The Role of The Serum Calcium on Erythropoietin Responsiveness in Anaemic Haemodialysis Chronic Renal Failure Patients and The Effect of Dialysis Frequency and Duration

Aljefery Mohammed Hashim
Merjan Teaching Hospital

Abstract

In this study we examine the effect of the serum calcium level (in mmol/dl) on the rHuEPO (EPREX) in 100 patients of different age group (20-65 years mean 42.5)of both sexes (35 female and 65 male) in haemodialysis program .We examine the responsiveness of anaemic CRF patients in HD program to the rHuEPO by the level of the PCV and compare the differences in the results according to the changes of the level of the serum calcium which may be fluctuated from patients to patients according to the different causes and from our result we can conclude that: the serum calcium level which examine in all patients in the study has a direct effect on erythropoietin treatment to anaemic HD CRF patients ($p<0.05$) and for this reason the calcium play a vital role in the anaemia management in this group of patients.

Key Words: anaemia; haemodialysis; erythropoietin responsiveness; serum calcium; frequency and duration of haemodialysis.

Introduction

In normal conditions serum calcium is equilibrated within the marrow limits. Parathyroid hormone (PTH) and calcitriol 1,25 dihidroxycholecalciferol; 1,25 $(OH)_2D_3$ are the main factors that maintain normal serum ionized calcium(Ca^{+2}) their interdependent effects provide fine control of serum calcium under control circumstances. PTH and vitamin D regulate the serum calcium by their action on bone, intestine and renal cells. Extracellular fluid calcium concentration (identical to blood concentration)is of paramount importance in variety of physiological functions such as cell contraction (myocardium, muscle)and coagulation. In addition, it is the most important action which controls hormone secretion. It regulates also its own homeostasis. Therefore, blood is the most important body fluid in which calcium concentration is measured (Agus & Goldforb, 1985).

It has been suggested that the changes in calcium concentration play a role in PTH suppression induced by calcitriol, and calcitriol also affects the proliferation of parathyroid cells.

Binding of $1,25(OH)_2D_3$ to its receptors in parathyroid glands may be modified in various circumstances. In uremic patients, the number of receptors is decreased when compared to the glands from patients after renal transplantation or with primary hyperparathyroidism(Adams, 1989).

Administration of $1,25(OH)_2D_3$ to patients with end stage renal failure decrease the set point for PTH suppression by ca+2 from 5.24+0.14 to 5.06 +1.5 mg/dl. In other words, the gland became more sensitive to circulating calcium(Benabe, 1987).

The prevalence of osteoporosis/ osteopenia is increased in haemodialysis patients and bone mineral density appears to correlate with high serum level of bioactive PTH (Polymeris *et al.*, 2012).

Chronic uraemia is characterized by decrease levels of plasma $1,25(OH)_2D_3$ due to decreased renal 1-hydroxylase activity and by decreased renal phosphate excretion. The consequence is an increased synthesis and secretion of parathyroid hormone-secondary parathyroidism-due to the low levels of plasma calcium ،low levels of plasma $1,25(OH)_2D_3$ and high levels of phosphate. The association between renal bone disease and chronic renal failure is well described. Epidemiological studies have indicated that an association also exists between secondary hyperparathyroidism and increased mortality and cardiovascular calcification in chronic uraemic patients. Treatment of secondary hyperparathyroidism in chronic uraemia focuses on avoiding hyperphosphataemia by the use of oral phosphate binders, which bind phosphate in the intestine and concomitant substitution by a 1alpha-hydroxylated vitamin D analog in order to compensate for the reduced renal hydroxylation . Additional treatment with aluminum containing phosphate binders to overcome phosphate absorption and retention was initiated already in the 1960s and used extensively until aluminum toxicity was disclosed in the mid-1980s. Instead calcium carbonate and calcium acetate where used as phosphate binders. Until recently the most commonly used active vitamin D drug was either the natural $1,25(OH)_2D_3$ or the 1alpha hydroxylated analog. 1 alpha$(OH)D_3$ which after hydroxylation in the liver is converted to $1,25(OH)_2D_3$, alpha $(OH)D_3$ was produced by LEO pharma 1973. The two vitamin D analog were used in different geographical areas(Brandi, 2008).

There is evidence that bone marrow erythropoietic cells express calcitroil receptors and that calcitroil induces proliferation and maturation of erythroid progenitor cells(Carozzi *et al.,* 1990). Thus deficiency in calcitriol as one of the causes of hyperparathyroidism could impair erythropoiesis. In support of this hypothesis, Albitar *et al* have observed an increase in hemoglobin (Hb) levels in renal patients treated with high dose of alfa-calcidol, that was not associated with any change in PTH levels .Others , however found that an increase in Hb levels in response to calcitroil therapy was associated with lower PTH levels (Goicochea *et al.,* 1998).

In uraemic patients , several studies have provided support for the hypothesis of possible suppression of erythropoiesis by the marked bone marrow fibrosis that is the major feature of sever hyperparathyroidism.

Zengraff *et al*, Barbour *et al* observed a more marked degree of anaemia in dialysis patients with sever oseitis fibrosa compared with dialysis patients who had no marrow fibrosa. In the later study, human recombinant erythropoietin (rHuEPO) requirements for the treatment of anaemia were significantly higher in the patients with marrow fibrosa than in those without marrow fibrosa(Tilman & Kai-Uwe, 2002).

Many studies have shown that dialysis patients with secondary hyperparathyroidism either require a higher dose of rHuEPO to correct anaemia than euparathyroid dialysis patients(Horl *et al.,* 2000; Tonelli *et al.,* 2001) or achieved significant improvements in anaemia after medical or surgical correction of secondary hyperparathyroidism(Kokof *et al.,* 1993; Goicoechea *et al.;* 1996; Argiles *et al.,* 1994; Shasha *et al.,* 1978; Neha *et al.,*

2010). Whether the direct antagonist effect of PTH on erythropoiesis exclusively or could be partially owing to low $1,25(OH)_2D_3$ level accompany high PTH in dialysis patients, is unknown because vitamin D level were not measured in the studies that examined PTH and anaemia (Deicher & Hori, 2005).

Patricia Jaoa Matiaz *et al* found that the role of vitamin D metabolism has also been involved in the improvement of erythropoiesis by a direct effect on erythroid precursor proliferation(Aucella *et al.,* 2003) and/or, marginally, by controlling secondary hyperparathyroidism(Schober *et al.,* 1991), by activate the vitamin D receptor in the parathyroid gland and the controlling the parathyroid gland secretion(Ritter *et al.,* 2006).

Schober *et al* found that the potential effects mediated by parathyroid hormone include alterations in extracellular and intracellular calcium and phosphate levels, the release of cytokines by osteoclasts or resorbed bone ,and decrease responsiveness of erythropoietic progenitor cells to exogenous erythropoietin(Neusser *et al.,* 1993). In another aspect and at the same consequences to these studies several earlier studies have shown that chronic erythropoietin (EPO) administration leads to a rise in Ca^{+2}(Vaziri *et al.,* 1995; VanGreet *et al.,* 1990; Miller & Cheung, 1994).

In this study we try to find the relationship between the serum level of calcium and the value of the PCV in chronic haemodialysis patients and how the changes of the serum calcium affect the PCV value and try to explain the role of calcium ion in the responsiveness to the erythropoietin treatment .

The method

In this study 100 patients (n=100) of chronic renal failure on haemodialysis program are divided into 4 groups equal in number [each group with (25 patients)−n=25]. The patients ages involved in this study ranging from 20 to 65 years of both sexes (35female and 65 male). The study continued for 6 months from 24 September 2011 to February 2012.For each patient we get the blood urea ،serum creatinin, serum calcium, PCV, body weight(post dialysis session) and the kt/v. The serum calcium measured by the calcium CPC method) Biolabo reagen ، Biolabo SA, France), the blood urea measured by Spinreact and the serum creatinin measured by cretinine Jaffe. Colorimetric-Kinetic)Berthelot .Enzimatico colorimetrico ،Spinreact,S.A/.S.A.U .Ctra.Santa Coloma.7 E-17176 Sant Esteve DE BAS(G) Spain).

1-The group one treated in haemodialysis program 4 hours 2 times / week with Eprex dose of 50 IU/Kg subcutaneously two time a week.

2-The group two treated as the same as the group one adding one microgram each day of one alpha (Alphacalcidol-1-hydroxyvitamin D3) of Leo pharmaceutical product, Ballerup−Denmark.

3-The group three treated with haemodialysis program 4 hours 3times / week with the same dose of Eprex as groups one and two adding to the treatment of this group a low dose of calcium carbonate (500 mg/day).

4-The last group (the group four) treated with the haemodialysis program of 4 hours 3 times/week with Eprex of dose and route same as the last three groups and one alpha 1 micg/day.

The data of the patients collected and statistically calculated and analyzed to get the mean, standard deviations (SD), P value and comparing the results for each group.

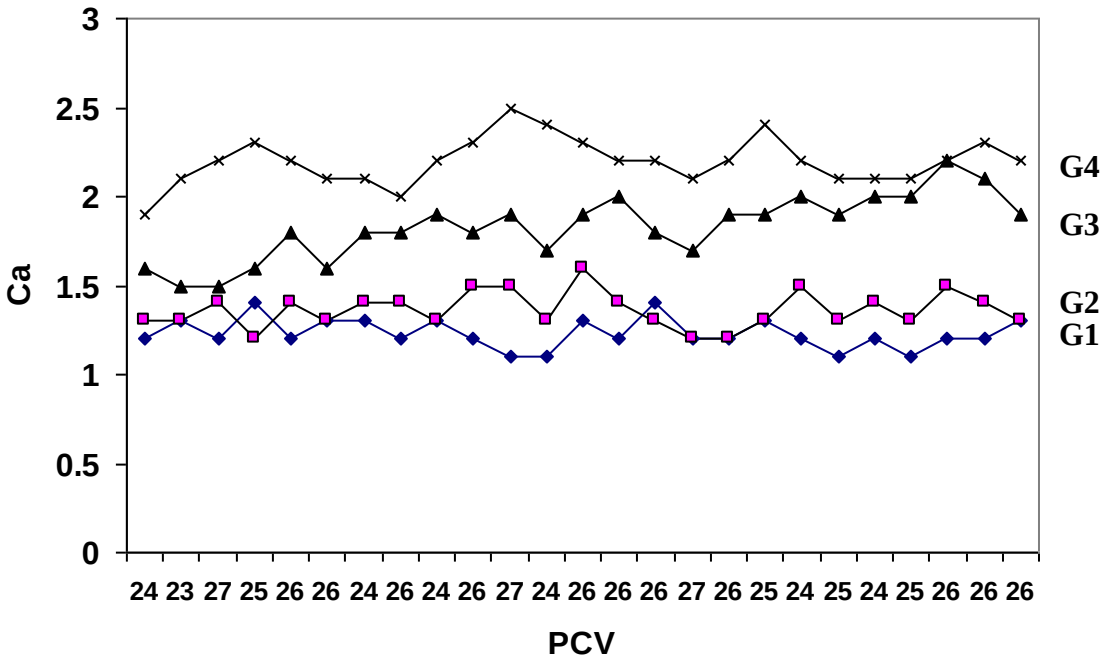
Result

The group four has the best value of the PCV in comparing to other three groups the mean are 32.76 in the same time the serum calcium when measured in this group also appear the better value when it compare to the result of the other three groups the mean are 2.198 mmol/dl (Graph 1), and when we compare the results of the group 4 and group 2 we find the PCV mean value in the group 2 are 28.6 and the mean value of the serum calcium for this group is 1.36 mmol/dl this indicate that the frequency of the dialysis causes a more decline of uraemic toxin in uraemic patients lead to better response to rHuEPO which in turn, in the presence of normal level of serum calcium lead to a better results in RBC production and better PCV results. (Graph 2).

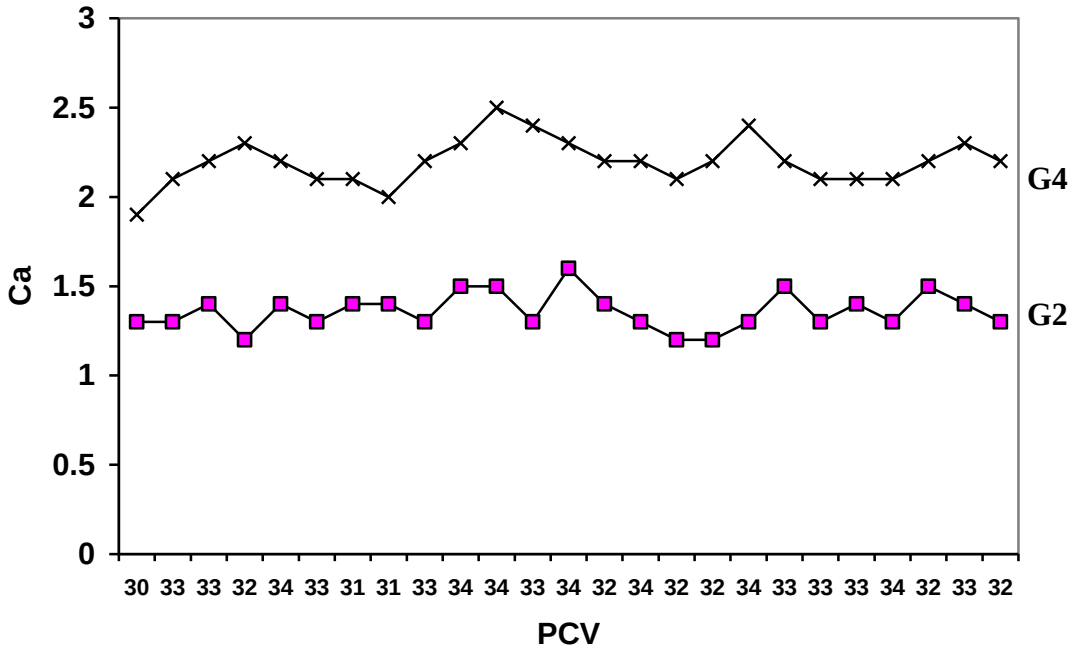
When comparing the results of the group three and the group one we can find the PCV mean value in group 3 is 32.08 while in group one is 25.36 and the serum calcium mean value for the group 3 is 1.832 mmol/dl and for group one is 1.226 mmol/dl .The difference in the values of the PCV in the two groups related to two factors the first is the frequency of the dialysis differ between the two groups the second factors regarding to the calcium carbonate dose which given daily to the patients in the group three. (Graph 3). This indicate the presence of the calcium ion is important for the better results of the PCV which occur because of the calcium have a vital role in erythroid progenitor maturation and production.

When we compare the group 4 and the group 1 there are a wide variety in the PCV value where PCV mean value in group 4 is 32.76 while its 25.36 in group 1 at a parallel with calcium mean value where its 2.198 mmol/dl in the group 4 and 1.226 mmol in group 1. There are two factor act, the first is the frequency of the dailysis, the other is the present of one alpha and both of them affect the EPO responsiveness in different ways. The other factor is the value of the Ca^{+2} where it has an important role in having a better results of the PCV value (Graph 4).

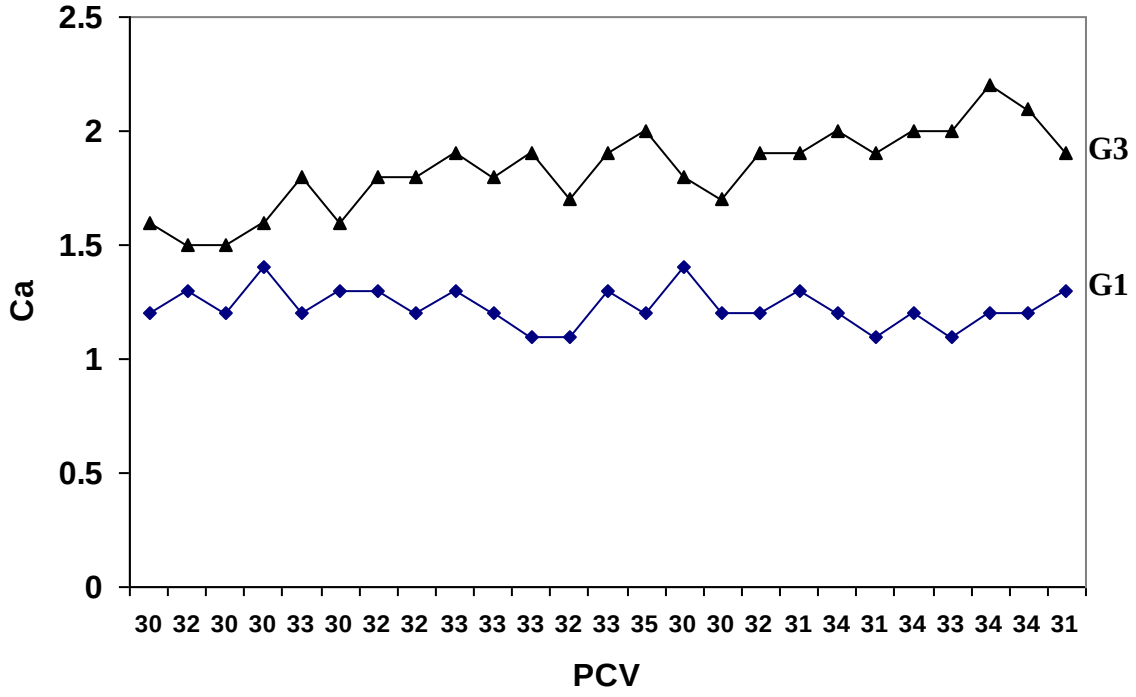
Our result indicate the better calcium value give the better PCV value when other factors present in the treatment of the patient like a better frequent analysis and Vit D3 and dealing with other multiple factors affecting the dialysis patients (Graph 5).



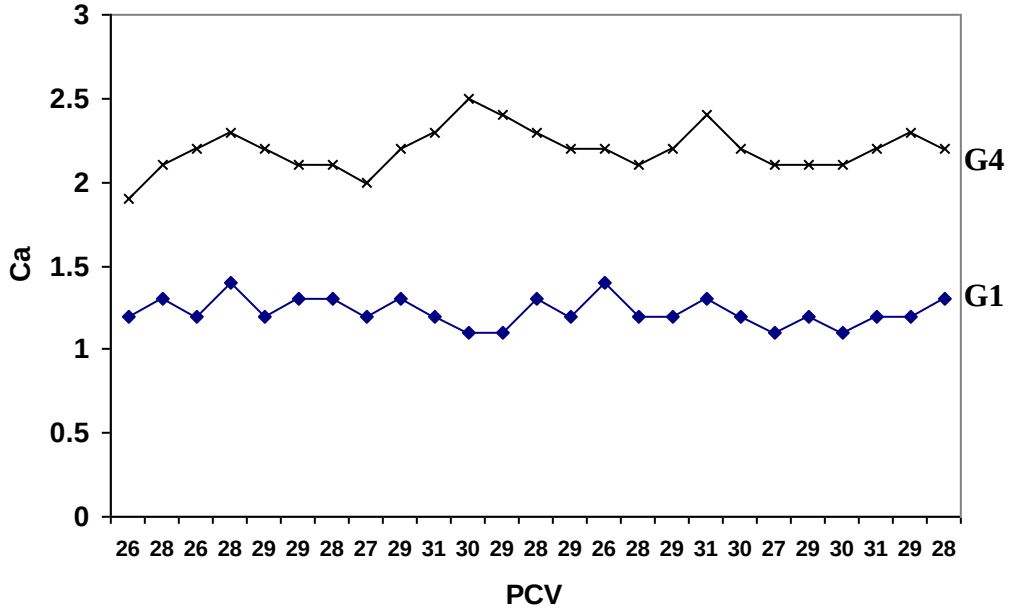
Graph (1) : The comparison of the results of the four groups which showed the different of the value of the PCV and serum calcium



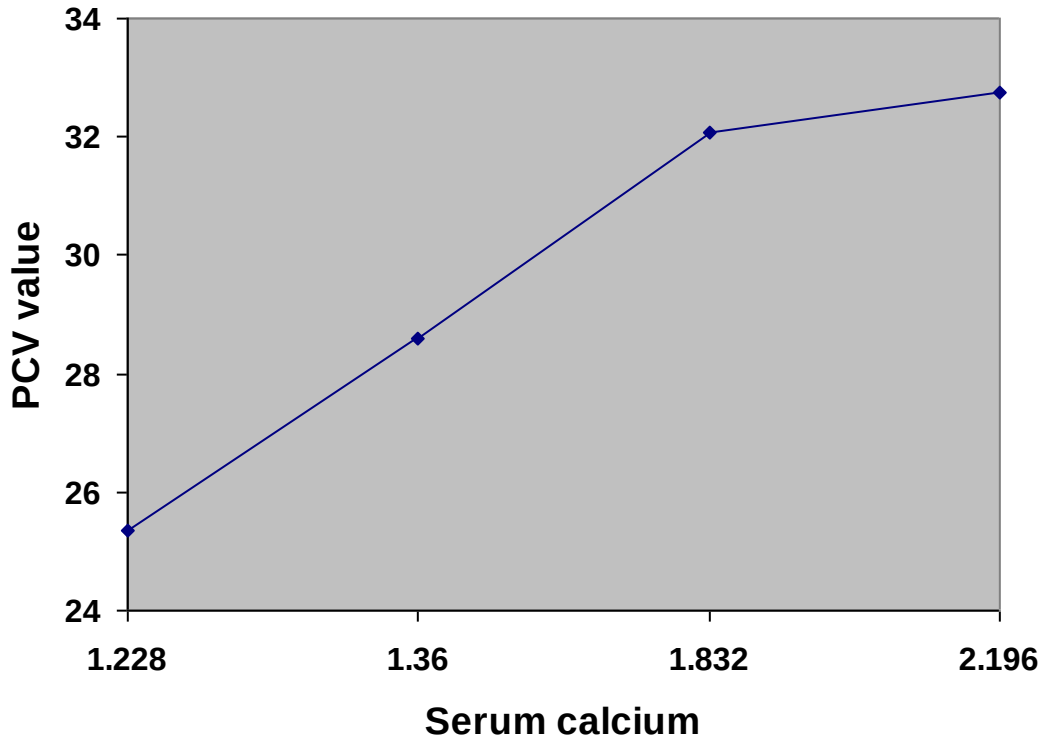
Graph (2) : Which present the difference in the value of the PCV which go with the difference of the serum calcium value.



Graph (3) : Show the differences between the G3 and G1.



Graph (4) : The great variations in the value of the PCV and the serum calcium



Graph (5) : Showed the proportional relationship between the serum calcium and the PCV values

Discussion

Miller BA and Cheung JY proved that there is a rise in intracellular calcium is another major effect of Epo-Epo receptor interaction. D Shudhaker Rao, *et al* showed there is a potential effects mediated by parathyroid hormone include alterations in extracellular and intracellular calcium and phosphate levels, the release of cytokines by osteoclasts or resorbed bone and decreased responsiveness of erythropoietic progenitor cells to exogenous erythropoietin.(Shudhaker *et al.*, 1993) The other hypothesis showed by Polymeris A *et al* (2012) explained that the prevalence of osteoporosis/ osteopenia is increased in haemodialysis patients and bone mineral density appears to correlate with serum level of bioactive PTH but not with 25OHD. From our results we can clarify that the calcium which play an important role in erythroid progenitor production and maturation to RBC which affected by the PTH secretion, and the last (PTH)can counteract by the vitamin D and their derivatives as 1alpha OHD3, this calcium ion can be affected by other factors as the frequency and/or the duration of the haemodialysis, and this fact produced by action of many interacted factors, and these factors communicated one to another's or we can say each one can complete the others and in the last maintaining the value of the serum calcium in a level can accomplished its action in the circle of maintaining the PCV value of the patients, but we hypothesized that each mechanism which act with or act against the serum calcium can act to a certain limit, and this limit varying in their effect accordingly .

In this study we hypothesized that the frequency and/or the duration of the hemodialysis act in different directions; the first direction , it act against the uraemic toxin level where the duration of the haemodialysis when its long ,the greater amount of the uraemic toxin can be excluded this mechanism gave the cells including bone marrow cells some extent of the freedom to produce RBC precursors and help in liberated of the Epo receptors and according to Miller BA and Cheung JY hypothesis it permit to increase the level of the calcium ion and by this way the PCV increased. The second direction we hypothesized that the calcium receptors and calcium transport channels can be affected by the uraemic toxin and this can reduced by reducing the effect of uraemic toxin by increase the duration and/or the frequency of the haemodialysis and by this effect the intracellular and extracellular calcium will be increased (Karpatil *et al.,* 2001).

It has been known for some time that an in vitro increased synthesis of RNA follows the addition of Epo to erythropoietic cells (Goldwasser 1981). This occurs essentially with Epo-responsiveness erythropoietic cells which have specific membrane receptors. As soon as Epo binds to these receptors one of the first effects observed is an increased cell membrane uptake of Ca^{+2}.(Sawer & Kranfz, 1984) This erythropoietic effect is similar to that seen in vitro when accessory hematologic cells are cultured in the presence of mitogens or ionophoric substances, in which case there is also increased uptake of Ca^{+2} into the cytoplasmic pool .

This event indicates Ca^{+2} as an early messenger of cellular activity, in that it leads to the stimulation of metabolic processes which involve certain cytoplasmic and nuclear protein.(Chantler, 1985).

It thus appears that cytoplasmic Ca^{+2} modifications induced by Epo represent an important step in the differentiation and maturation of erythroid precursors(Carrozi *et al.,* 1990).

In vivo and in vitro studies suggest that 1,25D directly affects the proliferation of erythroid precursors via increased membrane permeability of calcium(Aucella *et al.,* 2003; Neha *et al.,* 2010). In addition vitamin D has anti-inflammatory actions that could theoretically improve erythropoietin responsiveness.(Neha *et al.,* 2010)

Extremely high PTH levels have been considered a mechanism for decreased erythropoiesis via increased bone marrow fibrosis and erythropoietin resistance in chronic kidney disease patients(Rao *et al.,* 1993; Neves *et al.,* 2006) whether this represents exclusively a direct antagonistic effect of PTH on erythropoiesis or could be partially owing to the low 1,25 vitamin D levels that accompany high PTH in dialysis patients. (Neha *et al.,* 2010)

Lowering elevated parathyroid hormone levels by oral calcium supplementation and phosphate restriction, by administration of vitamin D3 derivative and, in the near future, by treatment with calcimimetics may prove efficient in some patients to fight extensive requirements of erythropoietic agents. (Deicher & Horl, 2005)

The combined treatment with calcium containing phosphate binder and active vitamin D induces an increase in plasma Ca^{+2}, because $1,25(OH)D_3$ induces a marked suppression of plasma PTH without causing serious side effect in patients on chronic haemodialysis. It possible to prevent hypercalcaemia by dosing monitoring plasma ca+2 levels and adjust the dose of 1 alpha$(OH)D_3$ accordingly.(Brandi 2008).

We can concluded that plasma calcium ion has a vital role in the anaemia treatment of chronic haemodialysis patients where, there are an inter-relationship between calcium

and Epo responsiveness where each can complete the effect of the others and itself the calcium ion affected by the vitamin D and their derivative (as 1-alpha(OH)D_3) which facilitated the effect of the calcium ion and we can say this triangle have a vital role in the anaemia management of chronic haemodialysis patients .

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