

Retinol and Alpha-Tocopherol Levels Among Hemodialysis Patients.

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Abstract

Plasma retinol, alpha tocopherol, total cholesterol and triglycerides were measured in 40 patients aged 27-65 years, under regular hemodialysis (HD) for 1.8-13 years at Ahmed Maher teaching Hospital and in 28 healthy age and sex matched control. Predialysis and postdialysis measurements were also, done for a subset of 13 hemodialytic patients.

Among hemodialytic patients ,all values (Plasma retinol ,alpha- tocopherol, total cholesterol and triglycerides)were significantly higher ($p < 0.05$) with respect to healthy control .The ratio of retinol to total cholesterol was significantly higher , while alpha-tocopherol to total cholesterol ratio was insignificant .In almost all hemodialytic patients, plasma retinol was at toxic level (> 100 ug /dl) except for one patient .

On the other hand ,alpha-tocopherol level in hemodialytic patients was ranged between deficiency (<650 ug/dl) in about 6 (15%) of them , marginal level (650-860 ug/dl) at 3 (8%) , while 9 (23%) had normal level (860-1080ug/ dl) and the rest 22 (54%) were in the optimum level (> 1080 ug/dl). Comparing predialysis and postdialysis measurements , the hemodialytic patients showed non significant difference concerning retinol level , while alpha tocopherol was significantly decreased in postdialytic state .In conclusion ; further studies are needed to answer, if hemodialytic patients are at risk for symptomatic vitamin A toxicity?. Even with normal or low plasma vitamin E, it is needed as an antioxidant accessory therapy in hemodialytic patients.

Keywords: hemodialysis , retinol , alpha-tocopherol , triglycerides total cholesterol .

Introduction

Contributions of the kidney to vitamin A metabolism involve Retinol Binding Protein (RBP) metabolism, hepatic release of retinol and retinoic acid synthesis (Chazot and Kopple,1997) .Chronic renal failure patients, have elevated plasma concentrations of total vitamin A and RBP-bound vitamin A. Elevated vitamin A is considered to be due to increased plasma RBP ,and have no clinical significance as long as the RBP/vit A ratio is normal (Mydlik ,1991) .

Chronic renal failure patients, who had no clinical features of vitamin A toxicity, have increased liver and skin vitamin A content. In the absence of increased serum prealbumin,an excess of

vitamin A /RBP complex would easily bind to tissues with RBP receptors , causing toxic effects. In contrast, features of vitamin A toxicity in a skin examination of hemodialysis (HD) patients was not found although, they had elevated serum levels of RBP and retinol (Chazot and Kopple,2004).

Vitamin E is the most important defense against peroxidation of unsaturated lipids in cells and of plasma lipoproteins. The normal concentrations of vitamin E are inadequate to cover the need for protection in HD patients (Usberti ,*et al.* 2002). In HD patients there was a decrease in concentration of red blood cell (RBC) tocopherol and there was marked decrease in lipid peroxidation in patients treated with

alpha tocopheryl acetate (Pastor, *et al.*1993; Ongajooth, *et al.* 1996 ; Cristol, *et al.* 1997).This may be due to the oxidative stress or a defect in the high density lipoprotein(HDL) – mediated transfer of tocopherol from plasma to the RBC membrane (Chazot and Kopple , 2004) .

The aim of this study was to evaluate plasma concentration of retinol and alpha-tocopherol in hemodialytic patients and their lipid pattern. Also,to detect the effect of hemodialysis on these parameters.

Subjects and methods

Forty stable hemodialytic patients ,24 (60%) males and 16 (40%) females were recruited from dialysis unit of Ahmed Maher teaching hospital , Cairo, Egypt. Patients were aged 49.5 ± 11.3 years with duration of HD for 4.5 ± 2.4 years (mean \pm SD) and were adequately dialysed with a mean Kt/V of 1.12 ± 0.23 according to the DOQI guidelines (2002). End stage renal disease (ESRD) was due to, hypertensive nephropathy (16) patients (pts), diabetic nephropathy (8)pts, pyelonephritis (4)pts, renal stones (4)pts, polycystic kidney (2)pts, hyperparathyroidism (1) pt , and to unknown causes in five . All patients were on bicarbonate – hemophan dialyzers (modified cellulose). Twenty eight persons who were age and sex matched healthy control were also, recruited for the study. Informed consent were obtained from all people under the study.

Fasting (12 hours) venous blood samples were collected, plasma was separated by centrifugation at 3000 rpm for 15 min. Plasma was used for determination of retinol and alpha- tocopherol using high pressure liquid chromatography (HPLC) according to Bieri *et al.* (1979). Also, total cholesterol (Allain, *et al.* 1974) and triglycerides (Wahlefeld, 1974) were determined using kits provided from biomerieux, France .Retinol and alpha-tocopherol were chosen out of the fat soluble vitamins ADEK, vitamin D was excluded due to complicated interactions and medications with renal failure (Jacobson, *et al.*1995).Vitamin K was also

excluded to avoid variations in levels of its measurements due to the prevalence of hepatic diseases, cirrhosis and bilharsiasis among Egyptian hemodialysis patients (Afifi, 2003).

Data handling and Statistical analysis

The collected data were computerized and analyzed statistically through SPSS-under windows version 11. Excel computer program was used to tabulate the results. Qualitative variables were expressed as percentages. Quantitative variables from normal distribution were expressed as mean \pm S.E. Independent t- test , and one –way analysis of variance (ANOVA) with the least significant differences (LSD) were used. The difference was considered signifycant at p-value < 0.05 levels (Armitage, 1971) .

Results

Plasma retinol level was markedly greater among HD patients than healthy control, it's level was about (8 fold) higher in HD patients compared to healthy persons [465.27 ± 26.72 μ g/dl versus 61.14 ± 3.48 μ g/dl , $p \leq 0.000$].

As it's ratio to cholesterol was significantly higher in HD patients than control [2.65 ± 0.19 versus 0.397 ± 0.02 , $p \leq 0.000$] table (2).

Also, plasma alpha tocopherol was significantly higher among HD patients compared to healthy control [1323.04 ± 150.32 μ g/dl versus 891.24 ± 36.21 μ g/dl, $p \leq 0.008$] , while there was no significant difference regarding alpha tocopherol cholesterol ratio between HD patients and healthy control [6.62 ± 0.55 versus 5.8 ± 0.27 , $p = 0.19$] table (2) .

As regards lipid pattern ,both triglycerides (TG) and total cholesterol (Tch) were significantly elevated in HD patients compared with control group . For TG [187.56 ± 23.25 mg/dl versus 105.58 ± 9.3 mg/dl, $p \leq 0.002$]. While cholesterol level was [204.98 ± 13.02 mg/dl versus 166.63 ± 6.7 mg/dl, $p < 0.05$] table (2).

There was no statistically significant difference between gender and all values

(retinal, alpha tocopherols, total cholesterol, triglycerides). Also, there was no correlations between the primary cause of renal failure, age of the patients or the duration of dialysis and all measured values.

We also, studied the effect of HD on plasma level of retinol, alpha tocopherol, lipid pattern and their ratios for subgroup of 13 patients table (3) . The results showed significant decrease in alpha tocopherol level in post dialysis condition compared to pre dialysis one [1284.23 ± 245.18 µg/dl

versus 1348.12 ± 243 .08 µg/dl ,p ≤ 0.005] . Retinol /cholesterol ratio was decreased after HD session than before session [2.48 ± 0.29 versus 2.35 ± 0.25, p < 0.05] .

After HD session, total cholesterol level was significantly decreased than beforeHD session [161.6 ± 10.37 mg/dl versus 201.54± 13.77, p ≤ 0.002].

While, there was no significant difference in plasma retinol level, alpha tocopherol cholesterol ratio and TG level before and after HD session.

Table (1): Base line data of hemodialytic patients :-

Age (mean±SE)	49.5± 11.3 years
Sex : Males	24 (60%)
Females	16 (40%)
Dialysis time (hours/session)	6 hours
Duration of dialysis(years±SE)	4.5 ±2.4
Kt/v (mean±SD)	1.12 ± 0.23

Table (2): Plasma retinol , alpha-tocopherol , Lipid pattern and Their ratios among control and hemodialytic groups

Parameters	Control group (n=28)	Hemodialytic patients (n=40)
	Mean ± SE	Mean ± SE
Retinol (ug/dl)	61.14 ± 3.48	465.27 ± 26.72*
Retinol/ cholesterol ratio	0.3966 ± 0.02	2.65 ± 0.19*
alpha-tocopherol (ug/dl)	891.24 ± 36.21	1323.04 ± 150.32*
alpha-tocopherol /cholesterol ratio	5.800 ± 0.266	6.62 ± 0.55
Triglycerides (mg/dl)	105.58 ± 9.30	187.56 ± 23.25*
Total cholesterol (mg/dl)	166.63 ± 6.71	204.98 ± 13.02*

* P<0.05 = significant

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Table (3): Effect of hemodialysis on plasma level of retinol, alpha-tocopherol, lipid pattern and their ratio for the subgroup (n=13)

Parameters	Predialysis level	Postdialysis level
	Mean ± SE	Mean ± SE
Retinol (ug/dl)	443.7 ± 32.09	430.8 ± 33.16
Retinol / cholesterol ratio	2.35 ± 0.25	2.48 ± 0.29*
alpha-tocopherol (ug/dl)	1348.12 ± 243.08	1284.23 ± 245.18*
alpha-tocopherol /cholesterol ratio	6.25 ± 0.64	7.69 ± 1.11
Triglycerides (mg/dl)	151.53 ± 36.00	187.35 ± 13.77
Total cholesterol (mg/dl)	201.54 ± 13.77	161.60 ± 10.37*

* P<0.05 = significant

Discussion

In previous surveys (Yatzidis *et al.*, 1975 ; Werb *et al.*,1979 ; Casey *et al.*, 1981 ; Farrington *et al.*, 1981 ; Gleghorn *et al.*, 1986) , although serum vitamin A level was elevated (> 2SD) in patients with renal failure , clinical symptoms of hypervitaminosis A were not mentioned .Our results presented dramatic elevation of plasma retinol level in HD patients compared to healthy persons. However, we didn't check clinical manifestations of hypervitaminosis A. Failure of the kidney to metabolize retinol to retinoic acid may cause diminished excretion via bile and urine, thus leading to accumulation of retinol in chronic renal failure (CRF) (Ellis, *et al.* 1980). The elevation of serum vitamin A in renal failure patients has also been thought to be related to a defect in RBP catabolism by the diseased kidney (Chazot and Kopple, 2004). As RBP to retinol ratio is approximately 1:1, in renal failure patients the RBP values increase over normal and is often in excess of that seen for vitamin A. This may explain the lack of reported clinical symptomatology in renal failure patients, because toxicity may be caused by retinol and /or its metabolites non specifically bound to plasma lipoproteins

rather than to RBP (Gleghorn *et al.*,1986). The lipid status may influence vitamin A level in patients undergoing chronic dialysis. A positive correlation between plasma vitamin A levels and both serum total cholesterol and triglycerides has been observed in 72 patients undergoing maintenance hemodialysis (MHD), (Werb *et al.*,1979 and Smith *et al.*,1992). Even, individuals without renal failure and with hypertriglyceridemia were reported to be at increased risk of vitamin A toxicity because of increased bioavailability of retinyl esters from lipoproteins (Ellis *et al.*,1980). Because hyper-triglyceridemia occurs commonly in patients with renal failure, it is possible that those individuals also, may be at increased risk for vitamin A toxicity (Chazot and Kopple ,2004) . In this study, total cholesterol and triglycerides were significantly elevated in HD patients than in healthy individuals adding to the increased risk for vitamin A toxicity in these patients.

Among HD patients, the major cause of death is cardiovascular disease, which accounts for about 50% of the mortality in those patients (Rock *et al.*, 1997). Vitamin E is the main antioxidant in biologic membranes, protecting phospholipids

membranes from oxidative stress (Farrell and Roberts, 1993). Vitamin E is also, an antiatherogenic agent. Rimm *et al.*, (1993) and Stampfer *et al.*, (1993), studies have found a reduced risk of coronary heart disease in individuals with higher intake of vitamin E. The mechanism of this protective effect is considered to be decreased oxidation of low density lipoprotein (LDL) cholesterol- a key step in the pathogenesis of the fatty streak , the first step in the development of the atheromatous plaque (Chazot and Kopple , 2004) .

In this study, alpha-tocopherol level was significantly higher in patients receiving chronic HD therapy than control persons. The same results have been reported in previous studies(Mydlik *et al.*,1991,Fydryk *et al.*,1998).Other studies demonstrated no significant difference in alpha tocopherol level between HD patients and healthy control (Warwick *et al.*, 2000) . While another studies reported decreased alpha-tocopherol level in HD patients (Rajbala *et al.*, 1997 and Kinra *et al.*, 2000) .

The oxidative stress associated with uremia is exacerbated by HD, since inflammatory cells (neutrophils, and monocytes) activated by contact with the dialysis membranes, release large amounts of reactive oxygen species (ROS) (Usberti *et al.*, 2002). The deleterious effects of ROS on carbohydrates, lipids and proteins have a pathological role in many inflammatory diseases, most of which are frequent in HD patients (Galli *et al.*, 1999a&b).

Although some studies (De Bevere *et al.*, 1982; Cristol *et al.*, 1997 and De Cavanagh *et al.*, 1999) found no difference in plasma vitamin E concentrations between predialysis and post dialysis samples . In this study, the HD procedure that exacerbates oxidative stress had significant effect on plasma alpha-tocopherol level. As, its level was significantly decreased after hemodialysis procedure. This may be due to increased consumption of alpha- tocopherol due to oxidative stress.

In contrast, the HD procedure had no significant effect on plasma retinol level. As it is a lipid soluble compound , so

significant dialytic clearance of plasma retinol is not expected .

In conclusion, further studies are needed to answer, if hemodialytic patients are at risk for symptomatic vitamin A toxicity? The commercially available multivitamins preparations containing vitamin A may be hazardous for hemodialytic patients. Also, even with normal or low plasma vitamin E, it may be needed as an antioxidant accessory therapy in hemodialytic patients.

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مستوى الرتينول والالفا - توكوفيرول فى مرضى الغسيل الكلوى (الدموى)

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أجريت هذه الدراسة لقياس مستوى الرتينول والالفا توكوفيرول فى مرضى الغسيل الكلوى (الدموى) المنتظم من بين المترددين على قسم الكلى الصناعية بمستشفى أحمد ماهر التعليمى بالقاهرة. وقد اشتملت هذه الدراسة على (40) مريضا يستخدمون جهاز الكلى الصناعية (الغسيل الدموى) بانتظام لمدة تتراوح بين 1,8 إلى 13 سنة وتتراوح أعمارهم بين 27 - 65 عام. وكذلك اشتملت الدراسة على 28 فردا كمجموعة ضابطة مماثلة فى العمر والجنس. تم قياس نسبة الرتينول ، الألفا - توكوفيرول ، الكولستيرول الكلى ، والدهون الثلاثية فى بلازما الدم للمرضى وللمجموعة الضابطة. وكذلك تم قياس هذه المؤشرات فى 13 من المرضى قبل وبعد جلسته الغسيل الكلوى (الدموى).

لقد أظهرت النتائج أن نسبة الرتينول فى البلازما كانت فى مستوى السمية فى كل المرضى عدا مريضا واحدا فقط. وكذلك وجدت زيادة ذات دلالة إحصائية فى كل المؤشرات بين المرضى بالمقارنة بالمجموعة الضابطة. ووجدت أيضا زيادة ذات دلالة إحصائية فى معدل الرتينول للكولستيرول الكلى بين المجموعتين (المرضى والضابطة) ولم توجد دلالة إحصائية بين معدل الالفا- توكوفيرول والكولستيرول الكلى. ولم تؤثر عملية الغسيل الكلوى (الدموى) على مستوى الرتينول بالبلازما تأثيرا ذا دلالة إحصائية ولكن على العكس بالنسبة للآلفا - توكوفيرول فقد حدث نقص فى مستواه بالبلازما بعد عملية الغسيل الكلوى (الدموى) وكان النقص ذا دلالة إحصائية.

نستخلص من هذه الدراسة أننا نحتاج إلى دراسات أخرى لتحديد ما إذا كان مرضى الغسيل الكلوى (الدموى) عرضه لخطر الإصابة بالاعراض الظاهرة لسمية فيتامين ا. كذلك فإن هؤلاء المرضى فى حاجة إلى فيتامين "هـ" كأحد مضادات الأكسدة حتى إذا كان فى مستواه الطبيعى بالدم.