

Vitamin D, inflammation and malnutrition in prevalent haemodialysis patients – is there a link?

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ABSTRACT

The malnutrition, inflammation and atherosclerosis (MIA) syndrome is associated with increased morbid-mortality in haemodialysis patients.

The aim of this cross-sectional study was to evaluate the relationship between hydroxyvitaminD₃ [25(OH)D₃] and 1,25-dihydroxyvitaminD₃ [1,25(OH)₂D₃] serum levels and serum markers of inflammation, malnutrition and anaemia in haemodialysis patients.

Laboratory data considered were C-reactive protein (CRP), serum albumin, ferritin, haemoglobin, calcium, phosphorus, intact PTH, [25(OH)D₃] and [1,25(OH)₂D₃] serum levels.

We studied 198 prevalent haemodialysis patients with mean age (±SD) of 62.5±15.3 years, 51.5% male, 27% diabetic, mean HD time 43.2±39.3 months. Half of the patients were taking vitamin D, 14% oral calcitriol and 86% IV paricalcitol. All were dialysed with high flux helixone filters, ultrapure water dialysate and on-line haemodiafiltration. Univariate and multivariate analysis were performed and a *p*<0.05 was considered significant.

25(OH)D₃ mean level was 22.56±15.96ng/ml and 1,25(OH)₂D₃ mean level 6.35±7.63pg/ml.

On univariate and multivariate analysis, 25(OH)D₃ was negatively correlated with CRP (*r*=-0.22; *p*=0.002; *p*=0.04), darbepoetin alfa dose (*r*=-0.23; *p*=0.004; *p*=0.04) and positively with albumin (*r*=0.24; *p*=0.001; *p*=0.01) and haemoglobin (*r*=0.15; *p*=0.04; *p*=0.02).

These results suggest that 25(OH)D₃ deficiency may play a role in increased inflammation, malnutrition and high morbid-mortality observed in haemodialysis patients.

Key-Words:

Haemodialysis; MIA syndrome; vitamin D.

INTRODUCTION

Chronic inflammation is very common in chronic kidney disease stage 5d¹ (CKD5d) and has been identified as playing a key role in atherosclerotic cardiovascular disease²⁻⁵. The development of inflammation in dialysis is multifactorial and includes factors both related and unrelated to dialysis^{1,6}. The lack of renal function *per se* may decrease the clearance of pro inflammatory cytokines such as interleukin 1, interleukin 2, interleukin 6 and tumour necrosis factor

and enhance overall inflammatory responses while the vascular congestion may increase the permeability of the gastrointestinal tract leading to endotoxaemia and in turn the release of pro inflammatory cytokines and the heightened risk of co morbid conditions, including hypercatabolic state or dialysis access infections, are also a constant in these patients⁵. Further, dialysis treatment itself can also carry additional risk factors for inflammation, including the exposure to dialysis membranes (less biocompatible ones) or to non ultrapure dialysate⁵.

Recent studies have suggested that inflammation plays a significant role in malnutrition aetiology (by the promotion of a catabolic state) and atherosclerosis in uraemic patients. This link has led to the so-called MIA (Malnutrition-Inflammation-Atherosclerosis) syndrome⁷⁻⁹. This syndrome describes the relationship between these three parameters based on the raised levels of pro inflammatory cytokines². The poor food intake that results from it can aggravate the anaemia, but the decreased haemoglobin of these patients may also be the result of inflammatory cytokine activation¹⁰. Several mechanisms have been proposed for cytokine-induced anaemia, including intestinal bleeding, impaired iron metabolism, suppression of bone marrow erythropoiesis and of erythropoietin production¹¹. Inflammation, malnutrition and atherosclerosis may explain a large part of the exceptionally high mortality rate in dialysis patients¹².

Unfortunately, there are no proven established guidelines for the treatment of chronic inflammation in CKD5d patients.

In addition to the known effects on bone metabolism and calcium-phosphate metabolism, vitamin D and its synthetic analogues, through the activation of its specific receptors, are responsible for other actions in several tissues¹³. These pleiotropic effects include anti proliferative and pro differentiative effects^{14,15} which play a role in many different fields, including oncology, dermatology and infectious diseases^{16,17}.

The role of vitamin D metabolism has also been involved in the regulation of blood pressure through inhibition of the renin-angiotensin pathway¹⁸ and in the improvement of erythropoiesis by the direct effect on erythroid precursor proliferation¹⁹. Further

to that, the active form of vitamin D can also restore EPO responsiveness by controlling elevated PTH.

Experimental studies with macrophages, vascular smooth muscle cells and vascular endothelial cells suggest that vitamin D has anti atherosclerotic effects^{20,21} and, *in vivo*, its administration improves immune functions^{5,22} and normalises inflammatory reactions^{13,23}.

These results show that therapy with activated forms of vitamin D may open up a new opportunity to reduce the chronic inflammatory status observed in uraemic patients.

The aim of this study was to evaluate the relationship between hydroxyvitamin D₃ [25(OH)D₃] and 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] serum levels and serum markers of inflammation (C-reactive protein, ferritin), malnutrition (albumin) and anaemia (haemoglobin and darbepoetin dose).

■ PATIENTS AND METHODS

■ Study design

This was an observational, cross sectional study of a cohort of chronic prevalent haemodialysis patients treated according to the KDOQI guidelines.

■ Population

We evaluated 198 prevalent HD patients with a mean age (±SD) of 62.5±15.3 years, 51.5% males, 27% diabetic, with mean HD time of 43.2±39.3 months. All patients were dialysed with high flux helixone membranes (Fresenius Medical Care®), ultrapure water dialysate (endotoxine free, measured by Chromogenic Kinetic LAL assay) and on-line haemodiafiltration (pre-dilution, 250ml/min of reinfusion).

Half (49.5%) of the patients were taking vitamin D receptor activators (VDRA), 14% oral calcitriol, (mean dose 1.1±0.5 µg/week) and 86% IV paricalcitol, (mean dose of 7.3±4.3 µg/week).

Biochemical analysis

Serum levels of 25(OH)D₃ and 1,25(OH)₂D₃, as well as serum levels of C-reactive protein (CRP), albumin, ferritin, haemoglobin (Hb), calcium, phosphorus, total intact parathyroid hormone (iPTH) and bone alkaline phosphatase (bAP) were measured at the same time and the dose of darbepoetin was collected.

Biochemical analysis including albumin, ferritin, Hb, calcium, phosphorus and bone alkaline phosphatase was performed using standard methods. iPTH was measured by immunochemiluminescence using a second generation assay and the normal range is 10 to 65 pg/ml. 25(OH)D₃ and 1,25(OH)₂D₃ were determined using radioimmunoassay provided by IDS (Baldon, UK). The assay, after an extraction procedure, is carried out with anti – 25(OH)D₃ and anti – 1,25(OH)₂D₃ ovine antibodies. This is followed by a separation phase with anti ovine IgG antiserum. Intra and inter assay variability are 5 and 8%. The normal range for 25(OH)D₃ is 10 to 60 ng/ml and for 1,25(OH)₂D₃ 20 to 46 pg/ml.

We defined for 25(OH)D₃ normal serum levels those >30 ng/ml, deficiency serum levels those between 15 and 30 ng/ml and insufficiency serum levels those <15 ng/ml. For 1,25(OH)₂D₃ we defined normal serum levels those ≥20 pg/ml and deficiency serum levels those <20 pg/ml.

Statistical analysis

Data are presented as mean±SD values for normally distributed variables or as frequencies for categorical variables.

Independent variables were compared using the Mann Whitney and the chi square tests. Correlations between variables were made by the Spearman test for univariate analysis and by logistic regression for multivariate analysis (confidence interval of 95%), with forward method. Variables entered in multivariate analysis were albumin, Hb, CRP and darbepoetin dose.

All tests were performed using the SPSS system 14.0 (SPSS Inc., Chicago, IL) and a p<0.05 was considered statistically significant.

RESULTS

The baseline clinical and biochemical characteristics are summarised in Table I.

Table I

Baseline clinical and biochemical characteristics of the population

Variable	Patients (n=198)
Age (years)	62.7±15.3
Male gender	102 (51.5%)
HD duration (months)	42.9±39.3
Diabetes	53 (27%)
Vitamin D therapy	98 (49.5%)
Calcium (mg/dL)	8.7±0.7 (6.8 - 10.7)
Phosphorus (mg/dL)	4.5±1.4 (1.7 - 8.7)
Calcium x Phosphorus (mg/dL) ²	48.6±15.5 (16.5 - 83.2)
Intact PTH (pg/mL)	260.1±338.9 (3 - 4461)
Bone alkaline phosphatase (µg/L)	16.8±13.2 (1.1 - 100.3)
Darbepoetin dose (µg/week)	37.1±35.9 (0.0 - 200)
Haemoglobin (g/dL)	12.45±1.3 (7.8 - 15.3)
C-reactive protein (mg/dL)	0.91±1.65 (0.1 - 17.4)
Albumin (g/dL)	4.22±0.37 (3.1 - 5.1)
Ferritin (mg/dL)	439.7±252.7 (1242 - 23.1)
25-(OH) D ₃ (ng/mL)	22.6±15.9 (2.4 - 152.8)
1,25-(OH) ₂ D ₃ (pg/mL)	6.4±7.6 (0.04 - 49.1)

Serum levels of 25(OH)D₃ and 1,25(OH)₂D₃ were both surprisingly low. Most of the patients (50.5%) were insufficient for 25(OH)D₃ and only 17.7% were in the normal range >30 ng/ml. 91.4% of the patients presented 1,25(OH)₂D₃ deficiency, defined by a serum level below 20 pg/ml. The serum levels of 25(OH)D₃ and 1,25(OH)₂D₃ were positively correlated (r=0.18, p=0.01).

Using the Pearson correlation, serum levels of CRP were negatively correlated with serum levels of albumin (r=-0.27, p<0.0001) and positively correlated with darbepoetin dose (r=0.29, p<0.0001). Albumin besides CRP was also negatively correlated with darbepoetin dose (r=-0.24, p=0.003). Hb was also negatively correlated with darbepoetin dose (r=-0.29, p<0.0001).

We evaluated hypothetical correlations between 25(OH)D₃ serum levels (normal, insufficient and deficient), 1,25(OH)₂D₃ serum levels (normal, deficient) and several clinical and laboratorial variables (Tables II and III). A significant negative association was found between 25(OH)D₃ and age

Table IIComparison of 25(OH)D₃ serum levels and some clinical and laboratory variables

25(OH)D ₃	Deficiency < 15 ng/ml (n=35)	Insufficiency 15 - 30 ng/ml (n=63)	Normal > 30 ng/ml (n=100)	p
Age (years)	67.5±15.2	61.6±15.2	57.1±13.9	0.003
HD duration (months)	50.8±44.5	39.7±38.3	37.9±29.8	NS
Diabetes	33.3%	22.0%	31.4%	NS
VDRA therapy	42.9%	55%	45.7%	NS
Calcium	8.8±0.7	8.8±0.7	8.6±0.7	NS
Phosphorus	4.4±1.4	4.5±1.4	4.6±1.5	NS
Intact PTH	274.2±554.5	257.5±167.4	242.3±150.4	NS
Bone alkaline phosphatase	15.6±14.8	16.5±10.9	20.1±16.1	NS
Haemoglobin	1.1±1.3	0.9±2.0	0.5±0.7	NS
C-reactive protein	12.3±1.3	12.5±1.3	12.7±1.3	NS
Albumin	4.1±0.4	4.2±0.3	4.3±0.3	0.02
1,25(OH) ₂ D ₃	4.9±7.7	7.6±7.9	5.5±6.1	0.01

VDRA – vitamin D receptor activators

Table IIIComparison of 1,25(OH)₂D₃ serum levels and some clinical and laboratory variables

1,25(OH) ₂ D ₃	Deficiency <20 pg/ml (n=181)	Normal >20 pg/ml (n=17)	p
Age (years)	62.4±15.6	65.7±12.8	NS
HD duration (months)	42.5±38.0	47.4±52.3	NS
Diabetes	28.2%	17.6%	0.03
VDRA therapy	50.8%	35.3%	<0.001
Calcium	8.7±0.7	8.8±0.6	NS
Phosphorus	4.5±1.4	4.0±1.4	NS
iPTH	261.5±352.7	245.4±121.4	NS
Bone alkaline phosphatase	17.0±13.5	15.1±9.7	NS
C-reactive protein	0.9±1.7	0.5±0.9	NS
Haemoglobin	12.4±1.3	12.5±1.2	NS
Albumin	4.2±0.4	4.3±0.3	0.03
25-(OH) D ₃	22.6±16.5	22.5±8.9	NS

and a positive association between 25(OH)D₃ and albumin. We also found a significant association between lower serum levels of 1,25(OH)₂D₃ and the presence of diabetes. Patients treated with paricalcitol had lower 1,25(OH)₂D₃ serum levels. Also for 1,25(OH)₂D₃ serum levels, a positive association with albumin was found.

On univariate analysis, 25(OH)D₃ was negatively correlated with age ($r=-0.31$, $p<0.001$), presence of diabetes ($r=-0.16$, $p=0.03$), CRP ($r=-0.22$; $p=0.002$), darbepoetin dose ($r=-0.23$, $p=0.004$), and positively with albumin ($r=0.24$, $p=0.001$) and with Hb

($r=0.15$, $p=0.04$). Serum levels of 1,25(OH)₂D₃ were also negatively correlated with darbepoetin dose ($r=-0.19$, $p=0.01$).

On multivariate analysis, 25(OH)D₃ showed a positive correlation with albumin ($p=0.01$) and Hb ($p=0.02$) and negative correlation with age ($p=0.01$), diabetes ($p=0.03$), CRP ($p=0.04$) and darbepoetin dose ($p=0.04$). 1,25(OH)₂D₃ was negatively correlated with darbepoetin dose ($p=0.03$).

There was no correlation between 25(OH)D₃ or 1,25(OH)₂D₃ and ferritin.

Table IV

Univariate analysis correlating 25(OH)D₃ and other variables

	25(OH)D ₃	
	r	p
Age	-0.31	<0.001
Diabetes	-0.57	0.027
Albumin	0.24	0.001
Haemoglobin	0.15	0.042
Darbepoetin dose	-0.16	0.004
C-reactive protein	-0.22	0.002
1,25(OH) ₂ D ₃	0.18	0.013

Table V

Univariate analysis correlating 1,25(OH)₂D₃ and other variables

	1,25(OH) ₂ D ₃	
	r	p
Darbepoetin dose	0.19	0.01
25(OH)D ₃	0.18	0.01

Table VI

Multivariate analysis correlating 25(OH)D₃ and other variables

Dependent variable	Independent Variable	β	95% CI	p	R ²
25(OH)D ₃	Albumin	0.19	2.04 to 14.28	0.01	0.121
	Haemoglobin	0.16	1.31 to 3.76	0.02	
	C-reactive protein	-0.13	-2.61 to -0.09	0.04	
	Darbepoetin dose	-0.16	-0.11 to -0.002	0.04	
					0.27

Table VII

Multivariate analysis correlating 1,25(OH)₂D₃ and other variables

Dependent variable	Independent variable	β	95% CI	p	R ²
1,25(OH) ₂ D ₃	Darbepoetin dose	-0.18	-0.076 to -0.004	0.03	0.032

DISCUSSION

This study showed that 25(OH)D₃ insufficiency or deficiency is associated to increased inflammation, anaemia and malnutrition observed in haemodialysis patients.

The most important cause of death in CKD5d is cardiovascular disease²⁴ and traditional cardiovascular risk factors alone do not fully explain the cardiovascular disease outcome in these patients²⁵. Inflammation, a non traditional risk factor, plays a key role in atherosclerotic cardiovascular disease^{7,8,25-27} and if this inflammatory status somehow decreased, our patients would probably live longer.

Uraemia is an inflammatory state and the degree of inflammation can be measured by serum levels

of acute phase proteins. These proteins are usually divided into positive (such as CRP, ferritin, fibrinogen) and negative (albumin, fetuin A, transferrin) inflammatory serum markers. Albumin and transferrin are also frequently used as nutritional markers in uraemic patients.

Epidemiological studies have showed that the CRP levels are increased in CKD5d patients²⁸ and these serum levels are independent predictors of all-cause and cardiovascular mortality^{3,4,27,29,30}.

Poor nutritional status also predicts a high risk for morbidity and mortality in haemodialysis patients³¹⁻³³ and albumin serum levels can reflect the inflammatory status as well as the nutritional status of these patients. Several mechanisms for this association (inflammation vs. malnutrition) have

been suggested and include appetite suppression^{34,35} and the promotion of a catabolic state by some pro inflammatory proteins. CRP independently predicts decrease in fat mass over time in haemodialysis patients⁹.

In our study, CRP and albumin were associated and this result probably illustrates the interrelationship between these two markers.

The increased levels of pro inflammatory cytokines and poor food intake can induce anaemia. Several studies have shown the benefits of Hb target of 11 to 12 g/dl³⁶, and levels <9 g/dl were associated with increased mortality^{37,38}. The most common factors that are associated with failure to achieve Hb target level, despite erythropoietin use, include iron deficiency, hospitalisations, catheters, hyperparathyroidism, hypoalbuminaemia and elevated CRP. In a cross sectional study, an association between erythropoietin resistance and inflammatory markers (CRP, IL 6) was found³⁹.

We found no correlation between Hb levels and inflammatory markers including CRP or albumin. In spite of that, we found a positive correlation between darbepoetin dose and CRP serum levels as well as a negative correlation between darbepoetin dose and albumin serum levels. So, more inflammation is associated to higher doses of therapy with an erythropoiesis stimulator in order to maintain Hb levels in the expected range.

There are many studies showing laboratory and epidemic association between inflammation and a worse outcome in CKD5d patients. We cannot say the same for studies that evaluate benefits of anti inflammatory therapies.

There are no clear guidelines for the treatment of chronic inflammation in ESRD patients, but several strategies, including minimising exposure to infections, treating chronic heart failure, dialysing patients with biocompatible membranes and the use of ultrapure water have been implemented by many groups.

As to diet supplementation, experimental studies with macrophages, vascular smooth muscle cells and vascular endothelial cells suggest that vitamin D has anti atherosclerotic effects^{20,21} and, *in vivo*, its

administration improves immune functions^{5,22} and normalises many inflammatory reactions^{13,23}. A recent study correlated 25(OH)D₃ and some markers of arteriosclerosis and endothelial dysfunction in CKD5d patients²⁰. Vitamin D was also implicated in the improvement of erythropoiesis by the direct effect on erythroid precursor proliferation¹⁸.

Although we are living in a sunny country, the majority of our patients had 25(OH)D₃ insufficiency and 1,25(OH)₂D₃ deficiency and these two levels were correlated.

The very low levels of calcidiol in this specific population were unexpected. The serum calcidiol concentration is dependent on dermal synthesis and, to a small percentage, the dietary intake of vitamin D^{40,41}. Living in a sunny country, we expected higher values of this vitamin. But we are studying an old population, with mean age of 62.5±15.3 years. The elderly have inadequate sun exposure, the skin of those older than 70 years of age does not convert vitamin D effectively and they also have a poor intake of food (“tea and toast diet”)⁴². Our results also showed that serum levels of 25(OH)D₃ were negatively associated and correlated with age and positively associated and correlated with serum levels of albumin, which, in addition to being an acute phase protein, is also a nutritional marker.

Deficiency in 1,25(OH)₂D₃ was expected as these are prevalent haemodialysis patients, with no renal function and with uraemic toxins in circulation⁴¹. As plasma calcitriol (1,25(OH)₂D₃) concentration is not only a function of the activity of renal tubular cells enzymes, but also of the availability of calcidiol (25(OH)D₃)^{23,24}, these results are consistent.

One interesting data was that patients treated with IV paricalcitol had lower 1,25(OH)₂D₃ serum levels. This could be explained by the fact that paricalcitol is 19-nor-1,25vitD and we are measuring serum levels of 1,25(OH)₂D₃. Besides, perhaps when we give IV paricalcitol to a patient, the organism acts by negative feedback, reducing 1,25(OH)₂D₃ production.

In our study we found a correlation between higher serum levels of 25(OH)D₃ and higher serum levels of albumin and Hb, and between higher serum levels of 25(OH)D₃ and lower serum levels of CRP and lower dose of darbepoetin. This association

between higher 25(OH)D₃ serum levels and lower CRP serum levels could mean that 25(OH)D₃ has anti-inflammatory properties.

In our population, 1,25(OH)₂D₃ serum levels were only correlated with lower doses of darbepoetin.

CONCLUSIONS

As the vast majority of the uraemic patients seem to be deficient (or at least insufficient) in 25(OH)D₃ and 1,25(OH)₂D₃ supplementation with these two forms of vitamin D is probably indicated. These results are in accordance with other observational studies, but must be confirmed by prospective randomised trials.

Conflict of interest statement. None declared.

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