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THE RELATIONSHIP BETWEEN HAPTOGLOBIN POLYMORPHISM AND OXIDATIVE STRESS IN HEMODIALYSIS PATIENTS

ODNOS IZMEĐU POLIMORFIZMA HAPTOGLOBINA I OKSIDATIVNOG STRESA KOD PACIJENATA NA HEMODIJALIZI

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Summary

Background: The relationship between haptoglobin polymorphism and oxidative stress in hemodialysis patients is not fully understood. In this study, total antioxidant capacity and ceruloplasmin ferroxidase activity were evaluated in relation to haptoglobin phenotype distribution in hemodialysis patients.
Methods: Serum samples collected from 161 patients and 84 healthy controls were haptoglobin-typed by electrophoresis. Ceruloplasmin ferroxidase activity and total antioxidant capacity were assayed using colorimetric methods.
Results: Irrespective of the haptoglobin phenotype, patients exhibited significantly lower total antioxidant capacity (1.42±0.29 vs. 1.55±0.28 mmol/L, P=0.002) and higher ferroxidase activity than controls. Frequency of Hp1-1 and Hp2-1 in patients was 15.5% and 36% as compared with 9.5% and 41.7% in controls. While ferroxidase activity was lower in Hp2-2 patients than in controls (142±61 vs. 179±47 U/L, P=0.002), it was higher in Hp2-1 (173±56 U/L) and Hp1-1 (170±54 U/L) patients than in controls (141±43 and 99±30 U/L respectively) (P=0.002 and 0.009). Ferroxidase activity in Hp2-2 patients was significantly lower than that of Hp2-1 or Hp1-1 patients (P=0.004 and 0.034). Total antioxidant capacity was significantly lower only in Hp2-2 patients (1.44±0.25) compared to that in Hp2-2 controls (1.65±0.22) (P=0.000).
Conclusions: These findings suggest that haptoglobin polymorphism can differentially impact oxidative stress levels in hemodialysis patients.

Keywords: chronic renal failure, ceruloplasmin ferroxidase activity, haptoglobin, hemodialysis, total antioxidant capacity

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Kratak sadržaj

Uvod: Odnos između polimorfizma haptoglobina i oksidativnog stresa kod pacijenata na hemodijalizi nije dovoljno istražen. U ovoj studiji, kod pacijenata na hemodijalizi određeni su ukupan antioksidantni kapacitet i aktivnost ceruloplazmin feroksidaze u odnosu na distribuciju fenotipa haptoglobina.
Metode: U uzorcima seruma uzetim od 161 pacijenta i 84 zdravih kontrolnih subjekata putem elektroforeze je određen tip haptoglobina. Aktivnost ceruloplazmin feroksidaze i ukupan antioksidantni kapacitet utvrđeni su kolorimetrijskim metodama.
Rezultati: Nezavisno od fenotipa haptoglobina, kod pacijenata je uočen značajno niži ukupan antioksidantni kapacitet (1,42±0,29 vs. 1,55±0,28 mmol/L, P=0,002) i veća aktivnost feroksidaze nego kod kontrolnih subjekata. Učestalost Hp1-1 i Hp2-1 kod pacijenata bila je 15,5% i 36%, u poređenju sa 9,5% i 41,7% kod kontrolnih subjekata. Dok je aktivnost feroksidaze kod Hp2-2 pacijenata u odnosu na kontrolu bila manja (142±61 vs. 179±47 U/L, P=0,002), kod Hp2-1 (173±56 U/L) i Hp1-1 (170±54 U/L) pacijenata bila je veća nego u kontrolnoj grupi (141±43, odnosno 99±30 U/L) (P=0,002 i 0,009). Aktivnost feroksidaze kod Hp2-2 pacijenata bila je značajno manja nego kod Hp2-1 i Hp1-1 pacijenata (P=0,004 i 0,034). Ukupni antioksidantni kapacitet bio je značajno niži samo kod Hp2-2 pacijenata (1,44±0,25) u odnosu na Hp2-2 kontrolne subjekte (1,65±0,22) (P=0,000).
Zaključak: Naši rezultati ukazuju na mogući diferencijalni uticaj polimorfizma haptoglobina na nivoe oksidativnog stresa kod pacijenata na hemodijalizi.

Ključne reči: hronična bubrežna insuficijencija, aktivnost ceruloplazmin feroksidaze, haptoglobin, hemodijaliza, ukupan antioksidantni kapacitet

List of abbreviations: HP, haptoglobin; HD, hemodialysis; TAO, total antioxidant capacity; Cp, ceruloplasmin.

Introduction

Antioxidants present in blood and other body fluids represent a major line of defense against the formation and accumulation of free radicals and reactive oxygen species. Among the major primary antioxidants in circulation are Hp, Cp, superoxide dismutase, glutathione peroxidase and catalase (1, 2). Additionally, some secondary antioxidants like vitamin E, vitamin C, β-carotene and uric acid function to remove newly formed free radicals (1–3). Currently, it is possible to assess the antioxidative capacity or the sum antioxidative potential of the various classes of endogenous and exogenous antioxidants by means of commercially available kits referred to as TAO measuring kits (3). Use of such kits is routinely indicated in cases where increased oxidative stress is suspected. Among the clinical conditions known to perturb the balance between free radical formation and accumulation on the one hand and the availability or potency of extracellular antioxidants on the other are hemofiltration and dialysis (4–6). Chronic renal failure (CRF) patients, irrespective of the underlying pathology, generally undergo one form or another of blood dialysis, mostly HD, as means of kidney function replacement therapy. HD results in marked changes in the concentration of serum proteins and other constituents due to filtration, dilution effects and alterations in the synthesis, metabolism or activity of many constituents (4–6). Increased production of free radicals and significant alterations in the availability or activity of various serum antioxidants are outcomes known to be associated with long-term HD (7, 8). Previous work has indicated that HD patients are under increased oxidative stress and possibly at high risk of developing oxidative stress-related complications (9–13).

Among the major serum antioxidants is Hp, which functions as a scavenger of free hemoglobin (14, 15). In humans, Hp synthesis is controlled by two alleles (Hp1 and Hp2) resulting in three major protein phenotypes: Hp1-1 type, Hp2-1 type and Hp2-2 type. The functional properties of Hp are type-dependent. Hp1-1 is a more potent antioxidant and binds more strongly with free hemoglobin compared with Hp2-2 (15). Hp1-1 prevents iron loss and the formation and accumulation of Fenton reaction-derived free radicals at a greater rate than Hp2-2 (14, 15). Hp polymorphism was shown to heavily bear on the prevalence of many diseases. Hp2-2 is over-represented in autoimmunity (16, 17); it is also a risk factor in some oxidative stress-related disease states like chronic renal failure (18). Hp2 homozygosity in diabetics has been shown to increase the risk for nephropathy and retinopathy (19–21). Another serum antioxidant is Cp, which is a liver-derived copper-containing free radical scavenger. Cp, via its ferroxidase activity, is vital in iron metabolism as it converts iron from the ferrous state to ferric iron, facilitating the release and binding of ferric iron to transferrin (22–24). Decreased Cp ferroxidase activity and aceruloplasminemia were both shown to lead to some oxidative stress-related disease states (25–27).

The bearing of Hp polymorphism on the antioxidative potential in HD patients is yet to be evaluated. In this study, the relationship between Hp polymorphism and the status of Cp ferroxidase activity as well as TAO capacity was evaluated in HD patients. Relevance of the cause of HD to the overall antioxidative capacity of HD patients was also investigated.

Materials and Methods

Sample collection

Blood samples were collected from 161 unrelated HD patients. Inclusion criteria consisted of diagnosis with end-stage renal failure and regular attendance of a dialysis center in Jordan. Patients were enrolled in the study irrespective of period on dialysis, type of medication, age, gender, or underlying disease state. Clinical data regarding the exact cause of HD was obtained from the patient's medical records with prior permission of the attending physician. Detailed information pertinent to the general characteristics of patients are given in *Table I* below. For the control group, blood samples were collected from 84 randomly selected, apparently healthy, unrelated individuals with no history of disease and not on any kind of medication; mean age was 42 ± 13 and male/female ratio was 57/27. All participants were informed of the goals of the study and asked to sign an institutionally-drafted form of consent on the understanding that his or her name will be kept confidential.

Table I General characteristics of patients included in the study.

Mean age ± SD (years) Age range (years) Gender (male/female)	46±13 19–80 109/52
Cause of renal failure (n, %) *Glomerulonephritis* *Hypertension* *Diabetes mellitus* *PKD* Other	 (52, 32%) (45, 27.8%) (30, 19%) (14, 8.8%) (20, 12.4%)
Dialysis modalities – Dialysis time/session (hours) – Sessions/week – Overall period on hemodialysis (years)	 3–4 3 3.1±2.6
Type of dialysis membrane (%)	Modified cellulose or synthetic
Dialysate buffer	Bicarbonate

Typing of Hp

Hp type distribution was determined using 8% vertical polyacrylamide gel electrophoresis (PAGE) as described previously (28). Briefly, Hp-hemoglobin complex solution was prepared by adding 5 μL of 10% hemoglobin A to 40 μL of the sample buffer (50% glycerol) followed by addition of 10 μL sample serum. Electrophoresis was run at room temperature for 4 hours at 130 volts. The gel was then removed from the apparatus and immersed in benzedine solution for 30 minutes to visualize the Hp bands. Benzedine solution was prepared by dissolving 0.2 g of benzedine in 250 mL boiling water. Just prior to staining, 1.5 mL glacial acetic acid and 0.6 mL H_2O_2 were added to the benzedine solution.

Measurement of Cp ferroxidase activity and TAO status

Cp Ferroxidase activity was measured using the O-dianisidine-dihydrochloride colorimetric method (29). For determination of TAO, commercially available TAO kits (Randox, UK) compatible for application in an automated chemistry analyzer (Express Chemistry analyzer, CIBA-CORNING, Minnesota, USA) were used according to manufacturer's instructions. Briefly, an aliquot of 5 μL sample, calibrator or control was separately mixed with 250 μL of metmyoglobin and incubated for 30 seconds. A volume of 50 μL of ABTS reagent was added and the subsequent drop in absorbance was measured at 600 nm 180 seconds later; values of TAO were calculated and expressed in mmol/L. The intra-assay and inter-assay CVs for Cp ferroxidase activity were 4.8% and 5.6% and those for TAO were 6.4% and 8.1%.

Data analysis

Data analysis was carried out using version 17.0 of the SPSS statistical software package (SPSS Inc, Chicago, IL). The Mann-Whitney U test for nonparametric variables was used to calculate the difference between various groups (patients vs. controls, different patient groups based on Hp type, sex, age, etc.) and results were expressed as mean ± SD; statistical significance was set at $P < 0.05$. Hardy-Weinberg equilibrium (HWE) was used to calculate Hp phenotypic and allelic frequencies and Chi-square test statistic was used to measure deviation from expected values at 1 degree of freedom and 0.05 level of significance.

Results

Results from this study demonstrate a number of interesting findings pertinent to the bearing of Hp phenotype on the oxidative stress in HD patients. Hp phenotype distribution in HD patients as a whole was remarkably distinct from that of the general population (*Table II*). In that, the frequencies of Hp1-1 and Hp2-1 were 15.5% and 36%, as compared with 9.5% and 41.7% in the control group and the general Jordanian population at large (30, 31). At levels of significance ≤ 0.05 and 1 *df*, the finding that the Chi-square value for the HD population is 5.76 strongly suggests that deviation from the HWE expectations cannot be attributed to chance alone.

Table II Hp phenotype distribution in healthy controls and hemodialysis patients.

Hp Type	Controls (n=84)		HD patients (n=161)	
	Observed	Expected	Observed	Expected
Hp1-1	8 (9.5%)	8 (9.2%)	25 (15.5%)	18 (11.2%)
Hp2-1	35 (41.7%)	36 (42.3%)	58 (36.0%)	72 (44.6%)
Hp2-2	41 (48.8%)	41 (48.5%)	78 (48.5%)	71 (44.2%)
Total	84	85	161	161
χ^2 (1df)	0.028		5.76	
Allele	Allele frequency distribution as per group			
Hp1	0.304		0.335	
Hp2	0.696		0.665	

n = number of individuals per group, χ^2 = Chi-square, df = degrees of freedom

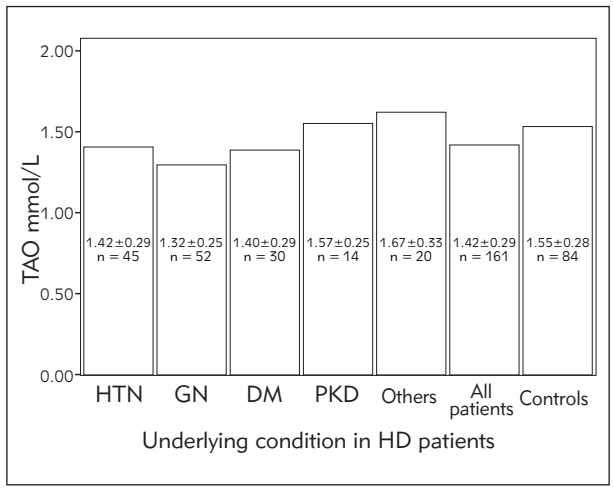

Figure 1 TAO levels in healthy controls and hemodialysis patients as grouped according to underlying condition (hypertension, HTN; glomerulonephritis, GN; diabetes mellitus, DM; polycystic kidney disease, PKD; and other miscellaneous forms); values are expressed as mean ± SD.

Consistent with previous findings (8–10), levels of TAO where significantly lower in patients than in controls (P=0.002) (*Figure 1*). Although levels of Cp ferroxidase activity were higher in patients than in controls, the differences were statistically insignificant (*Figure 2*). Furthermore, no significant differences were observed with regard to age or gender as they relate to levels of TAO and Cp ferroxidase activity (data not shown). When both parameters were evaluated in relation to Hp type, however, significant differences were found (*Table III*). Consistent with previous reports (30), healthy individuals with the Hp2-2 phenotype expressed Cp ferroxidase activity at significantly higher levels than healthy individuals with the Hp1-1 type (P=0.004). In contrast, HD patients with the Hp2-2 phenotype expressed significantly lower levels of Cp ferroxidase activity as compared with Hp2-1 or Hp1-1 patients (P=0.004 and 0.034). Additionally, while levels of Cp ferroxidase activity were lower in Hp2-2 patients than in healthy counterparts (P=0.002), they were higher in patients with Hp2-1 and Hp1-1 than in healthy counterparts (P=0.009 and 0.002).

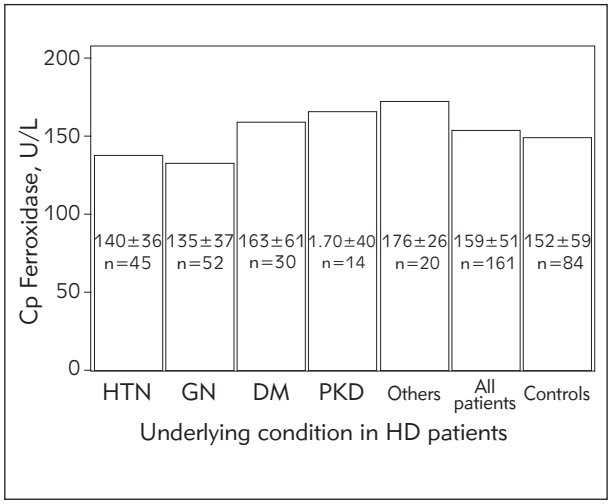

Figure 2 Cp ferroxidase activity in healthy controls and hemodialysis patients as grouped according to underlying condition (Hypertension, HTN; Glomerulonephritis, GN; Diabetes mellitus, DM; Polycystic kidney disease, PKD; and other miscellaneous forms); values are expressed as mean ± SD.

With respect to TAO capacity (*Table III*), healthy individuals with the Hp2-2 phenotype expressed significantly higher levels than their Hp1-1 (P=0.002) or Hp2-1 counterparts (P=0.003). In patients, however, no significant differences in the levels of TAO were observed among the various Hp types. By comparing patients with controls, levels of TAO were found to be significantly lower in patients with Hp2-2 as compared with controls (P=0.000). Although the levels of TAO were also lower in patients with Hp2-1 than in their controls counterparts, the differences were statistically insignificant.

Cp ferroxidase activity in patients with hypertension or glomerulonephritis was significantly lower than that in healthy controls (P=0.045 and 0.034) (*Figure 2*). In contrast, its levels in patients with diabetes were significantly higher than in controls (P= 0.048). As for patients with PKD and those with miscellaneous conditions, Cp ferroxidase activity was insignificantly higher than that in controls (P=0.085 and 0.061). With regard to TAO capacity, patients with hypertension, glomerulonephritis and diabetes expressed significantly lower levels than healthy controls (P=0.031, 0.002, and 0.045) (*Figure 1*). However, patients with PKD as well as those with miscellaneous conditions expressed slightly higher levels than healthy controls (P=0.560 and 0.281).

Table III Cp Ferroxidase and TAO levels in hemodialysis patients and controls according to Hp phenotype.

	HD Patients (n=161)			Controls (n=84)		
	Hp 1-1 (25)	Hp 2-1 (58)	Hp 2-2 (78)	Hp 1-1 (8)	Hp 2-1 (35)	Hp 2-2 (41)
Ferroxidase, U/L	170 ± 54 (NS*)	173 ± 56 (0.004**)	142 ± 61 (0.034***)	99 ± 30 (0.010*)	141 ± 43 (0.000**)	179 ± 47 (0.004***)
	(0.002^)	(0.009^^)	(0.002^^)			
TAO, mmol/L	1.45 ± 0.34 (NS*)	1.39 ± 0.33 (NS**)	1.44 ± 0.25 (NS***)	1.33 ± 0.10 (NS*)	1.47 ± 0.32 (0.002**)	1.65 ± 0.22 (0.003***)
	(NS^)	(NS^^)	(0.000^^^)			

* P value for 1-1 vs 2-1; ** for 2-1 vs 2-2; for *** 1-1 vs 2-2 for intra-group (controls and patients) analysis
^ P value for 1-1 vs 1-1; ^^ for 2-1 vs 2-1; for ^^^ 2-2 vs 2-2 for inter-group analysis of controls vs. patients
NS = not significant

Discussion

Several interesting findings regarding the relationship between Hp polymorphism and oxidative stress can be discerned from the results of the present study. It is clear that HD patients as a whole are under increased oxidative stress, as evidenced by the decreased TAO potential and increased Cp ferroxidase activity (*Figures 1* and *2*). Decreased TAO capacity is consistent with numerous previous studies, which have shown that CRF and hemodialysis are contributing factors to oxidative stress (4, 13, 32–35). Previous work has demonstrated that levels of iron storage (serum ferritin) rise in HD patients (36). Increased iron content in circulation and tissues contributes to the generation of free radicals through the Fenton reaction. Under such conditions, it is likely that Cp ferroxidase activity upregulates to handle the increased availability of ferrous iron, thus enhancing the efflux of ferric iron from stores and accelerating its uptake by transferrin in the circulation (23, 24, 37).

Our findings suggest that different Hp phenotypes have differential effects on the overall health status of HD patients. Disturbed patterns of Hp type distribution in HD patients as compared with controls, and the general population (30, 31), vis-à-vis increased representation of Hp1-1 at the expense of Hp2-1 (*Table II*), is evidence of that. For one thing, a Chi-square value of 5.76 as compared with the HWE expectations (at $df = 1$ and 0.05 level of significance) (*Table II*) can hardly occur due to chance alone. Instead, such a disturbed pattern of Hp type distribution is strongly suggestive of demographic changes in this particular population. In other words, there might be differential rates of mortality among HD patients depending on the Hp phenotype they express. Overall, results from this study are consistent with previous studies, which have demonstrated a link between Hp polymorphism and the occurrence of renal failure (18, 38, 39).

Findings presented here clearly indicate that Hp polymorphism has a significant influence on the level of oxidative stress in HD patients. For example, HD patients with Hp1-1 or Hp2-1 have higher levels of ferroxidase activity than Hp2-2 patients. Moreover, HD patients with Hp2-1 have higher levels of ferroxidase activity and lower levels of TAO as compared with Hp1-1 patients or with healthy counterparts. In fact, levels of TAO in Hp1-1 patients are slightly higher than those in healthy counterparts. These results suggest that HD patients with the Hp2-1 phenotype are under higher levels of oxidative stress as compared with HD patients expressing Hp1-1. This may partially explain the previously noted disturbed pattern of Hp type distribution and the consequent demographic changes in the HD patient population (18, 39).

The finding that Hp2-2 patients show significantly lower levels of TAO and Cp ferroxidase activity as compared with their healthy counterparts clearly suggests that they are under increased oxidative stress. Surprisingly, though, and unlike the case in Hp2-1 patients, the frequency distribution of Hp2-2 did not differ between patients and controls. Additionally, the decrease in TAO levels is not as significant as that in patients with Hp2-1. On the other hand, one should keep in mind that the Hp2-2 type is a poor antioxidant (14, 15). It is possible, therefore, that other antioxidants could be uniquely triggered in such individuals as means of compensating for the inheritance of two Hp2 alleles (Hp2 homozygosity). The pattern of increase in both Cp ferroxidase activity and TAO capacity in the healthy group as one moves from Hp1 to Hp2 homozygosity (*Table III*) is strongly supportive of this proposition (30). Details pertinent to the initiation and/or regulation of this proposed compensatory mechanism, though very significant, have yet to be investigated.

The underlying cause of CRF seems to have little effect on the overall status of oxidative stress in HD patients. In that, although HD patients with glomerulonephritis or hypertension have lower levels of ferroxidase activity as compared with other HD patient groups (diabetics, patients with PKD, small kidney disease or Alport's disease), no clear pattern can be discerned regarding the TAO status in the various HD patient subgroups. This is understandable given that, with the possible exception of diabetes (40), the underlying cause of CRF has little to do with antioxidative potential or oxidative stress.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

References

1. Jacob RA, Burri BJ. Oxidative damage and defense. Am J Clin Nutr 1996; 63: 985S–990S.
2. Prior RL, Cao G. In vitro total antioxidant capacity: comparison of different analytical methods. Free Radic Biol and Med 1999; 27: 1173–81.
3. Yeum KJ, Russell RM, Krinsky NI, Aldini G. Biomarkers of antioxidant capacity in the hydrophilic and lipophilic compartments of human plasma. Arch Biochem Biophys 2004; 430: 97–103.
4. Nguyen-Khoa T, Massy ZA, De Bandt JP, Kebede M, Salama L, Lambrey G, et al. Oxidative stress and haemodialysis: the role of inflammation and duration of dialysis treatment. Nephrol Dial Transplant 2001; 16: 335–40.
5. Warnner C, Buhaner U, Mattern R, Lang D, Passlick-Deetjen J. Effect of dialysis flux and membrane material on dyslipidemia and inflammation in haemodialysis patients. Nephrol Dial Transplant 2004; 19: 2570–5.
6. Mitrogianni Z, Barbouti A, Galaris D, Siamopoulos KC. Tyrosine nitration in plasma proteins from patients undergoing haemodialysis. Am J Kidney Dis 2004; 44: 286–92.
7. Dakshinumurty KV, Rao PV, Saibaba KS, Sheela RB, Sreekrishna V, Venakataramana G, et al. Oxidative stress in haemodialysis: postdialytic changes. Clin Lab 2003; 49: 255–61.
8. Filiopoulos V, Hadjiyannakos D, Takouli L, Metaxaki P, Sideris V, Vlassopoulos D. Inflammation and oxidative stress in end-stage renal disease patients treated with hemodialysis or peritoneal dialysis. Int J Artif Organs 2009; 32: 872–82.
9. Tiranathanagul K, Eiam-Ong S, Tosukhowong P, Praditporsilpa K, Tungsanga K. Oxidative stress from rapid versus slow intravenous iron replacement in haemodialysis patients. Nephrology (Carlton) 2004; 9: 217–22.
10. Upim LB, Himmelfarb J, McMonagle E, Shyr Y, Ikizler TA. Influence of initiation of maintenance haemodialysis on biomarkers of inflammation and oxidation. Kidney Int 2004; 65: 2371–9.
11. Ward RA, Mcleish KR. Oxidant stress in haemodialysis patients: what are the determining factors. Artif Organs 2003; 27: 230–6.
12. Wratten ML, Galaris D, Tetta C, Sevanian A. Evolution of oxidative stress and inflammation during haemodialysis and their contribution to cardiovascular disease. Antioxid Redox Signal 2002; 4: 935–44.
13. Morena M, Martin-Mateo M, Cristol JP, Canaud B. Oxidative stress, hemoincompatibility and complications of long-term dialysis. Nephrologie 2002; 23: 201–8.
14. Melamed-Frank M, Lache O, Enav BI, Szafranek T, Levy NS, Ricklis RM, et al. Structure–function analysis of the antioxidant properties of haptoglobin. Blood 2001; 98: 3693–8.
15. Langlois RL, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem 1996; 42: 1589–600.
16. Rantapää Dahlqvist S, Beckman G, Beckman L. Serum protein marker in systemic lupus erythematosus. Hum Hered 1988; 38: 44–7.
17. Goldenstein H, Levy NS, Levy AP. Haptoglobin genotype and its role in determining heme-iron mediated vascular disease. Pharmacol Res 2012; 66: 1–6.
18. Awadallah S, Hamad M. A study of haptoglobin phenotypes in patients with chronic renal failure. Ann Clin Biochem 2003; 40: 680–3.
19. Nakhoul FM, Zoabi R, Kanter Y, Zoabi M, Skorecki K, Hochberg I, et al. Haptoglobin phenotypes and diabetic nephropathy. Diabetologia 2001; 44: 602–4.
20. Nakhoul FM, Marsh S, Hochberg I, Leibu R, Miller BP, Levy AP. Haptoglobin phenotype as a risk factor for diabetic retinopathy. JAMA 2000; 284: 1244–5.
21. Fröhlander N, Johnson O. Haptoglobin groups in acute myocardial infarction. Hum Hered 1989; 39: 345–50.
22. Floris G, Medda R, Padiglia A, Musci G. The physiopathological significance of caeruloplasmin. Biochem Pharmacol 2000; 60: 1735–41.
23. Attieh ZK, Mukhopadhyay CK, Seshadri V, Tripoulas NA, Fox PL. Ceruloplasmin ferroxidase activity stimulates cellular iron uptake by a trivalent cation-specific transport mechanism. J Biol Chem 1999; 274: 1116–23.
24. Young SP, Fahmy M, Golding S. Ceruloplasmin, transferrin and apotransferrin facilitate iron release from human liver cells. FEBS Lett 1997; 411: 93–6.
25. Cairo G, Conte D, Bianchi L, Fraquelli M, Recalcati S. Reduced serum ceruloplasmin levels in hereditary haemochromatosis. Br J Haematol 2001; 114: 226–9.
26. Yoshida K, Kaneko K, Miyajima H, Tokuda T, Nakamura A, Kato M, et al. Increased lipid peroxidation in the brains of aceruloplasminemia patients. J Neurol Sci 2000; 175: 91–5.
27. Fox PL, Mazumdar B, Ehrenwald E, Mukhopadhyay CK. Ceruloplasmin and cardiovascular disease. Free Radic Biol Med 2000; 28: 1735–44.
28. Laemmili UK. Cleavage and structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680–5.
29. Schosinsky H, Lehman HP, Beeler MF. Measurement of Ceruloplasmin from its ferroxidase activity in serum by use of O-dianisidine-dihydrochloride. Clin Chem 1974; 20: 1556–63.
30. Janaydeh M, Hamad M, Awadallah S. The relationship between haptoglobin polymorphism, and serum ceruloplasmin ferroxidase activity. Clin Exp Med 2004; 3: 219–23.
31. Hamad M, Awadallah S. Age group-associated variations in Hp type distribution in Jordanians. Clinica Chimica Acta 2000; 300: 75–81.
32. Annuk M, Zilmer M, Fellstro B. Endothelium-dependent vasodilation and oxidative stress in chronic renal failure: Impact on cardiovascular disease. Kidney Int Suppl 2003, 84: S50–S53.

33. Lucchi L, Bergamini S, Iannone A, Perrone S, Stipo L, Olmeda F, et al. Erythrocyte susceptibility to oxidative stress in chronic renal failure patients under different substitutive treatments. Artif Organs 2005, 29: 67–72.

34. Rutkowski P, Malgorzewicz S, Slominska E, Renke M, Lysiak-Szydlowska W, Swierczynski J, et al. Interrelationship between uremic toxicity and oxidative stress. J Ren Nutr 2006, 16: 190–3.

35. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrol Dial Transplant 2003, 18: 1272–80.

36. Senol E, Ersoy A, Erdinc S, Sarandol E, Yurtkuran M. Oxidative stress and ferritin levels in haemodialysis patients. Nephrol Dial Transplant 2008; 23: 665–72.

37. Hübner-Woźniak E, Okecka-Szymańska J, Stupnicki R, Malara M, Kozdroń E. Age-related blood antioxidant capacity in men and women. Journal of Medical Biochemistry 2011; 30: 103–108.

38. Chen YC, Lee CC, Huang CY, Huang HB, Yu CC, Ho YC, et al. Haptoglobin polymorphism as a risk factor for chronic kidney disease: a case-control study. Am J Nephrol 2011; 33: 510–14.

39. Burbea Z, Nakhoul F, Rosenberg S, Zoabi R, Skorecki K, Hochberg I, et al. Role of haptoglobin phenotype in end-stage kidney disease. Nephron Exp Nephrol 2004; 97: e71–76.

40. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003; 17: 24–38.

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