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RESEARCH ARTICLE

Relationship of Sclerostin with PTH and Erythropoietin in-patient with CKD on dialysis in AL-Najaf Province.

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Abstract

Chronic kidney disease (CKD) is a condition that correlated with biochemical and clinical abnormalities due disturbances endocrine, which affects the function of the kidneys. Sclerostin, erythropoietin, and PTH have been shown to be associated with many complications related in patients with CKD.

The study involved 21 male patients with (CKD) mean age (45.21±2.8 years) comprising nine no dialysis CKD, twelve patients with dialysis (end stage of CKD), who were attending kidney disease Center of Al-Sadder Teaching Hospital at Al-Najaf province. The exclusion criteria include blood transfusion in the last one-month and dialysis less than three months.Nine healthy volunteer male mean age (44.1±3.7 years) as control group. Estimated biomarkers sclerostin, PTH, and Epo, in addition to the age, body max index (BMI), red blood corpuscle (RBC), heamoglobin (Hb), ferritin, calcium, phosphor levels for all subject. Lower level of RBC, Hb, and calcium, while higher level of phosphor and ferritin in patient comparison with control group. Significant difference ($P<0.05$) between no-dialysis and dialysis groups in these parameters. Sclerostin and PTH levels was higher significantly but Epo levels lower significantly in CKD patients than controls ($P<0.05$), and highest in those with dialysis more than no- dialysis.Duration of dialysis showed a significant positive correlation with age ($R^2 = 0.921$), BMI ($R^2 = 0.940$), Phosphor ($R^2 = 0.907$), and PTH ($R^2 = 0.874$). While it was observed a significant negative correlation with Sclerostin ($R^2 = 0.740$), Calcium ($R^2 = 0.863$), and erythropoietin ($R^2 = 0.714$). A significant negative ($P<0.01$) correlation between Sclerostin serum levels after adjustment dialysis duration and PTH ($r= –0.92$), while it`s a significant positively correlated with Erythropoietin ($r=0.7$)

In conclusion, there is a relationship between sclerostin with each of Epo and PTH level, which affected with duration of dialysis in CKD patients.
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Introduction:-

CKD is represented a major problem considerable burden on the patients affected **(1)**. CKD is a condition that associated with biochemical and clinical abnormalities which affects the function of the kidneys, and that may progress over time to kidney failure **(2)**. CKD is divided into five stages. The prevalence of CKD increases with progress age, the risk of mortality in CKD faraway prevail of the progression to end-stage or dialysis of kidney disease. When the kidneys dysfunction, for improvement patients life dialysis or a kidney transplant is required to support survival these patients **(3).**

Reduced renal function not briefly of disturbances in mineral metabolism phosphorus and calcium, but this is underlying mechanisms for endocrine disturbances advanced to other clinical important. Effectively all patients with end-stage renal disease will experience from Endocrine abnormalities **(4)**. In addition to abnormalities in erythropoiesis, there are a number of hormonal disorders as parathyroid hormone (PTH), Erythropoietin, sclerostin, and vitamin D precursors **(5),(6), (7).**Erythropoietin is an essential erythropoietic glycoprotein hormone that prompts erythrocyte production, synthesis and released from kidney responses for hypoxia or anemia **(8), (9)**. During the advancement of CKD erythropoietin synthesis reductions, its level existence insufficient to the degree of anemia **(10)**.Sclerostin is inhibitors of Wnt signaling produced by osteocytes and potentially important player in obstruct bone formation **(11**). Sclerostin may affect bone metabolism during CKD and the end-stage renal undergoing maintenance dialysis **(12), (13), (14).**

The intention of our study is to investigate three important biomarkers sclerostin, Erythropoietin, and PTH in CKD with dialysis and interpretation the interrelationship of these biomarkers and duration of dialysis.

Materials and Methods:-

Twenty one patients with (CKD) mean age (45.21±2.8 years) comprising nine no dialysis CKD (1-3 stage), twelve patients with dialysis (end stage of CKD) depending on glomerular filtration rate (GFR) who were attending for kidney function assessment in kidney disease and transplant Center of Al-Sadder Teaching Hospital at Al-Najaf province. The exclusion criteria include blood transfusion in the last one-month, duration of dialysis less than three months.

Nine healthy volunteers male mean age (44.1±3.7 years) as control group. Estimated biomarkers sclerostin, parathyroid hormone (PTH), erythropoietin (Epo) hormone, in addition to the age, body max index (BMI), red blood corpuscle (RBC), heamoglobin (Hb), ferritin, calcium, phosphor, were recorded for both patients and controls.

5 ml of intravenous blood were drawn from all subject patient and controls. (2ml) from the collected blood put in tubes with K3-EDTA was immediately perform RBCs and Hb using automated analyser Mythic 18 (RINGELSAN CO., Turkey), while other (3ml) was centrifuged 3000 rpm/5min to obtain serum stored in aliquots at _20°C for assay other the study parameters. Calcium and phosphor were done through automated analyser (bt 35i, Turkey) depended on the standard laboratory procedure. Human Sclerostin (Sensitivity 7.8pg/ml, Range 31.25 -2000 pg/ml), Human Parathyroid hormone (PTH) Sensitivity (15.6pg/ml, Range62.5 -4000 pg/ml). Human Erythropoietin (Epo) (Sensitivity 1.6 mIU/ml, Range 3.1- 100 mIU/ml, and ferritin (Sensitivity 2.5ng/ml, Range 5-160 ng/ml) levels by the quantitative sandwich enzyme immunoassay technique were measured using the commercially ELISA kit was depended on the manufacturer's instructions (Cusabio Biotech, Co., Ltd., P.R.C).

Statistical methods:-

The results were analyzed statistically using SPSS 17.0. Student T-test using for obtaining Mean and standard error (SE) of the all parameter were calculated. Statistically significant $p<0.05$.

Results:-

The demographic and biochemical parameters of the study subject CKD patients and control groups were summarized in Table (1). Of the twenty-one patients mean age was (45.21±2.84) years compared with nine healthy controls (44.11±3.71) years. CKD Patients were significantly ($P < 0.05$) lower than controls of mean BMI (22.89±0.31 vs. 24.01±0.27 kg/m^2), RBC (3.07±0.11 vs. 4.70±0.1710^6/mm^3), and Hb (10.43±0.43 vs. 13.80±0.38 g/dl). Serum Ferritin levels were higher significantly in CKD patients (352.06±19.5vs.114±3.22 ng/ml). Mean serum calcium level was significant decreased (7.11±0.31 vs. 8.98±0.16 mg/dl), and significantly increased serum phosphor level (5.63±0.22 vs. 4.12±0.28 mg/dl) compared with controls respectively. Sclerostin and PTH levels in patients with CKD was higher significantly than controls (57.35±5.78 vs.26.67±0.41 pg/ml) and (249.78±19.9 vs. 48.41±0.74 pg/ml), furthermore serum erythropoietin levels lower significantly (10.34±0.76 vs. 15.06±0.86mIU/ml).

Table (1) comparison between in all parameters of the patients with CKD and control groups					
Parameters	**Mean ± SE**		**Mean ± SE**	**Min- Max**	**p-value**
	Control n= 9	**Min- Max**	**CKD Patients n= 21**		
Age (year)	44.11±3.71	28 – 60	45.21±2.84	26 – 60	0.78
BMI (kg/m^2)	24.01±0.27	22.8 – 25.2	22.89±0.31 *	20 – 25	0.03
RBC (10^6/mm^3)	4.70±0.17	4 – 5.42	3.07±0.11 *	2.45 – 3.82	0.00
Hb (g/dl)	13.80±0.38	12.3 – 15.6	10.43±0.43 *	7.8 – 13	0.00
Ferritin (ng/ml)	114±3.22	93 – 125	352.06±19.5 *	240 – 532	0.00
Calcium (mg/dl)	8.98±0.16	8.3 – 9.7	7.11±0.31 *	5 – 8.8	0.00
Phosphor (mg/dl)	4.12±0.28	2.8 – 5.7	5.63±0.22 *	4.5 – 7.7	0.00
PTH (pg/ml)	48.41±0.74	44 – 52	249.78±19.9 *	114 – 410	0.00
Sclerostin (pg/ml)	26.67±0.41	25 – 28.7	57.35±5.78 *	27 – 94.6	0.00
Erythropoietin (mIU/ml)	15.06±0.86	11.7 – 19.5	10.34±0.76 *	26 – 60	0.00
*significant differences (p<0.05) between control group and CKD patients					

Descriptive Analyses of dialysis and no- dialysis CKD patients was showed in table (2). Duration of dialysis in dialysis groups was (20.91 ± 3.25 month). Age and BMI were no significantly difference in both groups of dialysis and no- dialysis CKD patients. RBC, Hb, and calcium levels were lower significantly (P < 0.05) in dialysis groups as comparison with no-dialysis groups (2.86 ± 0.11 vs. 3.33 ± 0.15 10^6/mm^3), (8.95 ±0.28 vs. 11.85 ± 0.44 g/dl), and (5.92 ± 0.19 vs. 8.29 ± 0.10 g/dl) respectively. Dialysis patients were higher significantly of Ferritin and phosphor of dialysis than with no- dialysis patients (411.5 ± 25.65 vs. 292.5 ± 9.21 ng/ml) and (6.20 ± 0.32 vs. 5.07 ± 0.12 mg/dl) respectively. Sclerostin and PTH serum levels were higher significantly in dialysis (78.89 ± 4.73 vs. 35.81 ± 1.94 pg/ml) figure (1) and (310.44± 22.65 vs. 189.11-± 15.85 pg/ml) figure (2). Erythropoietin serum level was lower significantly in dialysis than with no-dialysis groups (7.80 ± 0.64 vs. 12.98 ± 0.55 mIU/ml) figure (3).

Table (2) Descriptive Analyses of dialysis and no- dialysis CKD patients			
parameters	**Mean ± SE**		**p- value**
	No-dialysis n= 9	**dialysis n= 12**	
Age (year)	43.67 ± 4.30	46.75 ± 3.2	0.5
BMI (kg/m^2)	23.21 ± 0.56	22.58 ± 0.25	0.2
RBC (10^6/mm^3)	3.33 ± 0.15	2.86 ± 0.11 *	0.01
Hb (g/dl)	11.85 ± 0.44	8.95 ± 0.28 *	0.00
Calcium (mg/dl)	8.29 ± 0.10	5.92 ± 0.19 *	0.00
Phosphor (mg/dl)	5.07 ± 0.12	6.20 ± 0.32 *	0.00
Ferritin (ng/ml)	292.5 ± 9.21	411.5 ± 25.65 *	0.00
Duration (month)	0.00	20.91 ± 3.25 *	0.00
significant differences (p<0.05) between dialysis and no- dialysis CKD patients			

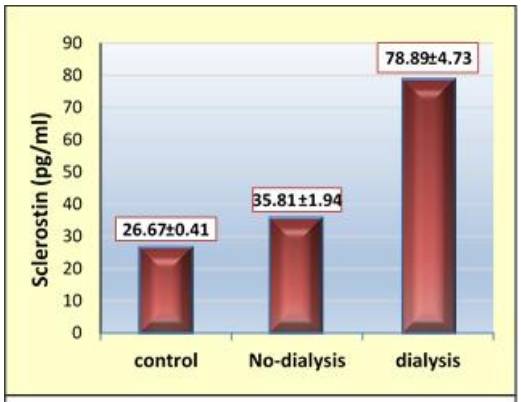

Figure (1) serum sclerostin level in controls, dialysis and no-dialysis patients. Data are expressed as mean ± SE

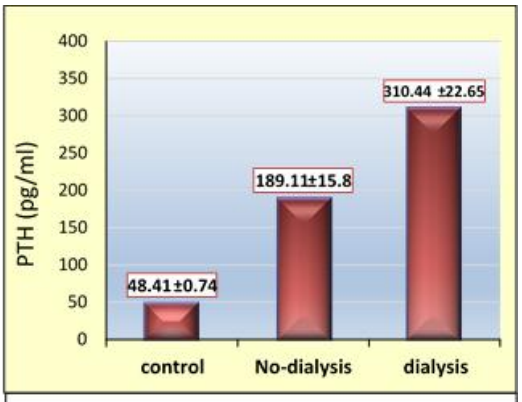

Figure (2) serum PTH level in controls, dialysis and no-dialysis patients. Data are expressed as mean ± SE

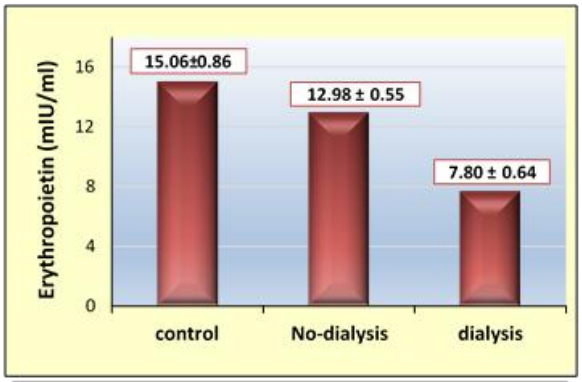

Figure (3) serum Erythropoietin level in controls, dialysis and no-dialysis patients. Data are expressed as mean ± SE

Result of correlation factor and linear model of regression, duration of dialysis showed a significant positive correlation with age ($y = 0.794 x - 16.203$, $R^2 = 0.921$), BMI ($y = 0.079 x + 20.916$, $R^2 = 0.940$), Phosphor ($y = 0.094 x + 4.227$, $R^2 = 0.907$), and PTH ($y = 6.917 x + 165.897$, $R^2 = 0.874$) figures (4), (5), (6), and (7) respectively. While it was observed a significant negative correlation with Sclerostin ($y = -1.292 x + 105.933$, $R^2 = 0.740$), Calcium ($y = -0.060 x + 7.173$, $R^2 = 0.863$), and erythropoietin ($y = -0.172 x + 11.379$, $R^2 = 0.714$) figure (8), (9), and (10) respectively.

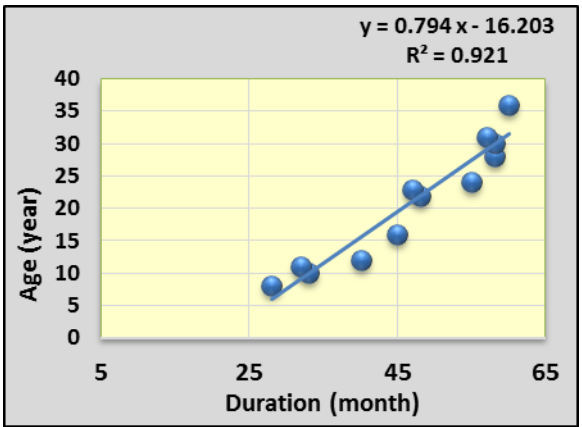

Figure (4) correlation and regression between duration of dialysis and age (P< 0.01) n= 12

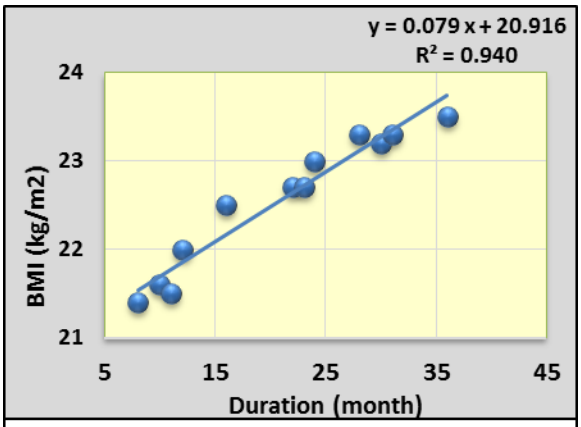

Figure (5) correlation and regression between duration of dialysis and BMI (P< 0.01) n= 12

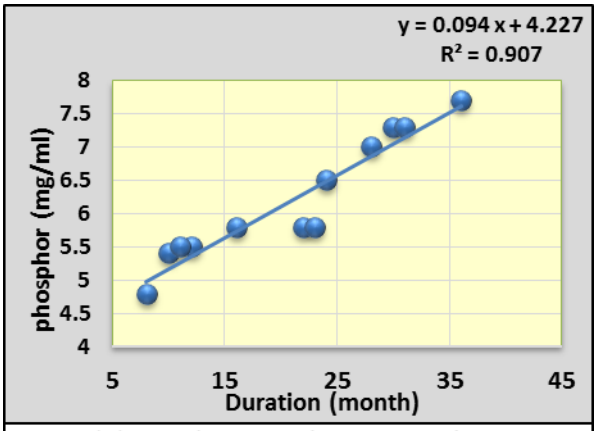

Figure (6) correlation and regression between duration of dialysis and phosphor (P< 0.01) n=

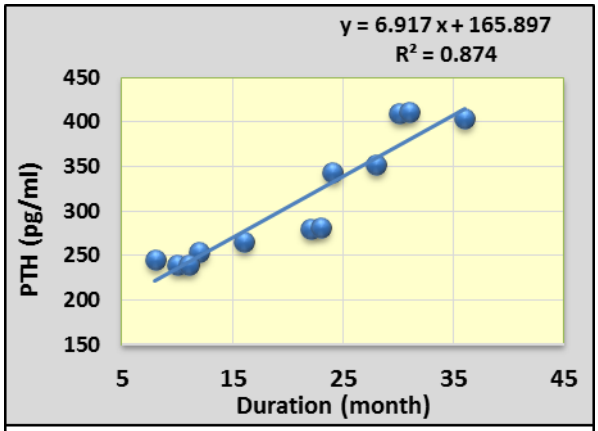

Figure (7) correlation and regression between duration of dialysis and PTH (P< 0.01) n= 12

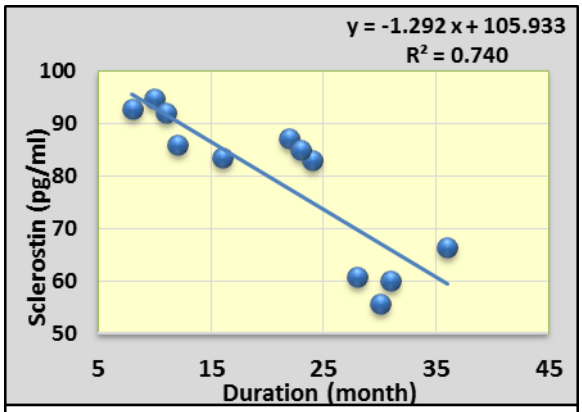

Figure (8) correlation and regression between duration of dialysis and Sclerostin (P< 0.01) n=

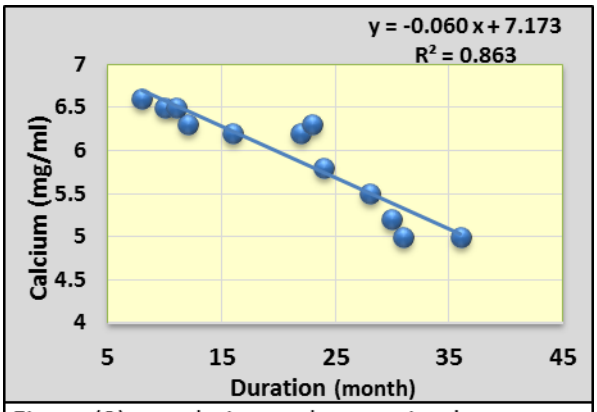

Figure (9) correlation and regression between duration of dialysis and calcium (P< 0.01) n= 12

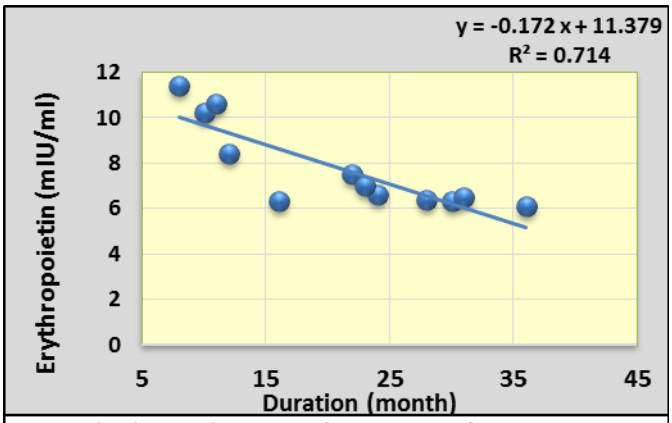

Figure (10) correlation and regression between duration of dialysis and erythropioetin (P< 0.01) n= 12

Table (4) Pearson Correlation (2-tailed) between sclerostin and parameters in dialysis patients. A significant negative (P< 0.01) correlation between Sclerostin serum levels after adjustment dialysis duration and Age (r= –0.85), BMI (r= –0.80), ferritin (r= –0.71), Phosphor (r= –0.91), and PTH (r= –0.92), furthermore it`s a significant positively correlated with RBC (r=0 .75), Hb (r=0.81), Calcium (r=0.93), and Erythropoietin (r=0.72).

Table (4) Pearson Correlation (2-tailed) between sclerostin and parameters in dialysis patients.

		AGE	BMI	RBC	Hb	Ferritin	Calcium	Phosphor	PTH	Sclerostin	Erythropoietin	duration
AGE	Pearson Correlation	1										
	Sig. (2-tailed)											
BMI	Pearson Correlation	.994(**)										
	Sig. (2-tailed)	.000										
RBC	Pearson Correlation	-.846(**)	-.863(**)									
	Sig. (2-tailed)	.001	.000									
Hb	Pearson Correlation	-.767(**)	-.800(**)	.722(**)								
	Sig. (2-tailed)	.004	.002	.008								
Ferritin	Pearson Correlation	.913(**)	.919(**)	-.767(**)	-.708(**)							
	Sig. (2-tailed)	.000	.000	.004	.010							
Calcium	Pearson Correlation	-.898(**)	-.890(**)	.720(**)	.793(**)	-.805(**)						
	Sig. (2-tailed)	.000	.000	.008	.002	.002						
Phosphor	Pearson Correlation	.930(**)	.916(**)	-.720(**)	-.751(**)	.878(**)	-.981(**)					
	Sig. (2-tailed)	.000	.000	.008	.005	.000	.000					
PTH	Pearson Correlation	.902(**)	.892(**)	-.681(*)	-.793(**)	.783(**)	-.984(**)	.965(**)				
	Sig. (2-tailed)	.000	.000	.015	.002	.003	.000	.000				
Sclerostin	Pearson Correlation	-.856(**)	-.846(**)	.752(**)	.817(**)	-.716(**)	.937(**)	-.918(**)	-.927(**)			
	Sig. (2-tailed)	.000	.001	.005	.001	.009	.000	.000	.000			
Erythropoietin	Pearson Correlation	-.934(**)	-.937(**)	.901(**)	.691(*)	-.898(**)	.747(**)	-.790(**)	-.736(**)	.726(**)		
	Sig. (2-tailed)	.000	.000	.000	.013	.000	.005	.002	.006	.007		
duration	Pearson Correlation	.960(**)	.969(**)	-.767(**)	-.842(**)	.921(**)	-.929(**)	.952(**)	.935(**)	-.860(**)	-.845(**)	
	Sig. (2-tailed)	.000	.000	.004	.001	.000	.000	.000	.000	.000	.001	

** Correlation is significant at the 0.01 level (2-tailed).

Discussion:-

This study is the first of its kind in Iraq, which investigated three important biomarkers serum sclerostin level, PTH and erythropoietin in patients with CKD who are undergoing dialysis and not dialysis, moreover explored the relationship between these biomarkers with duration of dialysis, calcium, phosphor and ferritin levels.

The demographic and biochemical parameters of the study subject table (1) revealed a significant decrease in values of BMI, RBC, Hb, calcium, and erythropoietin, while values of sclerostin, PTH, ferritin, and phosphor were showed higher significantly in CKD patients than controls these finding agree with Evenepoel *et al* **(15)**. This abnormality of the important parameters are very common because patients with CKD undergoing of many complications resulted from stress oxidative that associated with fails to function adequately CKD disease such as anemia, disorder metabolism, endocrine abnormalities, and bone disease etc. **(16),(17)**.

RBC and Hb are significantly lower in dialysis groups compared to no-dialysis groups table (2). This result corresponding with researchers **(18), (19)**, who have also explained that Anemia is one important risk factor that causes death of CKD patients. Alves *et al* **(20)** the inadequacy of the dialysis cause of erythropoietin resistance, due the mechanical procedure of dialysis incurrence destruction to erythrocytes, and conduce to blood loss. Estridge and Reynolds **(21)**, suggested this might be result to decrease in the synthesis of erythropoietin hormone, which is produced from kidneys in response to many factors such as hypoxia, and active vitamin D3, this hormone responsible of erythropoiesis in the bone marrow and regulating bone calcium and phosphorus. Erythropoietin deficiency in CKD patients due to the tissue damage or relative to tissue oxygenation by the underlying disease and inflammation. Our study supported this finding; it has showed Erythropoietin level a significantly lower in dialysis than with no-dialysis figure (3), and linear model of regression exhibit erythropoietin a significant negative correlation with duration of dialysis figure (10).

Calcium are significantly lower but Ferritin and phosphor are significantly higher in dialysis groups compared to no-dialysis groups table (2). Agreement with study of Mohammed **(22)**, who was indicated to lower of calcium level after dialysis in patients with CKD as compared to control groups while higher than the pre-dialysis stage, but phosphor level, becomes low in post dialysis compared to control and predialysis groups.

Some metabolic dysregulation may accompany CKD affected by the kidney disease itself or due to dialysis treatment. CKD may cause insufficiency of some essential elements that may be caused by increased losses throughout dialysis sessions, malnutrition, decreased intestinal absorption such as impaired calcium absorption **(23), (24).**Although rise phosphor level before dialysis result to the inorganic phosphate in the blood of the filtered during active transport by renal tubule. This active transport strongly repressed by disorder synthesis of essential metabolic regulators such as erythropoietin or parathyroid hormone or active vitamin D in kidneys and serious changes are apparent when in dialysis of CKD **(5), (25).**This finding were supported our results, which have showed duration of dialysis positive correlated with phosphor figure (6), while negative correlated with calcium figure (9).

In CKD, high levels of serum ferritin are reflecting the iron excess; Iron is not utilized because there is less erythropoiesis. Thus, to store by form ferritin protein that binds this increased amount of iron **(26).** In addition to increased frequently required blood transfusions to end-stage of CKD patients by dialysis, revealing of iron overload, lead to development of oxidative stress by activation of oxidative through the dialysis membrane and hemoincopatibility of the dialysis system causes chronic inflammation and production of free radicals**(27), (28), (29)**. These free radicals stimulated and increased serum hepcidin levels contribute to the dysregulation of iron homeostasis in CKD patients **(30).**

Our results indicate that Sclerostin and PTH are higher significantly in dialysis than with no-dialysis group figures (1) and (2),These result were conforming with**(31), (32)**, who have finding patients with CKD-end stages had increased level of circulating sclerostin and that GFR, age, and sex were independently associated with circulating sclerostin.

In addition, Pearson Correlation table (4) showed a significant negative correlation between sclerostin level with PTH and phosphor, while it was a significant positive correlation with calcium. Moreover, linear model of regression in our study showed that duration of dialysis a significant positive correlation with age, BMI, Phosphor, and PTH figures (4), (5), (6), and (7) respectively. While it was observed a significant negative correlation with Sclerostin, and Calcium, (figure (8), and (9), respectively.

Many researchers reveals higher sclerostin levels in end stage of CKD compared with early stage of CKD after adjusting for age, sex, and BMI, and that sclerostin reverse association with parameters of bone such as phosphor, calcium, and PTH **(33), (34), (6), (35)**. They reported that suppression of osteocytes Wnt/-catenin signaling and increased expression of sclerostin happened in early stage of CKD in mice. They suggested that repression of the Wnt/-catenin pathway is an early event in the development of renal osteodystrophy. Cejka *et al* **36)** found that increased sclerostin levels in patients with CKD were not due to decreased renal elimination, and serum sclerostin levels a positive correlation with BMD in uremic and dialysis patients.

In another side, a significant negative correlation between sclerostin level and duration of dialysis figure (8). Despite this negative relationship of sclerostin with PTH level, PTH remains high level in dialysis group and positive correlated with duration of dialysis figure (7), may be causes PTH resistant or present relationship between sclerostin, PTH, and phosphor these result confirmed with study of Thambiah **(37)**. Postulated high level sclerostin in CKD patients on dialysis, this is resulted to decrease clearance or extra production has not yet been completely evaluated. Sclerostin accruement may be participate response to PTH resistance in CKD with other factors such as calcitriol deficiency. Other studies showed that serum phosphate was independently associated with sclerostin level in patients with CKD, the diet phosphate stimulated bone sclerostin expression independently of PTH in a model of CKD-a dynamic bone disease (ABD)**(31), (38)**. Moreover, study of Nasri and Kheiri **(39)** revealed to increase activity of parathyroid glands with increase duration of dialysis. Thereby increase in PTH level after adjusting for calcium and phosphor level; may be belong that parathyroid glands were high stimulated by hyperphosphatemia and High level of phosphate promote parathyroid cell proliferation and increase synthesis and release of PTH directly and indirectly lead to reduction in serum calcitriol and calcium levels **(40)**.

Our result showed that BMI positive correlation with PTH and duration of dialysis, but it is a negative correlation with sclerostin level these found agreement with Kopple *et al* **(41)** who have reveals that BMI is a measure for explaining the nutritional status in dialysis patients. Higher BMI in treated patients with CKD by dialysis have increased survival over a 1-year period.

In conclusion, dialysis treatment remains an important major method of treatment for improving. In addition, there is a close relationship between sclerostin level with each of erythropoietin and PTH hormones, which affected with duration of dialysis in CKD patients may be help the development of treatment procedure.

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