. The effect of different treatment modalities are still unsetteled. Plasma Zn represent only a small part of the total body Zn (about 0.5%). Thus determination of intracellular Zn in the peripheral blood cells might be more reliable. The present study was designed to assess the actual Zn status in uraemia and to find whether the treatment modalities of CRF (conservative and dialytic) could influence Zn status. Also to determine the effect of single dialysis session, type of dialysis and dialysate buffer on the Zn status.

This study included ten healthy controls and fourty CRF patients divided in three subgroups on different treatment modalities [10 conservative treatment, 15 on intermittent perioneal dialysis (IPD) and 15 on haemodialysis (HD)].

Znic was measured by atomic absorption sepectrophotometry in plasma and platelets.

Statistically significant decrease of plasma Zn and significant increase of platelet Zn were found in CRF patients on different treatment modalities as compared to controls (P<0.01), but there was no significant difference in this respect between the three uraemic subgroups. There was no difference as regard serum protein and albumin levels in uramic subgroups compared to controls. Moreover plasma Zn was significantly increased (still less than control) and platelet Zn was significantly decreased (P<0.01) after a single dialysis session in both IPD and HD subgroups, but the changes of both parameters (before and after dialysis) were insignificant in IPD patients compared to HD patients.

Significant negative correlation was found between platelet Zn and creatinine clearance in the three uraemic subgroups (r = -0.81 P<0.01 in conservative patients, r= -0.72 P<0.01 in IPD and r= -0.76 P<0.01 in HD) while no correlation could be detected between the duration of dialysis and each of platelet & plasma Zn and between and plasma Zn and each of platelet Zn, serum creatinine and clearance. Plasma Zn showed transient significant rise in HD patients using bicarbonate (11.6± 1.1 μ mol/L) as compared to those using acetate buffer (9.1± 1.3 μ mol/L), P<0.01). We can conclude that intracellular measurements of Zn (platelet) is of value in diagnosis and monitoring of Zn status in uraemics. Different treatment modalities does not influence Zn haemostasis. with no superiority of particular type of dialysis in this respect. The effect of a single dialysis session and the use of bicarbonate versus acetate buffer was just a transient rise of plasma Zn due to haemoconcentration and better correction of acidosis during dialysis.

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Introduction

Zinc (Zn) is present in a large number of proteins and enzymes, e.g. metallo-thionine and DNA-binding proteins. It is essential for gene expression, growth hormone activity, cellular signal transduction, neuro-transmitter receptors and membrane functions [24]. It had been reported that uraemic patients have abnormal Zn metabolism [16]. The change in Zn metabolism may be responsible for certain features of uraemia [18]. These features include gonadal dysfunction [14], hyperprolactinaemia [13] and neuropathy [21].

The causes and degree of Zn deficiency in uraemia are controversal, it may be due to decreased Zn absorption [1] due to specific Zn transport defect or the absence of intestinal Zn ligands as picolinic acid [16].

Zn is usually measured in plasma or serum as it is easily accessible and relatively simple to process. However, plasma values represent only a small percentage (1-2%) of body content.

Determination of plasma Zn does not provide conclusive evidence of Zn deficiency [20]. Thus diagnosis of mild and moderate Zn deficiency is still an unsolved problem and increased attention should be devoted to the intracellular measurement in erythrocyte (which contain 85%), platelet and leucocytes which represent tissue Zn [6]. Red blood cells (RBCs) have slow turnover, thus Zn content does not reflect current Zn status and platelet Zn is a more reliable index of short term changes [19].

This study was designed to assess the actual Zn status in uraemia and to find out whether treatment modalities of CRF (conservative and dialytic) influence Zn haemostasis. Also to determine the effect of single dialysis session, type of dialysis and dialysate buffer (bicarbonate and acetate) on the Zn status.

Materials and methods

This study was carried out in the Internal Medicine (Nephrology Unit) and the Clinical Pathology Departments, Zagazig University Hospitals.

Subjects

We included fifty subjects in this work, they were divided into:

I. Control group: It comprised ten adult healthy volunteers (six males & four females), their ages ranged from 19-65 years with a mean of 41.3± 11.3 years. They had normal renal functions.

II. Chronic renal failure (CRF) group: It included fourty patients divided into three subgroups according to the line of treatment:

- Conservative subgroup: Ten patients (six males & four females) with CRF, their ages ranged from 31-63 years with a mean of 43.71± 13.3 years. They were on conservative treatment for five months to two years. The etiology of CRF was glomerulonephritis in four patients and chronic pyelonphritis in six patients. The mean serum creatinine was (4.6± 0.72 mg%).
- Peritoneal dialysis subgroups (IPD): It included 15 CRF patients (nine males & six females), their ages ranged from 16-56 years with a mean of 44.4± 12.1 years. Duration of dialysis ranged from 6-30 months with a mean of 14.5± 1.5 months (8 of them were dialysed for more than one year). IPD was performed twice weekly with thirty liters each session. None of IPD patients was previously treated by haemodialysis. The etiology of CRF was chronic pyelonephritis in ten patients and chronic glomerulonephritis in five patients.
- Haemodialysis subgroup (HD): It comprised 15 patients (eleven males & four females), their ages ranged from 16-66 years with a mean of 39.5± 14.2 years. HD was performed twice weekly, each session lasted for 4-6 hours, 8 of them used bicarbonate buffer and the other seven patients used acetate buffer. Only cuprophane low-flux (surface area 1-1) E_2 dialysers were used. The duration of dialysis ranged from 7-42 months, with a mean of 18± 3.1 months, (8 of them were dialysed for more than one year). The etiology of CRF was chronic pyelonephritis in eleven patients and chronic glomerulo-nehritis in 4 patients.

All patients in this study were selected to be free from diabetes, hepatic diseases and malignancy and were on recommended diet for uraemics. Those receiving erythropoeitin or vit D therapy were also excluded.

All cases were in stable condition and had no advanced complications. Patient data are shown in table 1.

Table 1. Clinical data of the different groups:

	Control group	*Conservative group*	*IPD group*	*HD group*
Age (years)	41.3±11.3	43.7±13.3	44.4±12.1	39.5±14.2
Sex (M/F)	6/4	6/4	9/6	11/4
Duration of dialysis (months)	-	-	14.5±1.5	18±3.1
Hb% (gm%)	14±1.3	9.5±1.01	8.5±0.99	8.92±1.03
Total protein (g/L)	8.1±0.91	7.7±0.72	7.6±0.63	7.9±0.58
Serum albumin (g/L)	4.5±0.44	3.9±0.331	3.8±0.40	4.1±0.39
S. creatinine (mg%)	0.9±0.03	4.6±0.72	8.2±0.9	6.57±0.61
Creatinine clearance (ml/min)	93±6.3	11.4±0.88	6.4±0.46	8.4±0.62

Methods

All subjects in this study were submitted to the following:

A. Thorough history and clinical examination.
B. Routine investigations including:
   - Urine analysis.
   - Complete blood count, packed cell volume, platelet count and platelet indices using sysmex cell counter model 3000.
   - Liver function tests especially total proteins and serum albumin and screening tests for HbsAg and HCV antibodies.
   - Kidney function tests: blood urea, serum creatinine and creatinine clearance by standard methods.
   - Fasting and 2h blood glucose.
   - X-ray chest and abdominal sonogram.
C. Specific test: Measurement of plasma and platelet zinc. After separation of platelet, zinc content was measured in both plasma and platelet by atomic absorption spectro-photometery [15].

Principle of assay

The samples were centrifuged (1200g 10 min) and their supernatents were carefully aspirated. We then added 3 ml of undiluted nitric acid to the tubes and the samples were heated at 135 C in a block heater until only a residue remained. This treatment with acid was repeated twice.
After the samples were digested, we added exactly 1 ml of a 10 ml/L solution of nitric acid to dissolve the mineral salts. Zinc content of both platelet and plasma samples was determined by atomic absorption spectrophotometry (Perkin-Elmer, Model 3110) and appropriate zinc standards.
N.B: Samples for plasma and platelet Zn were obtained before and after the dialysis session in patients under dialysis treatment, but once in conservatively treated patients and control subjects.

Results

A statistically significant increase in platelet count and platelet Zn (P<0.01), and statistically significant decrease in plasma Zn and packed cell volume (PCV) (P<0.01) were found in the three uraemic subgroups (conservative, IPD & HD) as compared to controls, while there was no significant difference in the previous parameters between the three uraemic subgroups.
Serum albumin showed no significant difference between control group and all uraemic subgroups and between each of the three uraemic subgroups as shown in table 2.
A statisticaly significant increase in plasma Zn and PCV (P<0.01) and significant decrease in platelet Zn (P<0.01) in IPD and HD subgroups after single session of dialysis was found. Platelet count was significantly increased after single dialysis session only in HD patients (P<0.01) as shown in table 3.
The change (Δ) in the levels of platelet and plasma Zn after single dialysis session (the difference in the between pre and post dialysis values) were not significant in IPD patients compared to HD subgroup as shown in table 4.
Plasma Zn was significantly increased in HD subgroup using bicarbonate buffer as compared to those using acetate buffer (P<0.01). The rise was transient after single dialysis session, while there was no significant difference in the platelet Zn in acetate buffered HD patients as compared to bicarbonate buffered HD patients as in table 5.
A statistically significant negative correlation between platelet Zn and creatinine clearance was observed in the all uraemic subgroups (r=-0.81, P<0.01 in conservative patients, r = - 0.72, P<0.01 in IPD and r=-0.76, P<0.01 in HD patients). No significant correlation could be detected between plasma Zn and each of platelets Zn and creatinine clearance in the three uraemic subgroups similarly between duration of dialysis and each of platelet and plasma Zn in dialytic subgroups as shown in table 6.

Table 2. Comparison of the mean values ±SD of platelet count, plasma & platlet zinc packed cell volume (PCV) and serum albumin in different groups

	Control group	*Conservative group*	*IPD*	*HD*	*F*	*P*
Platelet count ($x10^9$)	243.1±112.2	340.3±176.2 a	356.1±180.1 a	372.1±204.1 a	7.693	<0.01
Plasma Zn (μmol/L)	14.1±1.6	9.62±1.3 a	9.93±1.4 a	10.06±2.1 a	14.332	<0.01
Pletelet Zn(nmol/10^9 cells)	190.8±94.3	329.7±215.4 a	329.9±218.9 a	309±206.2 a	9.82	<0.01
PCV (%)	43.9±4.2	31.6±4.3 a	31.5±4.2 a	30.2±4.2 a	18.37	<0.01
Serum albumin (g/L)	4.5±0.44	3.9±0.331	3.8±0.401	4.1±039	0.071	NS

a = Significant in comparison to control group. b = Significant in comparison to conservative group.
c = Significant in comparison to IPD group.

Table 3. Comparison of the mean values ±SD of platelet count and zinc, plasma zinc and PCV in IPD and HD group before and after one dialysis session

	IPD				*HD*			
	Before	after	T	p	before	after	t	p
Platelet count ($x10^9$)	356.1±180.1	361.9±193	1.145	NS	372.1±204.1	411.2±197.4	6.921	<0.01
Plasma Zn (μmol/L)	9.43±1.4	10.9±1.2	9.22	<0.01	10.06±2.1	11.02±2.4	6.934	<0.01
Platelet Zn(nmol/10^9 cells)	329.9±218.9	299.5±211.9	10.82	<0.01	309±206.2	282.5±200.3	7.63	<0.01
PCV (%)	31.5±4.2	34.2±3.4	5.056	<0.01	30.2±4.3	36.3±3.2	5.99	<0.01

Table 4. Mean values ±SD of changes (∠) in the levels of platelet and plasma zinc in IPD and HD groups after single dialysis session

	IPD	*HD*	*t*	*P*
∠ of plasma zinc (μmol/L)	0.97±0.2	0.96±0.3	0.451	NS
∠ of platelet zinc (nmol/10^9 cells))	349±7.4	265.5±5.3	1.763	NS

Table 5. Comparison of the mean value ±SD of plasma and platelet zinc in HD subgroup using acetate versus bicarbonate buffer

	HD using acetate (n=8)	*HD using bicarbonate (n=7)*	*t*	*P*
Plasma zinc (μmol/L)	9.1±1.3	11.6±1.1	4.328	<0.01
Platelet zinc (nmol/10^9 cells)	321±105.1	307±111.2	2.093	NS

Table 6. Correlation coefficient between platelet and plasma zinc and the different parameters in the three uraemic subgroups

Variable	*Conservative group*		*IPD*		*HD*	
	r	p	r	p	r	P
Plasma Zn versus platelet Zn	-0.36	NS	-0.33	NS	-0.27	NS
Plasma Zn versus platelet count	-0.24	NS	-0.23	NS	-0.31	NS
Plasma Zn versus creatinine clearance	0.33	NS	0.34	NS	0.28	NS
Plasma Zn versus duration of dialysis	-	-	0.31	NS	0.18	NS
Platelet Zn versus platlet count	-0.22	NS	-0.17	NS	-0.19	NS
Platelet Zn versus creatinine clearance	-0.81	<0.01	-0.72	<0.01	-0.76	<0.01
Platelet Zn versus duration of dialysis	-	-	0.14	N.S.	0.26	N.S

Discussion

Zn is an essential component of a large number of metalloenzymes and is important for normal metabolism in man [6]. It had been reported that patients with chronic renal failure have an abnormal zinc metabolism [16]. These changes in Zn metabolism may account for certain feature of uraemia [18].

In the present study serum albumin and protein did not show any difference in the three uraemic subgroups as compared to controls, which exclude the effect of hypoalbuminaemia in Zn haemostosis. Plasma Zn levels were significantly lower is uraemic patients under different treatment modalities (conservative, IPD and HD) compared to control, indicating deficiency of circulating Zn in uraemics. Similar results were obtained by others [11,20,25]. Paniagua et al. [16] attributed low circulating Zn level in uraemia to low Zn intake, a specific Zn transport defect or absence of specific Zn ligands. Other studies suggested impaired absorption [8]. Several factors are thought to be involved in malabsorption as chronic enteritides and excessive losses (five times higher than the uptake of Zn may occur) [2]. Impaired tubular reabsorpation caused by the uraemic milieu was also suspected [23].

The low plasma Zn levels in the three uraemic subgroups in this study denote failure of any treatment modality to correct circulating Zn level. The absence of correlation between plasma zinc and duration of dialysis in dialytic subgroups and non significant difference of plasma Zn between the uraemic subgroups and each other indicate failure of dialytic therapy (IPD & HD) to correct plasma Zn. This is in agreement with the results obtained by Kaminska - Galwa et al. [7] who found low plasma Zn level in uraemics, which was similar in both short and long term dialysis groups.

It seems impossible to diagnose Zn deficiency by plasma level only, as it accounts for 1-2% of the entire pool, the largest amount of Zn is present intracellularly. Erythrocytes contain (85%) [9], but it has slow turn over and does not reflect current Zn status, while platelets have an average life span 7-10 days and its Zn content could be a more reliable index of short term changes in Zn status [19,20].

In this study platelet Zn was significantly higher in the three uraemic subgroups compared to controls. This result was supported by previous results obtained by Schmitt [20] who found that Zn is elevated in erythrocytes and platelets of uremics. These findings may be explained by abnormal shift of Zn from plasma to the intracellular compartment [22].

These results point to change in distribution of Zn in uraemics resulting in decreased plasma Zn and increased intracellular zinc (platelet). So in uraemic patients we can not depend on plasma zinc level to diagnose Zn deficiency. This concept could be illustrated by a study done by Chen & Yang [3] who found that platelet Zn was similar in uremics and controls but in the same study plasma Zn was markedly low indicating severe deficiency.

In view of the above results intracellular Zn is a more reliable index for diagnosis and monitoring of Zn status than plasma Zn.

Platelet Zn was negatively correlated with creatinine clearance in our uraemic subgroups but plasma Zn was not, indicating the close relation between tissue Zn (platelet) and degree of renal impairment. However there was no correlation between plasma and platelet Zn in the different studied subgroups. These results were supported by those of Iotova et al. [6]. Hinks et al. [5] recommended measurement of erythrocyte Zn content in order to determine the long term Zn status. For assessment of acute changes, leukocytes might be more suitable not only because of their shorter life span, but because they are nucleated, metabolically more active and thus more representive of other body compartments [4,17].

After a single dialysis session we found that plasma Zn was significantly higher in both dialytic subgroups compared to their values before the session, however, levels were still significantly lower than controls. This elevation was in agreement with the result obtained by Lin et al. [11]. It may be due to haemoconcentration as packed cell volume (PCV) was also significantly higher after the dialysis session although it was still lower than that of controls [6]. Platelet Zn was significantly decreased after the dialysis session compared to their values before dialysis in the two dialytic subgroups in our study. Similar results were obtained by Iotova et al. [6] and this transient change may be attributed to changes that occur during dialysis as redistribution of Zn from platelet to plasma or to the increased platelet count as we found in our patients during hemodialysis secondary to the effect of anticoagulants. We also found that there was no significant difference between the change in Zn status (plasma & platelet) in patients under HD in comparison to those under peritonial dialysis, indicating no superiority of either type of dialysis for correction of Zn status in CRF. Meanwhile plasma but not platelet Zn was transiently and significantly increased in patients on bicarbonate haemodialysis compared to those using acetate buffer. This finding was confirmed by Iotova et al. [6], denoting a more beneficial effect of bicarbonate buffer for correction of acidosis with subsequent improvement of Zn absorption.

We can conclude that intracellular measurment of Zn (platelet) is of value for diagnosis and monitoring of Zn deficiency states in uraemics. Furthermore treatment modalities of CRF do not influence Zn haemeostasis, with no superiority for a particular type of dialysis in this respect. A single dialysis session and the use of bicarbonate buffer caused transient elevation of plasma zinc, due to hemoconcentration and better correction of acidosis respectively that occur during dialysis.

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