

# The effect of Vitamin D administration on treatment of anemia in end-stage renal disease patients with Vitamin D deficiency on hemodialysis: A placebo-controlled, double-blind clinical trial

Afsoon Emami Naini, Zahra Parin Hedaïati, Danial Gholami<sup>1</sup>, Amir Hossein Pezeshki<sup>2</sup>, Firoozeh Moïnzadeh

Departments of Nephrology and <sup>1</sup>Internal Medicine, <sup>2</sup>Isfahan Medical Students Research Center (IMSRC), Isfahan University of Medical Sciences, Isfahan, Iran

**Background:** Chronic kidney disease is a progressive and irreversible loss of renal function. Anemia is one of the main complications of end-stage renal disease (ESRD) which is linked closely with other complications of the disease. The primary therapy for anemia in these patients is erythropoietin (EPO). The goal of this study was to find the effect of Vitamin D administration in addition to the appropriate dose of EPO in ESRD patients with Vitamin D deficiency. **Materials and Methods:** This was a double-blind clinical trial on 64 ESRD patients undergoing hemodialysis in Amin and Noor Hospitals of Isfahan, Iran. The patients were divided into two groups of control and intervention. The intervention group was given Vitamin D supplements and the control group received placebo. The required dose of EPO to reach the target hemoglobin (Hb) was measured and statistically analyzed. **Results:** A total number of 32 females and 32 males were included in this study. All the patients in the treatment group were aged between 18 and 76 and the patients in the control group were aged between 21 and 76 years old. There was a significant statistical relationship between Vitamin D administration and the required dose of EPO in both groups ( $P = 0.013$ ). However, there was no correlation between the concentration of Hb and serum Vitamin D levels. **Conclusion:** Based on the main finding of this study, the relationship between Vitamin D administration and required dose of EPO seems that the predicted dose of Vitamin D prescribing strategy in Kidney Disease Outcomes Quality Initiative guidelines is not adequate to achieve normal serum Vitamin D in ESRD patients.

**Key words:** Anemia, chronic kidney disease, end stage renal disease, erythropoietin, Vitamin D deficiency

**How to cite this article:** Naini AE, Hedaïati ZP, Gholami D, Pezeshki AH, Moïnzadeh F. The effect of Vitamin D administration on treatment of anemia in end-stage renal disease patients with Vitamin D deficiency on hemodialysis: A placebo-controlled, double-blind clinical trial. *J Res Med Sci* 2015;20:745-50.

## INTRODUCTION

Anemia is one of the most important complications of end-stage renal disease (ESRD), especially in patients on regular hemodialysis.<sup>[1]</sup> In these patients, anemia can lead to other morbidities such as a decrease in the quality of life and increased cardiovascular complications and also increased mortality rate. This is why an understanding of the mechanisms and factors involved in anemia and its treatment on ESRD patients is crucial.<sup>[2-5]</sup>

The main cause of anemia in these patients is lowered erythropoietin (EPO) secretion from kidneys but there are also other suggested factors involved in the development and severity of it such as Vitamin D deficiency. The primary treatment of anemia in ESRD patients are EPO stimulating agents (ESA) including injectable EPO.<sup>[1]</sup> Although a gradual hyporesponsiveness might develop in 10% of these patients so that they may need an increased dosage of EPO to maintain their hemoglobin (Hb) concentration in the aimed range.<sup>[6-9]</sup>

Even though there are several different mechanisms suggested for EPO hyporesponsiveness such as Iron

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

**For reprints contact:** reprints@medknow.com

### Access this article online

Quick Response Code:	Website: <a href="http://www.jmsjournal.net">www.jmsjournal.net</a>
	DOI: ****

**Address for correspondence:** Dr. Danial Gholami, Department of Internal Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.  
E-mail: danial\_gholami92@yahoo.com

**Received:** 18-03-2015; **Revised:** 27-04-2015; **Accepted:** 11-09-2015

deficiency, B12 deficiency, secondary hyperparathyroidism, chronic inflammation, and malignancies; but none of these can thoroughly explain this phenomenon in ESRD patients.<sup>[10-13]</sup> On the other hand, it has been proved that an increased hyporesponsiveness to EPO is associated with increased rate of mortality.<sup>[14-17]</sup>

In recent years, a growing number of studies have suggested that Vitamin D deficiency could be one of the risk factors involved in this hyporesponsiveness.<sup>[18-20]</sup> The prevalence of Vitamin D deficiency (serum concentration smaller than 30 ng/dl) in ESRD patients is a lot higher than in the other populations, so that 75% of these patients are estimated to suffer from Vitamin D deficiency. This could be due to lowered renal hydroxylase enzyme, decreased sun exposure, and malnutrition.<sup>[20-22]</sup> It is also known that Vitamin D along with EPO has a synergic effect, the stimulation of bone marrow precursor cells, and red blood cell formation. Furthermore, several tissues express the Vitamin D receptor and are hence able to transform it into the active form, so the level of Vitamin D could be effective on the function of these tissues.<sup>[21,23,24]</sup>

Although previous studies have worked on the correlation between Vitamin D administration in ESRD patients and required doses of EPO; there is no available double-blind, placebo-controlled clinical trial on the effect of Vitamin D deficiency on the required dose of EPO in ESRD patients on hemodialysis yet.

## MATERIALS AND METHODS

### Participants

This study is a double-blind, randomized, control clinical trial (placebo control) on ESRD patients on hemodialysis in Amin and Noor and Hospitals (Isfahan, Iran) between the years 2013 and 2014. The patients were all between 18 and 80 years of age suffering from anemia and Vitamin D deficiency and were referred to the above hospitals. The project was also registered at IRCT: IRCT201505152417N17.

### Study design

The ESRD patients needing hemodialysis in Amin and Noor hospitals were entered in the study. All of them were tested for Hb concentration and those with a measured amount lower than 11 g/dl were accepted for the study as anemic cases. Considering other causes of anemia, serum ferritin, iron, and total iron-binding capacity were checked. All of these anemic patients were tested for Vitamin D level and the levels smaller than 30 ng/dl were considered as Vitamin D deficient patients. The patients with anemia (Hb con. <11 g/dl) and Vitamin D deficiency<sup>[25]</sup> were assigned to two groups using random sampling method. The angiotensin-converting enzyme inhibitor and angiotensin II receptor blockers prescribed

patients were compared in two groups and sex- and age-matching was done. The minimum volume of each group was calculated to be 32 patients. The intervention group were administered a 50,000 IU Vitamin D pear weekly for 12 weeks and then every three weeks until each patient had taken 650,000 IU Vitamin D in the course of the study. The treatment was performed in a 4-month period and the control group was given placebo pearls in exactly the same pattern. The Vitamin D and placebo pearls were both prepared by Zahravi Pharmaceutical Co. (Tabriz, Iran). The Vitamin D level was measured for both groups at the beginning of the study and after 4 months, though the study and the Hb concentration was tested monthly. The weekly and cumulative EPO dosage was also recorded for each patient. The treatment approach for anemia was decided using Kidney Disease Improving Global Outcomes guidelines for both groups.<sup>[23]</sup>

### Criteria for entering the study

1. Patients must have been between 18 and 80 years of age.
2. Patients must have suffered from ESRD and on hemodialysis for 3 months at least.
3. Patients must have had an Hb con. lower than 11 g/dl.
4. Patients must have had Vitamin D deficiency (25[OH] Vitamin D <30 ng/ml).

### Criteria to be omitted from the study

1. Patients with serum Calcium of over 9.5.
2. Patients with serum Phosphate of over 5.5.
3. Noncompliant patients.
4. Patients who were treated by medications that could affect their Hb level such as immunosuppressants.

### Statistical methods

The results analyzed and showed in tables and graphs using SPSS version 22 (SPSS Inc, Chicago, IL) and descriptive results were obtained. A multivariate analysis of variance (ANOVA) was done for Hb levels, serum Vitamin D values, and EPO dosages before and after the study in two groups. Pearson statistical test was used to determine the correlation between age, sex, body mass index (BMI), and EPO dosages. Moreover, also two-way ANOVA was done to compare EPO dosages according to patient's sex in two groups before and after the intervention.  $P < 0.05$  was considered as statistically significant.

### Ethical considerations

This study was approved by Research Ethics Committee of the Research Department (research project number 292280). Considering that this study is a clinical trial, a specific questionnaire was filled and approved by the Research Department. Patients were completely informed about the process of the study, possible side effects, and other considerations, and were then volunteered to participate the study.

## RESULTS

### The patients characteristics and demographic data

In this double-blind clinical trial, 64 ESRD patients on hemodialysis in Isfahan's Amin and Noor Hospitals in 2013 and 2014 were studied into two groups of intervention and control [Figure 1]. The sex ratio and age range was the same in both groups. The age was between 20 and 78 years old and 24 patients (37.5%) were between 51 and 60 years [Table 1].

### Effects of Vitamin D administration on hemoglobin levels, erythropoietin dosage, and serum (OH) Vitamin D levels

The Hb and Vitamin D levels were measured in both groups before and after the treatment and the data are shown based

on gender in Table 2. Before the study, seven patients in the control group and five patients in the intervention group had serum (OH) Vitamin D levels under 10 ng/dl and the others had 20-30 ng/dl serum Vitamin D levels but after the study, none of them were under 10 ng/dl. Average of Hb levels of patients in the control group before and after the study was 9.19 and 10.14, respectively, and in the intervention group it was 9.93 and 11.09, respectively. Vitamin D toxicity was not seen in any of the participants during and after the study.

Using three variant ANOVA and co-variance, the effects of sex, BMI, and age on Hb and Vitamin D levels, and the required dose of EPO were controlled in both groups

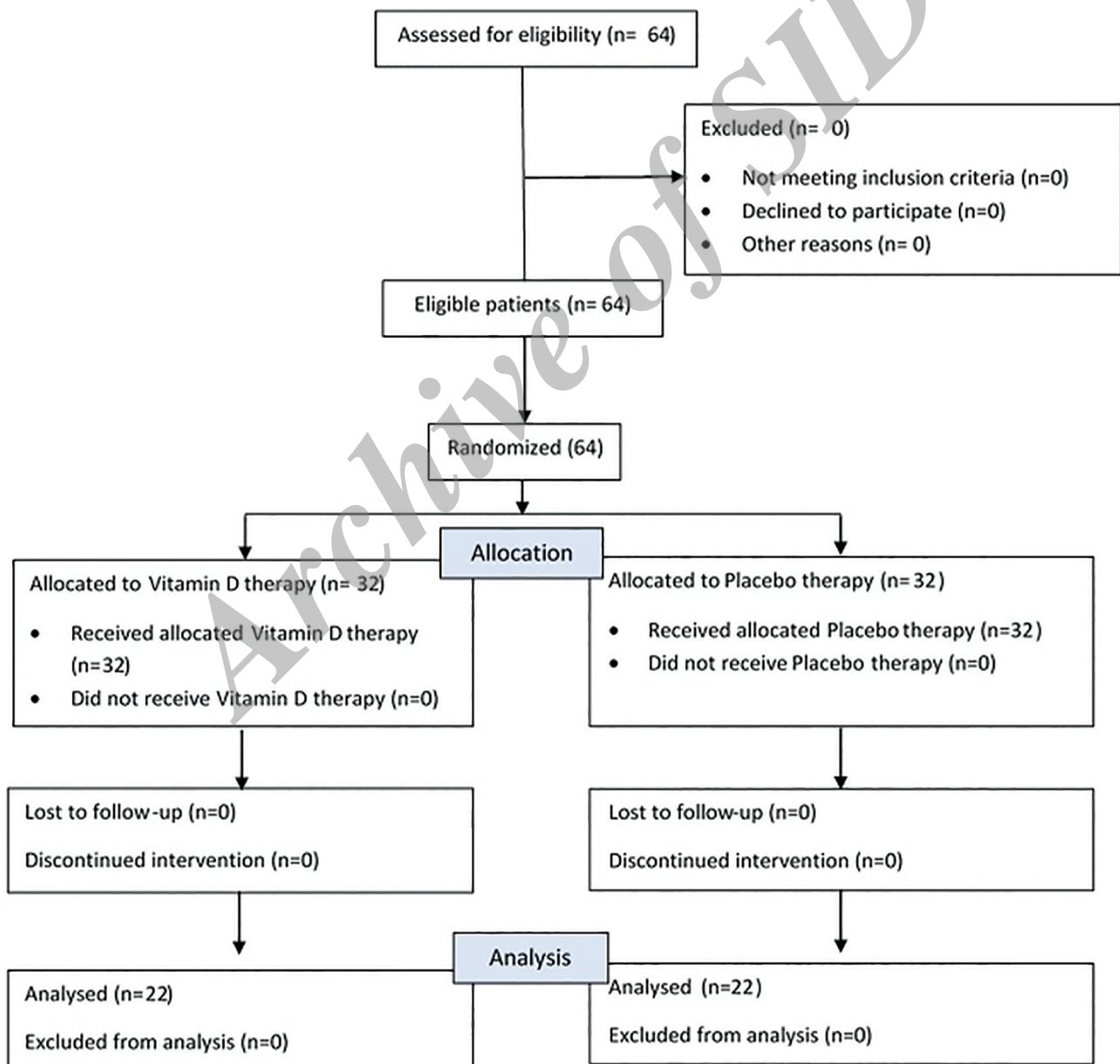


Figure 1: Clinical trial diagram of patients

and no significant correlation was detected between these three variants and the Hb and Vitamin D levels (power of statistical test: 80%).

Although a significant correlation was found between BMI and the required dose of EPO in both groups ( $P = 0.004$ ,  $R = 0.5$ ), the higher the BMI, more the required dose of EPO [Table 3].

A comparison among the average Hb concentration change, the intervention, and control groups showed no significant difference; with 80% power of statistical test [Table 4].

The change in Vitamin D level also demonstrated a statistically significant difference between the two groups ( $P < 0.001$ ). Average of Vitamin D levels had a 58.04 ng/ml increase in the intervention group; the amount of which was only 1.25 in the control group [Figure 2].

A comparison between the required doses of EPO in the two groups showed a decrease in the treatment group ( $P < 0.001$ ) while its changes was not statistically significant in the control group [Figure 3].

## DISCUSSION

The goal of this study was to approve the effect of Vitamin D administration on the dose of EPO need in ESRD patients to achieve the target Hb concentration while on hemodialysis, the serum level of Vitamin D was elevated to an average of 79 ng/ml in all of the patients in the treatment group

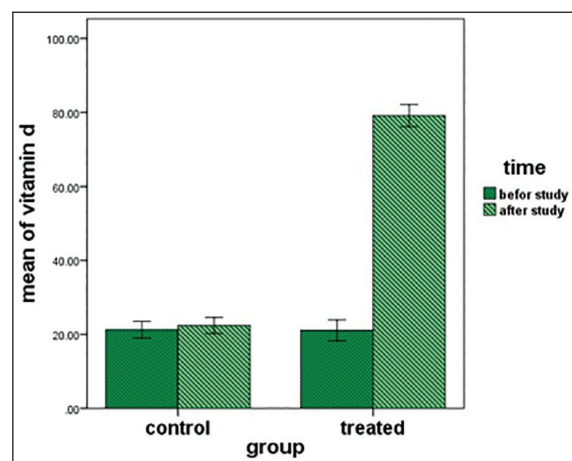
after taking 650,000 IU of Vitamin D. The correlation between BMI and EPO dosages is because of this fact that in patients with more weight, the need for almost all drugs is more than the patients with lower weight. After the administration of Vitamin D, the treatment group showed a significant decrease in the required dose of EPO while this same parameter had a slight but not statistically significant increase in the control group. Since several different factors are considered to be involved in hyporesponsiveness of ESRD patients to ESA treatments, such as inflammation, secondary hyperparathyroidism, etc., here the effect of Vitamin D administration in the treatment and control group was predictable.

In a study by Kumar *et al.*<sup>[25]</sup> a group of ESRD patients were administered by 200,000-350,000 IU of Vitamin D and during the course of the study, 44% of the patients had reached a Vitamin D level of over 30 ng/ml, while in the study by Saab *et al.*<sup>[26]</sup> the administration of 350,000 IU throughout 6 months could help at least 95% of the patients to achieve the target level of over 30 ng/ml. The difference between these two results is suggested to be due to the different seasons of study and the effect of sunlight on Vitamin D level. Nevertheless, we decided to give Vitamin D to the patients as suggested by Kumar *et al.*; and this seemed to be a sufficient amount for all the patients to surpass the target level of Vitamin D. In addition, the

**Table 1: Basic characteristics of the study population**

Variables	Mean/SD		Minimum		Maximum	
	Control	Treated	Control	Treated	Control	Treated
Age (year)	62/21	60/19	21	18	78	76
BMI (kg/m <sup>2</sup> )	26.2/6	27.4/8	18.1	17.2	35.1	34.8
Hb (mg/dl)	9.19/1.42	9.93/1.65	7.2	7.4	11.2	12.2
DM	14	10				
HTN	12	17				

BMI = Body mass index; SD = Standard deviation; DM = Diabetes mellitus; HTN = Hypertension



**Figure 2: Vitamin D levels before and after the study in both groups**

**Table 2: Serum 25(OH) Vitamin D levels of the patients before and after the study in both groups**

Group	Sex	Before study				After study				n	P
		Mean	SD	CI		Mean	SD	CI			
				Lower bound	Upper bound			Lower bound	Upper bound		
Control	Male	20.9	6.53	17.22	24.58	21.74	5.98	18.06	25.41	16	>0.05
	Female	21.64	6.5	17.96	25.31	23.06	6.49	19.39	26.74	16	
Treated	Male	22.12	7.72	18.45	25.80	79.24	7.59	75.57	82.92	16	<0.001
	Female	20.04	8.52	16.36	23.71	79	9.41	75.32	82.68	16	
P		>0.05				<0.05					

SD = Standard deviation; CI = Confidence interval

Vitamin D elevation response to this administration was the same in male and female patients. The Hb level in both groups was maintained at 10-11 in both groups throughout the study.

Kumar *et al.* found that the required dose of EPO in hemodialysed ESRD patients decreases with the administration of Vitamin D. Furthermore, Kiss *et al.* discovered correlations between Vitamin D level and Hb concentration and the required dose of EPO in ESRD patients, while Porter *et al.* found no such relationship which is in contrast to the findings of the current study.<sup>[1,27]</sup>

The mechanism involved in the effect of Vitamin D on the required EPO dosage in ESRD patients is unknown but a number of possible mechanisms could be suggested. First, both *in vitro* and *in vivo* studies have shown the

effect of Vitamin D on bone marrow precursor cells and their efficacy in the formation of red blood cells. In conjunction with that, this vitamin is known to decrease inflammation in the tissues which might decrease the hyporesponsiveness to ESAs to some extent. And at last, the fact that many types of tissues express the Vitamin D receptors such as parathyroid and erythroid precursor cells, both of which are known as the tissues involved in ESA hyporesponsiveness, suggests that the modulation of these tissues with Vitamin D might increase the response to EPO treatment.<sup>[1,26,27]</sup>

The strong point of this study is its methodology which makes it the first, double-blind, case-control clinical trial on this matter.

Several downsides could also be mentioned such as the study having been done in a hemodialysis center which means that there were no patients on peritoneal dialysis in the study. In addition, the number of cases was small and we suggest that studies with larger sample populations of ESRD patients might be helpful to further clear the matter.

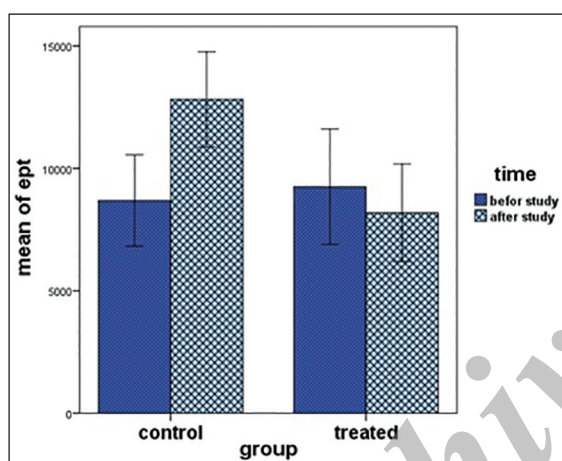


Figure 3: Dosages of erythropoietin in both groups before and after the study

Table 3: The association between the required doses of erythropoietin and patients BMI

Variables	Degree of freedom	F	P	Difference of levels
Corrected model	4	5.568	0	-
BMI	1	8.848	0.004	-
Time	1	2.385	0.125	1531.25
Group	1	2.822	0.096	-1677.597
Time - Group	1	6.842	0.01	-

BMI = Body mass index

## CONCLUSION

We state that the administration of Vitamin D in ESRD patients could help reduce the required dose of EPO during the courses of hemodialysis. Also, the administration of this vitamin-based supplements on the current guideline helps almost all patients to achieve its sufficient level. Basically, the treatment with Vitamin D in ESRD patients could be helpful due to its very small side effects and its probable efficacy in reducing other complications such as anemia.

## Acknowledgments

This study was supported by Isfahan University of Medical Sciences (Research project number; 292280).

## Financial support and sponsorship

This study was funded by Isfahan University of Medical Sciences.

## Conflicts of interest

There are no conflicts of interest.

Table 4: Hb levels of the patients before and after the study in both groups

Group	Sex	Before study				After study				n	P
		Mean	SD	CI		Mean	SD	CI			
				Lower bound	Upper bound			Lower bound	Upper bound		
Control	Male	9.23	1.35	8.58	9.88	10.07	0.81	9.43	10.72	16	>0.05
	Female	9.16	1.48	8.51	9.80	10.21	0.92	9.56	10.85	16	
Treated	Male	9.79	1.6	9.15	10.44	10.58	1.13	9.93	11.23	16	>0.05
	Female	10.07	1.71	9.42	10.71	11.16	1.16	10.51	11.80	16	
P		>0.05				>0.05					

SD = Standard deviation; CI = Confidence interval

## AUTHOR'S CONTRIBUTION

All authors participated in design, experiments, and gathering information and all of them have read and approved the content of the manuscript.

## REFERENCES

- Kiss Z, Ambrus C, Almasi C, Berta K, Deak G, Horonyi P, *et al.* Serum 25(OH)-cholecalciferol concentration is associated with hemoglobin level and erythropoietin resistance in patients on maintenance hemodialysis. *Nephron Clin Pract* 2011;117:c3-c378.
- Regidor DL, Kopple JD, Kovesdy CP, Kilpatrick RD, McAllister CJ, Aronovitz J, *et al.* Associations between changes in hemoglobin and administered erythropoiesis-stimulating agent and survival in hemodialysis patients. *J Am Soc Nephrol* 2006;17:1181-91.
- Foley RN, Parfrey PS, Harnett JD, Kent GM, Murray DC, Barre PE. The impact of anemia on cardiomyopathy, morbidity, and mortality in end-stage renal disease. *Am J Kidney Dis* 1996;28:53-61.
- Ishani A, Solid CA, Weinhandl ED, Gilbertson DT, Foley RN, Collins AJ. Association between number of months below K/DOQI haemoglobin target and risk of hospitalization and death. *Nephrol Dial Transplant* 2008;23:1682-9.
- Molnar MZ, Czira M, Ambrus C, Szeifert L, Szentkiralyi A, Beko G, *et al.* Anemia is associated with mortality in kidney-transplanted patients — A prospective cohort study. *Am J Transplant* 2007;7:818-24.
- Macdougall IC. Poor response to erythropoietin: Practical guidelines on investigation and management. *Nephrol Dial Transplant* 1995;10:607-14.
- van der Putten K, Braam B, Jie KE, Gaillard CA. Mechanisms of Disease: erythropoietin resistance in patients with both heart and kidney failure. *Nat Clin Pract Nephrol* 2008;4:47-57.
- Johnson DW, Pollock CA, Macdougall IC. Erythropoiesis-stimulating agent hyporesponsiveness. *Nephrology (Carlton)* 2007;12:321-30.
- Rosert J, Gassmann-Mayer C, Frei D, McClellan W. Prevalence and predictors of epoetin hyporesponsiveness in chronic kidney disease patients. *Nephrol Dial Transplant* 2007;22:794-800.
- Drüeke T. Hyporesponsiveness to recombinant human erythropoietin. *Nephrol Dial Transplant* 2001;16 Suppl 7:25-8.
- Macdougall IC, Cooper AC. Hyporesponsiveness to erythropoietic therapy due to chronic inflammation. *Eur J Clin Invest* 2005;35 Suppl 3:32-5.
- Kwack C, Balakrishnan VS. Managing erythropoietin hyporesponsiveness. *Semin Dial* 2006;19:146-51.
- Brancaccio D, Cozzolino M, Gallieni M. Hyperparathyroidism and anemia in uremic subjects: A combined therapeutic approach. *J Am Soc Nephrol* 2004;15 Suppl 1:S21-4.
- Kausz AT, Solid C, Pereira BJ, Collins AJ, St Peter W. Intractable anemia among hemodialysis patients: A sign of suboptimal management or a marker of disease? *Am J Kidney Dis* 2005;45:136-47.
- Zhang Y, Thamer M, Stefanik K, Kaufman J, Cotter DJ. Epoetin requirements predict mortality in hemodialysis patients. *Am J Kidney Dis* 2004;44:866-76.
- Locatelli F, Conte F, Marcelli D. The impact of haematocrit levels and erythropoietin treatment on overall and cardiovascular mortality and morbidity — The experience of the Lombardy Dialysis Registry. *Nephrol Dial Transplant* 1998;13:1642-4.
- Kilpatrick RD, Critchlow CW, Fishbane S, Besarab A, Stehman-Breen C, Krishnan M, *et al.* Greater epoetin alfa responsiveness is associated with improved survival in hemodialysis patients. *Clin J Am Soc Nephrol* 2008;3:1077-83.
- Ertürk S, Kutlay S, Karabulut HG, Keven K, Nergizoglu G, Ates K, *et al.* The impact of vitamin D receptor genotype on the management of anemia in hemodialysis patients. *Am J Kidney Dis* 2002;40:816-23.
- Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266-81.
- Blair D, Byham-Gray L, Lewis E, McCaffrey S. Prevalence of Vitamin D [25(OH)D] deficiency and effects of supplementation with ergocalciferol (Vitamin D2) in stage 5 chronic kidney disease patients. *J Ren Nutr* 2008;18:375-82.
- Jean G, Terrat JC, Vanel T, Hurot JM, Lorriaux C, Mayor B, *et al.* Daily oral 25-hydroxycholecalciferol supplementation for vitamin D deficiency in haemodialysis patients: Effects on mineral metabolism and bone markers. *Nephrol Dial Transplant* 2008;23:3670-6.
- Del Valle E, Negri AL, Aguirre C, Fradinger E, Zanchetta JR. Prevalence of 25(OH) vitamin D insufficiency and deficiency in chronic kidney disease stage 5 patients on hemodialysis. *Hemodial Int* 2007;11:315-21.
- National Kidney Foundation. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis* 2003;42 4 Suppl 3:S1-201.
- Holick MF. Vitamin D for health and in chronic kidney disease. *Semin Dial* 2005;18:266-75.
- Kumar VA, Kujubu DA, Sim JJ, Rasgon SA, Yang PS. Vitamin D supplementation and recombinant human erythropoietin utilization in Vitamin D-deficient hemodialysis patients. *J Nephrol* 2011;24:98-105.
- Saab G, Young DO, Gincherman Y, Giles K, Norwood K, Coyne DW. Prevalence of Vitamin D deficiency and the safety and effectiveness of monthly ergocalciferol in hemodialysis patients. *Nephron Clin Pract* 2007;105:c132-8.
- Porter A, Gilmartin C, Srisakul U, Arruda J, Akkina S. Prevalence of 25-OH Vitamin D deficiency in a population of hemodialysis patients and efficacy of an oral ergocalciferol supplementation regimen. *Am J Nephrol* 2013;37:568-74.