Research Article
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Tartrate resistant acid phosphatases and osteodystrophy in chronic renal failure as related to oxidative stress: - *tartrate resistant acid phosphatase is a proxidant*

Abstract

Objective: Oxidative stress has emerged as a constant feature of Chronic Renal Failure (CRF), this possibly helps to promote the progression and complications of CRF. This study aims to evaluate the relation of tartrate resistant acid phosphatase (TRACP) to oxidative stress in patients with End Stage Renal Disease (ESRD).

Methods: Forty Iraqi patients with ESRD underwent hemodialysis and thirty seven healthy individuals were included in the present study. Several biochemical parameters such as lipid peroxidation through the concentration of malondialdehyde (MDA), [copper], [iron], [transferrin], [uric acid] and ceruloplasmin ferroxidase activity were measured in sera of control and ESRD patients.

Results: The results revealed presence of a significant increase ($P< 0.001$) in both [MDA] and uric acid level in sera of ESRD patients in comparison with that of the control group. Meanwhile a non-significant increase ($P>0.05$) was found in copper concentration with a significant decrease ($P<0.001$) in serum [iron], total iron binding capacity (TIBC),unsaturated iron binding capacity (UIBC), and [transferrin] of the patients group in comparison with that of the control group .Moreover no difference was observed in the saturation percentage of transferrin with iron between both groups and non-significant decrease in both the activity and the specific activity of ceruloplasmin ferroxidase ($P > 0.05$) in sera of the patients group.

Conclusion: we concluded that the increased of TRACP activity takes part in the measured oxidative stress (as indicated by the increased MDA level) in Iraqi patients with ESRD.

Keywords: TRACP, oxidative stress, end stage renal disease, lipid peroxidation, antioxidant

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Introduction

Oxidative stress occurs when there is, excessive free radical production and / or low antioxidant, and results in chemical alterations of biomolecules, causing structural and functional modification,[1] which leads to pathological condition, such as uremia; a condition contribute to cell and tissue injury.[2] Whereas, the uremic syndrome is characterized by retention of a host of compounds which in healthy individuals are secreted into urine by the healthy kidneys. Part of these retained compounds are those produced through the oxidative processes as a result of the inflammatory statues of uremic patients, however, and even the concentration of oxidative compounds further increased by disturbances of urinary clearance.[3] Hemodialysis treatment may also induce some deleterious effects of free radical, or lipid peroxidation by activated phagocytes at the contact of dialysis membrane and dialysate endotoxins.[4] Increasing appreciation of the causative role of oxidative injury in many disease states, places great importance on the reliable assessment of lipid peroxidation.[5] Lipid peroxidation involves the oxidative deterioration of polyunsaturated fatty acids in bio-membranes and generates a variety of aldehydes' products including malondialdehyde (MDA).[6]

Iron is one of the most essential trace elements in the body.[7] It can cause tissue injury and oxidative stress by catalyzing hydroxyl radical production and lipid peroxidation. Intravenous iron preparations are routinely administered to treat anemia in patients with CRF.[8] Another metal that plays an important role in oxidative stress is Copper; which is the third most abundant trace element in the human body following zinc and iron, and is essential to all organisms.[9] It also catalyzes the formation of hydroxyl radical from hydrogen peroxide.[10] TRACP, likes other metalloproteins containing a redox-active iron, is able to catalyze the generation of reactive oxygen species,[11] which include superoxide anions, hydroxyl radicals, peroxy radicals, hydrogen peroxide, hydroperoxides, and peroxintrite anions. Superoxide itself may not be directly injurious, but the highly reactive hydroxyl radicals formed through Fenton's reaction can break peptide bonds and cause disruption of RNA, DNA, and protein structures.[12] Several antioxidants aim at modifying the oxidative status in CRF patients.[13] Antioxidants are compounds and reactions disposing of toxic oxygen species, scavenging them, suppressing their formation, or opposing their actions.[14] They fall into two classes enzymatic such as superoxide dismutase, catalase, and glutathione peroxidase and non-enzymatic such as glutathione, vitamin E, vitamin C, ferritin, transferrin, and albumin.[15] Transferrins are a family of iron-binding proteins are found in the physiological fluids of many vertebrates.[16]

In addition to its role in regulating the iron fluxes between the sites of absorption, storage, and utilization, transferrin also attributed a very important antioxidant function in plasma,[17] since it can be expected to sequester any free iron and thus prevent cell damage such as lipid peroxidation from any hydroxyl radical generated by an iron catalyzed Haber-wells reaction.[18] Ceruloplasmin (EC 1.16.3.1) is a

α_2 serum glycoprotein, which contains > 95% of the copper found in plasma.[19] This protein is a member of the multicopper oxidase family,[20] each molecule can bind up to six atoms of copper,[21] and an evolutionarily conserved protein group that utilizes copper to couple substrate oxidation with four-electron reduction of oxygen to water (19) .The primary physiologic role of CP involves plasma redox reaction. It can function either as an oxidant, or an antioxidant depending on other factors, such as the presence of free ferric ions and ferritin binding sites.[22] Acting as a ferroxidase, CP plays an important role in the movement of iron. By oxidizing the ferrous form of iron to the ferric form, cp promotes iron loading on to transferrin which only binds the ferric form of the metal.[23] In addition, CP is considered as an effective antioxidant because of its ability to oxidize highly toxic ferrous iron to the relatively non toxic ferric form and helps to prevent oxidative damage of proteins, lipids, and DNA.[24] In human, there is another compound which play a role in the oxidative stress formation that is uric acid. Uric acid (weak acid), is the major product of the catabolism of the purine nucleoside (adenosine and guanosine).[25] Adenosine and guanosine are converted to xanthine, a common intermediate compound, that is oxidized to uric acid by xanthine oxidase (an enzyme that contains molybdenum, FAD and non heme iron).[25] In addition to uric acid, the reaction catalyzed by xanthine oxidase produces $O_2^{\cdot -}$ and H_2O_2.[26] The present study aims to evaluate the role of TRACP in the process of bone mass changes in ESRD and its relation to oxidative stress.

Patients and samples

A total of 40 Iraqi patients with chronic renal failure at end stage renal disease attending Al-Karama and Specialist Surgeries Hospitals in Baghdad city were included in this study. They were all undergoing hemodialysis treatment for (2-24) months at the time of the study. Patients diagnosed as having hepatitis were excluded. As a control, 37 age & gender matches' healthy individuals were included in the present study. Six milliters (ml) of venous blood were collected from the healthy donors and the patients (before hemodialysis). Blood samples were centrifuged at (2000 g) for 10min after blood coagulation the serum was collected and stored at -20°C until being used. The current study protocol had been approved by College of Science/ University of Baghdad/ Ethics committee.

Methods

Determination of serum copper concentration: -Serum copper was determined using flame atomic absorption spectrophotometer type GBC 933 plus at λ^{32} 4.7nm.

Determination of serum iron:- The iron was determined using colorimetric method that based on the dissociated of iron from transferrin complex by a solution of guanidine acetate.[27,28]

Determination of total iron-binding capacity:- Excess iron was added to the serum to saturate the transferrin. The unbound iron was precipitated with basic magnesium carbonate. After centrifugation the total iron- binding capacity in the supernatant was determined using (Biomaghreb Kit).[28]

Determination of [transferrin]: - [Transferrin] can be estimated indirectly from the TIBC value by using the following equation:

$$[\text{Transferrin}]\ (g/dL) = 0.7 \times \text{TIBC}\ (\mu g/dL)$$

The percentage of saturation of transferrin with iron was determined by the following equation:

$$\%\,\text{Saturation} = \frac{\text{Serum iron}}{\text{TIBC}} \times 100$$

Determination of ceruloplasmin ferroxidase activity: - Serum ceruloplasmin ferroxidase activity was estimated by the end point measurement method.

Determination of uric acid: - Serum concentration of uric acid in the current study was determined by enzymatic method. [29]

Determination of lipid peroxidation level: Measurement of [MDA] by thiobarbituric acid reactivity is the most widely used method for assessing lipid peroxidation. MDA, an end product of lipid peroxidation reacts with thiobarbituric acid to form a colored substance. MDA was estimated according to the modified method of Satoh.[30]

Statistical methods

The results were expressed as the mean ±standard deviation .Statistical and correlation analysis were undertaken using student t-test, and Pearson's correlation coefficients. A ($p < 0.05$) was accepted as statistically significant, highly significant when ($p < 0.001$), no significant when ($p > 0.05$). SPSS was used for the statistical analysis.

Results

The mean values presented in (Table 1) showed the presence of a highly significant increase ($P < 0.001$) in lipid peroxidation level and uric acid concentration in sera of patients with ESRD in comparison with that of the control group. While a non-significant increase ($P > 0.05$) in serum copper concentration of patients with ESRD was detected in comparison with that of the control group. Meanwhile a highly significant decrease in serum [iron], TIBC, UIBC, and [transferrin] in ESRD patients was observed upon comparison with that of the corresponding control group ($P < 0.001$). Moreover no differences in the saturation percentage of transferrin with iron was found in sera of the patients group in comparison with that of the control group. On the other hand a non-significant decrease ($P > 0.05$) in both activity and specific activity of ceruloplasmin ferroxidase were observed in sera of the patients in comparison with that of the control group. Serum levels of uric acid were reported to be quite variable and higher in males than in female.[19] Therefore in order to check the variation in iron level according to the gender, the studied groups of the current study were divided into two subgroups: male and female groups. The results in (Table 2) showed the presence of a highly signification decrease ($P < 0.001$) in serum iron, TIBC, UIBC, and transferrin of male and female ESRD patients in comparison with that of their corresponding control group. While no differences in saturation percentage of transferrin with iron was observed in both male and female ESRD patients in comparison with that of their corresponding control group. Also a non-significant increase ($P > 0.05$) was found in uric acid concentration in the serum of male ESRD patients in comparison with that of its corresponding control group ,while a highly significant increase($P < 0.001$) in this compound concentration in the sera of female patients when compared with that of its corresponding control group.

TRACP and oxidative stress

Previously TRACP activity was measured in our laboratory using the same type of patients and control groups.[32] The patients groups there were classified , according to the level of this enzyme activity into group with a high TRACP activity(Group 1) & another group with a decreased, or normal TRACP activity (Group 2) .And in order to check the role of TRACP in the generation of the reactive oxygen species (ROS) in ESRD patients, the correlations were tested, between the previously measured TRACP activity and the level of

MDA (as a marker of lipid peroxidation) with some of pro-oxidants and antioxidants parameters that were measured in the current study between the results presented in (Table 3) showed the presence of a significant correlation between TRACP activity and MDA level in sera of the first group, while a significant negative correlation had been found between the iron concentration with both TRACP activity and MDA level in sera of the same group.

Table 1 Mean values± SD of lipid peroxidation level, copper concentration, [iron], TIBC, UIBC, [transferrin], saturation percentage of transferrin, activity and specific activity of Ceruloplasmin, and [uric acid] in the sera of control and patients with ESRD

Parameter	Control	ESRD	P value
Lipid peroxidation n mol/L	10.96±3.84	18.69±6.67	< 0.001
[Copper] mmol/L	5.98±0.962	6.44±0.777	> 0.05
[Iron] mg/dL	116.92±32.4	86.56±39.2	< 0.001
TIBC mg/dL	345.59±54.44	247.33±90.13	< 0.001
UIBCmg/dL	226.34±56.6	154.31±71.79	< 0.001
[Transferrin] mg/dL	239.71±38.03	171.27±63.13	< 0.001
%Saturation of transferrin	34.06±10.10	35.08±11.21	> 0.05
[Uric acid] mg/ dL	4.461±.508	7.18±2.284	< 0.001
Ceruloplasmin activity (U/L)	1103.60±324.61	1012.4±357.18	> 0.05
Ceruloplasmin Specific activity (U/mg)	1.525±0.475	1.483±0.584	> 0.05

Table 2 Mean values ± SD of [iron], TIBC, UIBC, [transferrin] and saturation percentage of transferrin and uric acid concentration in the sera of male and female control and patients with ESRD

Race	Group	[Iron] µg/dL	TIBC µg/dL	UIBC µg/dL	[Transferrin] µg/dL	%Saturation of transferrin	[Uric acid] mg/dL
Male	Control	125.29±34.3	341.1±55.7	210.42±50.5	146.37±76.2	235.4±38.6	37.64±9.6	5.0±1.66
	ESRD	90.41±51.6	241.53±107.5		169.08±75.11	36.6±14.02	6.65±2.56
Female	Control	115.99±27.0	344.91±41.5	237.34±64.2	1248.3±79.7	247.3±35.7	34.4±9.05	3.8±0.92
	ESRD	80.27±29.9		168.2±71.6		173.78±55.7	34.03±11.64	7.85±1.72

Table 3 Correlation coefficients of TRACP activity and MDA on some pro-oxidant and antioxidant parameters in sera of patients with ESRD (group 1, n=10) and (group 2, n=28)

	Group 1 (n=10)		Group 2 (n=28)	
	TRACP	MDA	TRACP	MDA
MDA	0.715*	1	0.289	1
TRACP	1	0.715*	1	0.289
Copper	-0.152	0.196	-0.095	0.15
Iron	-0.753*	-0.730*	-0.183	0.029
Transferrin	-0.569	-0.347	-0.13	0.1
CP	0.122	-0.081	-0.205	-0.219
Uric aid	-0.484	-0.095	0.127	0.087

* Significant correlation (P < 0.05)

Discussion

Throughout this study serum malondialdehyde was measured as a marker of lipid peroxidation in ESRD patients & the control groups as described in material and method section. A highly significant increase in serum MDA was observed in the patients with ESRD in comparison with that of the control group. Table 1. This result was in agreement with the result obtained by Chroreshi et al. who reported that in acute and chronic renal failure serum MDA levels increased while serum superoxide dismutase (SOD) decreased significantly.[33] Also with Selvaraj et al. who reported that concentration of lipid peroxidation was significantly higher in nondiabetic un-dialyzed chronic renal failure patients when compared with the controls.[34] Grinshtein et al.[35] concluded presence of a significant increase of lipid peroxidation levels in patients with chronic renal failure. A conclusion based on the increased values of malondialdehyde levels in red blood cells and blood serum and those of diene conjugates in red blood cell membrane they measured in these patients.[35] The increase in MDA level that was observed in the present study in sera of patients with ESRD indicated that there was imbalance between their pro-oxidants and antioxidant mechanisms.[1] Fenton reaction, a reaction requires the participation of metal ions such as Fe and Cu and which leads to the formation of hydroxyl radicals has been proposed to mediate most of the oxidative modifications.[36]

In the present study, no difference was observed in copper concentrations between patients with ESRD and controls. This result is in agreement with what was reported about serum copper level

in chronic hemodialysis patients of being similar to that in healthy subjects.[37] While this result disagreed with the result obtained by Lin et al.[38] who found that plasma [copper] was significantly increased in uremic patients on hemodialysis.[38] Also this result disagreed with the result obtained by Richard et al.[39] who found that copper level in sera of patients with chronic renal failure was significantly decreased.[39] Anemia is a frequent consequence of progressive renal disease.[40] This agreed with the obtained results of [iron] and [transferrin] in the present study which indicated that the used patients with ESRD in the present study had anemia, where serum [iron] and [transferrin] were found to be low and the percentage of saturation of transferrin with iron was within the normal value. The major factor underlying the development of anemia in patients with renal failure is a decrease in the levels of erythropoietin.[42] Another reason for the anemia in these patients is due to retention of uremic toxins which may shorten the half-life of red blood cells and diminish the response of the bone marrow to erythropoietin. In addition to other aggravating factors that contribute to the anemia in the case of chronic renal failure including bleeding, iron or other nutritional deficiencies, bone marrow fibrosis secondary to hyperparathyroidism, and aluminum excess.[42] Some studies reported that increased losses of iron and transferrin in the urine may account for the iron deficiency anemia in patients with the nephrotic syndrome.[42]

One of the functions that ceruloplasmin have, is the oxidation of ferrous iron to ferric iron, that is a critical step for the binding action of iron to transferrin in plasma.[42] Ceruloplasmin in common with ferritin and transferrin is an acute-phase protein which its concentration is altered by inflammation.[42] The result of the present study demonstrated a non-significant decrease ($P > 0.05$) in ceruloplasmin activity in patients with ESRD. This finding can be supported by the result obtained by Paul et al. who reported that there was no difference in ceruloplasmin levels between healthy individuals and hemodialysis patients.[43] Low ceruloplasmin levels may be caused by protein deficiency states including nephrotic syndrome, protein-losing enteropathy, malabsorption, and malnutrition.[9] On the other hand Kirschbaum had reported that ceruloplasmin level was increased in hemodialysis patients compared to the normal.[42] The observed increased level of [uric acid] in sera of ESRD patients in comparison with that of control group in the current study, was in line with the fact that hyperuricemia is a common feature of renal disease of all etiologies; as the Glomular Flow Rate(GFR) falls serum [uric acid] increases due to reduced renal excretion.[44,45] The hyperuricemia was found to be generally mild in renal failure patients, due to either increased excretion of uric acid via non renal (gastrointestinal) routs, diminished biosynthesis, and / or enhanced degradation of uric acid.[46] Kang et al. reported that hyperuricemia is associated with renal disease, and it is usually considered as a marker of renal dysfunction rather than as a risk factor for the progression of the disease.[44] While Nakagawa et al. had reported that uric acid is not a simple marker of this disease, but is a cause of renal disease.[47]

Previously uric acid has been shown to act as a water-soluble antioxidant.[48] The antioxidant properties of urate or its synergistic effects with other antioxidants have been attributed to its ability to scavenge hydroxyl and superoxide radicals and peroxynitrite and to its chelation action of the transition metal ions.[49] Furthermore Filipe et al.[50] reported that urate may behave as a pro-oxidant. They showed in their *in vitro* study that urate is antioxidant at high concentration but pro-oxidant at low concentration. Depending on Cu^{2+} concentration, the switch between the pro- and antioxidant behavior of urate occurs at different urate concentrations, where at high Cu^{2+} concentration, in the presence of urate, superoxide dismutase and ferricytochrome C protect LDL from oxidation but no protection was observed at low Cu^{2+}.[50] In the present study, among the studied patients group, a significant positive correlation had been found between TRACP activity and MDA in sera of ESRD patients who have a significant increase in TRACP activity. This result was in agreement with the fact that TRACP contain a binuclear iron center, and the redox active iron is able to catalyze the generation of reactive oxygen species by Fenton reaction.[51] Garret et al. demonstrated in their *in vitro* study that the active TRACP is capable of generating reactive oxygen species through Fenton's reaction, since the ferrous ion in its center reacts with hydrogen peroxide to produce highly destructive hydroxyl radicals.[52]

Conclusion

The copper, transferrin and uric acid in addition to the ferroxidase activity of ceruloplasmin seemed to have no role in the previously measured oxidative stress in the sera of patients with ESRD,[53] while the increased activity of TRACP appeared to have a role in the measured oxidative stress (as indicated by the increase of MDA level) in Iraqi ESRD patients.

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Conflict of author

The author deflects there is no conflict of interest

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