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Pin1 Gene Expression and Some Biochemical Parameters in Iraqi Population with Chronic Kidney Disease

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Abstract: A widespread general medical problem is chronic kidney disease (CKD). It is a primary source of illness and mortality globally and is becoming more acknowledged as a global public health concern, particularly in developing nation. The purpose of this study was to assess the clinical importance of *Pin1* gene mRNA expression in samples taken from CKD patients in Iraq and some biochemical parameters. Blood samples from 120 individuals were collected and sorted into the following three groups: Group 1 consists of 40 samples from CKD patients on dialysis, Group 2 of 40 samples from CKD patients who are not on dialysis, and Group 3 of 40 samples from controls who appear to be in good condition. Reverse transcription polymerase chain reaction (RT- PCR) was used to evaluate the messenger RNA (mRNA) expression levels of *Pin1* in peripheral blood, and GAPDH was used as a housekeeping gene. Serum parathyroid hormone (PTH) was determined by using ELISA Kit assay. The renal routine test includes serum urea and serum creatinines were detected using an automatic biochemical analyzer (SIMENS ATELLICA, USA). Results revealed that non-Dialysis CKD patients had significantly higher level of *Pin1* gene expression (P >0.01) than Dialysis CKD patient and control. The level of serum parathyroid hormone (S.PTH) was show highly significant increase with (p>0.01) in non-dialysis CKD patient than other group (dialysis and control group) while serum urea (S.Uea) and serum creatinin (S.cr) were show high significant increase with (p>0.01) in dialysis group compare to other study groups. It was concluded a high level of expression of *Pin1* gene in chronic kidney disease with SHP and high protein level of PTH.

Keywords: mRNA, PIN-1 gene, PTH, RT-PCR, CKD, Iraqi population.

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Introduction

Chronic kidney disease (CKD) is a degenerative illness that affects more than 800 million individuals worldwide, or 10% of the total population. People with diabetes mellitus, women, and, people of color, and those with high blood pressure are more likely to have chronic renal disease (1).CKD is described as renal tissue damage and gradual loss of glomerular filtration (GFR) for more than three months at a rate of less than 60 mL/min/1.73 m2.While hemodialysis treatment increases the patient's life expectancy, it also negatively impacts the patient on the physical, emotional, social, and spiritual levels most of the time since it can lead to impairment of mobility, reduced recreation, decreased autonomy, and other factors (2).Additional research has been done on the illness's origins in Iraq (3,4).The peptidyl-prolyl isomerase

family of proteins includes *Pin1* (Peptidyl-Prolyl Cis-Trans Isomerase NIMA Interacting-1).A particular catalytic Ser/Thr-pro amino acid motif controls the conformation of cell cycle proteins, which in turn impacts cell proliferation and differentiation. This alters the target proteins' biological activity, phosphorylation, and turnover (5). During the progression of cancer, *Pin1* induced conformational alterations may act as a vital catalyst for the potentiation of numerous oncogenic signaling pathways. According to several researches, *Pin1* expression plays an oncogenic role in a few common tumors; its overexpression is frequent and distinct in human cancers (6). PTH is the main hormone that the parathyroid glands generate to control calcium and phosphate levels. PTH is initially synthesized as a 115-amino acid polypeptide called pre pro PTH, which is subsequently proteolytically broken down at the Golgi complex within the rough endoplasmic reticulum into pro PTH with 90 amino acids and PTH with 84 amino acids. The major hormone that is stored, secreted, and physiologically active is PTH, which has a molecular weight of around 9500 Dalton (7). A frequent side effect of CKD, PTH output is elevated in secondary hyperparathyroidism (SHP), which results in improper bone and mineral metabolism due to parathyroid gland hypertrophy. In CKD patients, SHP is correlated with cardiovascular morbidity and mortality (8).

Urea is the final byproduct of the liver's nitrogen and protein metabolism. It is a tiny molecule that dissolves in water and is made up of two nitrogen atoms. The glomerulus filters the urea and reabsorbs it in nearby and distant nephrons. A sizeable portion of the filtered urea is absorbed in the nearby tubule's medulla collecting duct. The breakdown of creatinine phosphate into creatinine occurs in muscle tissue. This breakdown product is filtered via glomerulus and a little quantity of it is eliminated through the proximal tubules during glomerular filtration (9). This study aims to evaluate the level of *Pin1* gene expression as well as a number of biochemical markers in CKD patients from Iraq.

Materials and methods

A total of 120 fresh blood samples were collected from the Dialysis Center in Madinat Al-Amamin Al-Kadhimin Teaching Hospital/ Baghdad (From June 2022 to February 2023). All the study experiments were performed at the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies / University of Baghdad. The sample categorized into three groups. Dialysis CKD patient group, Non-dialysis CKD patient group and Control group, each group contains 40 samples. . A tube containing EDTA was used to collect 3 ml of peripheral whole blood from each participant's left cubital fossa veins. These blood samples were preserved using Trizol, and 2ml of the serum was used for a biochemical test.

Biochemical test

SunLong Biotech, China's ELISA Kit assay was used to measure serum PTH, and a SIMENS ATELLICA, USA's automatic biochemical analyzer was used to measure serum Urea and Creatinine.

Gene Expression of *Pin1*

Total RNA was reverse-transcribed to complementary DNA (cDNA) using the EasyScript One-Step gDNA removal and cDNA Synthesis SuperMix Kit (TransGen Biotech Co, China) in a reaction total volume of 20 µl, according to the manufacturer's instructions. The quantitative real-time PCR (qRT-PCR) for *Pin1* gene expression was carried out using the

QIAGEN Rotor gene Q Real-time PCR (Qiagen, Germany), 2 µl of cDNA, 1 µl for each of the forward and reverse primers (10 µM), indicated in (Table 1), and 10µl of the PerfectStartTM Green qPCR SuperMix kit (TransGen Biotech Co, China).Primer 3 Plus software was used to create *Pin1* primers. Reactions were carried out twice for each sample of study groups, and each run also included a no template control (NTC) as a negative control. The thermal profile was as follows: hold at 94 C° for 60 seconds (1 cycle), then 40 cycle: denaturation at 94 C° for 5 seconds, annealing at 58 C° for *Pin1* and *GAPDH* for 15 seconds, and extension at 72 C° for 20 seconds, finally dissociation from 65 C° to 95 C° (5 seconds for 1 degree).Melting curve analyses demonstrated the specificity of the amplified product. In order to determine the relative expression of the *Pin1* gene in the samples from the studied group, the expressions were assessed in relation to the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) reference gene using the 2^{-Ct} technique (10). The data were expressed as the fold change in *Pin1* gene expression in study groups (dialysis and non-dialysis CKD patients) relative to healthy controls and normalized to the expression levels of the reference gene (*GAPDH*).

Table (1): The studies designed primers.

Primer	Sequence (5′→3′ direction)	primer size bp	Product size bp	Ta °C
Pin1 (Gene Expression)				
Forward	AGAAGATCACCCGGACCAAG	20	185	58
Reverse	GGCGTCTTCAAATGGCTTCT			
***GAPDH*- Glyceraldehyde 3-phosphate dehydrogenase**				
Forward	GAAATCCCATCACCATCTTCCAGG	24	160	58
Reverse	GAGCCCCAGCCTTCTCCATG	20		

Statistical analysis

The data was presented as means± SEM .The program that has been used for the statistical analysis was SPSS 26 (SPSS Inc., Chicago, USA). Statistics were judged significant at p value < 0.05. One-way ANOVA was used to statistically analyze the differences between the mean values of the control participants and CKD patients.

Results and discussion

Biochemical test

The current study included measuring some biochemical tests of CKD patients' groups and control group. The results of Serum Blood Urea showed that there was a high significant difference between these three groups with p-value (0.001), and the dialysis CKD patient had highest difference from control in comparison to non-dialysis CKD patient. The means of non-dialysis CKD patient, dialysis CKD patient and control group were (87.10 ±4.71), (127.48 ± 5.15) and (25.95 ±0.79) mg/dl respectively (Table 2).

This result agreed with previous study (11) that explained the patients with CKD have higher urea levels. Because kidneys are responsible for excreting urea, blood urea nitrogen levels are directly related to renal excretory function. Due to the kidney's inability to eliminate urea in CKD, the blood's content of urea rises (12). The injury to the kidney that causes tubular necrosis and the lack of filtering function is the cause of this failure to excrete urea. Kidney damage from medication is also a possibility. Due to the slow rate of renal excretion, the

dehydration that results from CKD also affects the level of urea by elevating it(13). The result of serum creatinine showed that there was a high significant difference between these three groups with p-value (0.001), and the dialysis CKD patient had highest difference from control in comparison to non-dialysis CKD patient. The means of non-dialysis CKD patient, dialysis CKD patient and control group were (2.93 ± 0.32), (9.63 ± 0.44) and (0.320 ± 0.02) mg/dl respectively. These findings were supported by a prior study that assessed creatinine in CKD patients (14). The decrease in GFR and the production of urea from protein and nucleic acid degradation are the causes of the elevated levels of urea and creatinine. The glomerulus is supposed to clear the creatinine that results from the breakdown of phosphocreatine in the muscles, but in cases of CKD, it increases in an opposite connection to GFR (15). The kidneys filter creatinine, which is not reabsorbed and expelled but rather is affected by diet and muscle mass (16). The result of serum parathyroid hormone also showed that there was a high significant difference between these three groups with p-value (0.001), and the non- dialysis CKD patient had highest difference from control in comparison to dialysis CKD patient. The means of non- dialysis CKD patient, dialysis CKD patient and control group were (108.63 ± 14.69), (75.64 ± 6.27), and (31.43 ± 1.78) pg/ml respectively. The significant elevation in the

PTH level of CKD patients of the present study was agreed with (17, 18).The renal PTH breakdown mechanisms are damaged in CKD, which significantly lengthens the half-life of PTH fragments in the circulation. Moreover, kidney damage prevents PTH from being metabolized in the peritubule. Reduced cell responsiveness to PTH action, which results in increased PTH generation, is another mechanism contributing to hyperparathyroidism. According to reports, the calcium response to PTH infusions is reduced; therefore more PTH secretion is required to maintain normal calcium levels. PTH resistance can also result from a variety of factors, such as inadequate vitamin D activity in conjunction with hyperphosphatemia (19).

Table (2): Comparison between Difference Groups in Biochemical parameters.

Group	Mean ± SE		
	B.Urea (mg /dl)	S.Creatinine (mg/dl)	S.PTH (pg/ml)
Group 1: Non-Dialysis	87.10 ±4.71	2.93 ± 0.32	108.63 ± 14.69
Group 2: Dialysis	127.48 ± 5.15	9.63 ± 0.44	75.64 ± 6.27
Group 3: Control	25.95 ±0.79	0.320 ± 0.02	31.43 ± 1.78
P-value	0.0001**	0.0001**	0.0001**

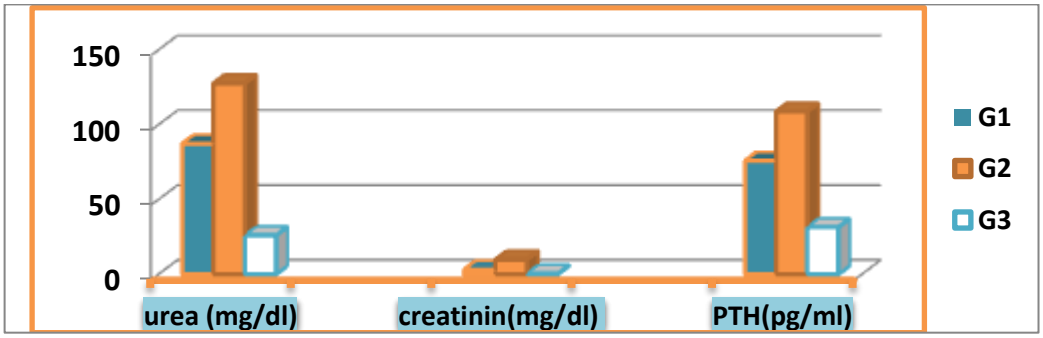

Figure (1): The Comparison between difference groups in serum levels of Urea, Creatinine and PTH

Pin1 Gene Expression

The use of housekeeping genes in molecular investigations is based on the hypothesis that their expression stays stable in the cells or tissues under study (20). One of the housekeeping genes most frequently used in gene expression data companions is *GAPDH* (21). Robert *et al*. (22) investigated the expression of 1,718 genes in 72 various types of normal human tissue using qRT-PCR utilizing *GAPDH* as a reference gene. They found that using *GAPDH* as a normalization method in qRT-PCR is an extremely trustworthy tactic when applied in clinical studies. Additionally, the 2-Ct value and the ratio of 2-Ct of the various study groups to that of the control were used to examine the variance of the overall change in *GAPDH* expression in the various study groups, as shown in (Table 3). The 2^{-Ct} value in Dialysis patient was 6.61E-06, for Non-dialysis CKD patient it was 6.75E-06, while in control it was equivalent to 6.36E-06. The computed ratio for gene fold expression was 1.03, 1.06 and 1.00 respectively. These small variations in gene fold expression between the study groups renders *GAPDH* gene a useful control gene. The level of *Pin1*gene mRNA was various in non-dialysis CKD patient group in comparison to dialysis and controls group with a fold change (5.801), (1.57) and (1.00) respectively. (Table 4) summarizes expression level of *Pin1* gene mRNA in controls and CKD patients groups (non-dialysis and dialysis) by the $2^{-\Delta\Delta Ct}$ method. The findings of this study conflict with those of other studies by (23) that showed low protein levels of *pin1* in the Chinese Han population in Northwest China with CKD and the other study by Nechama *et al*. (24), which discovered reduced *Pin1* activity and elevated PTH mRNA levels and stability in parathyroid extracts from rats with CKD. Both *Pin1* overexpression and *Pin1* knockdown increased the quantity of PTH mRNA in transfected cells. The *Pin1* gene was therefore expressed more frequently in Iraqi patients in this study, which is the first to investigate *Pin1* gene expression in human CKD patients with SHP. The discrepancy between these studies in the level of *Pin1* gene expression distribution may be due to different diseases, sample sizes are small or ethnic admixtures.

Conclusion

According to these findings, *Pin1* gene overexpression and elevated PTH protein levels are present in Iraqi CKD patients with SHP.

Table (3): Comparison between study groups regarding *GAPDH* fold expression levels.

Group	Means Ct of *GAPDH*	2^{-Ct}	experimental group/ Control group	Fold of gene expression
G1:Non-dialysis group	17.176	6.75E-06	6.75E-06/6.36E-06	1.06
G2:Dialysis group	17.206	6.61E-06	6.61E-06/6.36E-06	1.03
G3:control Group	17.261	6.36E-06	6.36E-06/6.36E-06	1.00

Table (4): Fold of Pin 1 expression Depending on 2-ΔΔCt Method.

Groups	*Pin 1* Ct value	ΔCT	ΔΔCT	$2^{-\Delta\Delta Ct}$	experimental group/ Control group	fold	p-value
Non-Dialysis	28.91	11.93	-2.53	5.784	5.784/ 0.997	5.801	0.0001**
Dialysis	32.89	15.86	-0.65	1.57	1.579/0.997	1.574	
Control	31.53	14.27	0.0032	0.997	0.997/ 0.997	1	

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