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Assessment of some neurological complexities in kidney failure disease: evaluation of neuroproteins and neuropeptides

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Abstract

Introduction: Chronic kidney disease (CKD) patients suffer from several neurological complications due to the retention of uremic toxins, electrolytes, and water and the resultant metabolic disturbances. Therefore, the aim of this research is to assess neuropeptide Y (NPY), amyloid beta (Aβ), P-Tau-181, acetylcholinesterase (AChE) and peptidyl-prolyl cis-trans isomerase1 (Pin1) that play important role in neural dysfunction.

Methods: This study was performed on 20 CKD patients and 20 healthy controls who were referred to the hospital. The circular levels of NPY, Aβ, and P-Tau-181 were determined by specific ELISA kits. While, the levels of Pin1 and AChE were determined by western blot analysis in blood samples.

Results and discussion: The obtained results show that NPY was significantly lower ($P<0.001$) and p-tau-181 and Aβ-40 were significantly higher in CKD patients than control subjects ($P<0.001$). CKD patients showed no significant changes in circular AChE content while activity of this enzyme increased remarkably that confirmed cholinergin abnormalities in hyperuricemia condition. Also, based on western blot analysis, the Pin-1 level is significantly lower in CKD patients ($P<0.05$).

Conclusion: In this research, elevated p-tau181, AChE, and Aβ-40 and reduced Pin1 and NPY were associated with renal impairment. Although increased in the activity of AChE is associated with dementia and cognitive impairment, AChE may directly interact with Aβ and cause its deposition. Accordingly, kidney dysfunction could be considered an effective risk factor for dementia development.

Keywords: Chronic Kidney Disease; Neuroproteins; Neuropeptide Y; Dementia related biomarker; hyperuremia imposed neural damages

1. Introduction

Kidney failure disease can be divided into two categories, including acute kidney disease (AKD) and chronic kidney disease (CKD). AKD is a frequent diagnosis with an incidence that varies from 5.0% to 7.5% in hospitalised patients and that reaches up to 50–60% in critically ill patients [1, 2]. CKD refers to a wide spectrum of disease conditions whereby the renal structure and functions are impaired. This leads to a decrease in the glomerular filtration rate

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below 60 mL/min/1.73 m^2, and the resultant retention of uremic toxins in the body. It could be considered as a global health issue, affecting more than 15% of the adult population in developed nations [3]. Once renal damage is initiated, factors including proteinuria, hyperglycemia, hypertension, metabolic disturbances, and lifestyle factors contribute to the progression of the disease to end-stage renal disease. With progression of the disease and retention of metabolic wastes, electrolytes, and water in the body, CKD leads to oedema, cardiac failure, arrhythmia, bone disease, changes in pigmentation, insulin resistance, thiamine and calciferol deficiency, liver infection, dyslipidemia, and hyperhomocysteinemia [4]. In CKD, circulating uremic toxins accumulate due to renal dysfunction, so the gut microbiome is changed by the influx of urea and other retained toxins. The altered gut microbiome resulted in an increase in bacteria that produce uremic toxins such as indoxyl sulphate (IS) and p-cresyl sulphate (PCS), which are strong inducers of oxidative stress. In addition, cerebrospinal fluid and brain levels of compounds such as creatinine and methylguanidine are substantially elevated in uremic patients. Interestingly, these high toxin concentrations (up to 10-fold higher in CKD patients than in controls) were found in brain regions that play a determinant role in cognition, such as the thalamus, mammillary bodies, and cerebral cortex [5, 6]. Therefore, the resulting uremic toxin disturbance in CKD patients affects the nervous system, which results in neurological complications [5]. The pathogenesis of the neurologic complications of CKD and its uremic syndrome is multifactorial. Accumulation of uremic toxins due to increased production and inability of the kidney to excrete endogenous and exogenous toxins (e.g., guanidine compounds) results in neurotoxicity, blood-brain barrier (BBB) injury, ischemic/microvascular changes, neuroinflammation, oxidative stress and apoptosis, disturbance of brain neurotransmitter amino acid balance, vascular autoregulation dysfunction, brain oedema, and brain metabolism dysfunction [7]. Hyperammonemia also disrupts cerebral homeostasis and induces neural cell death, which leads to the development of neurological abnormalities [8].

Hyperammonemia refers to a condition characterised by elevated levels of ammonia in the bloodstream. When ammonia levels rise, particularly in cases where the liver is unable to properly metabolise ammonia, it can enter the brain and disrupt its normal functioning. Hyperammonemia disrupts the delicate homeostasis of the brain and has toxic effects on neural cells, ultimately leading to the development of neurological abnormalities. Understanding the mechanisms and consequences of hyperammonemia is crucial in the diagnosis, management, and treatment of conditions associated with elevated ammonia levels, aiming to mitigate the neurological consequences and improve patient outcomes. Therefore, CKD patients suffer from several neurological complications, including anxiety, depression, motor abnormalities (restless-leg syndrome; RLS), sleep disturbances, and cognitive dysfunctions [9]. According to previous results, acetylcholinesterase (AChE) plays a crucial role in cholinergic neurotransmitter systems and is responsible for terminating the nerve impulses at cholinergic synapses by splitting the neurotransmitter acetylcholine into choline and acetate. Cognitive decline is known to be caused by cholinergic deficiency, including a decrease in the activity of AChE in the brain [10, 11]. A raise in the activity of AChE in the cortex and hippocampus of the brain has been implicated in dementia and cognitive impairment in Alzheimer's disease (AD)

patients [12], which may thus explain the mechanism underlying the cognitive decline in CKD. Also, neuropeptide Y (NPY) has a modulatory role in learning and memory and is involved in the pathophysiology of neurodegenerative diseases. It is a neurotransmitter that affects different organs, from the central nervous system to the cardiovascular (CV) system, the bone, and the kidney [12, 13]. Moreover, phosphorylated tau (p-tau) and amyloid-β (Aβ) which are biomarkers of the most popular cognitive decline named Alzheimer disease (AD) [14-16], reduced kidney function could be associated with increased these biomarkers. Peptidyl-prolyl cis-trans isomerase NIMA-interacting-1 (Pin1) is also one of the main players in the tau phosphorylation and aggregation processes [17, 18]. Thus, the present study was undertaken to assess some neuroactive proteins and peptides that mainly play a role in dementia-related disease to survey the possible mechanism for the cognitive decline observed in CKD. By considering the crucial role of AChE, NPY, Pin1, P-tau-181, and Aβ in neural functions and cognitive ability, assessment of them could elucidate the possible mechanism involved in neuropathological signs of CKD.

2. Material and methods

2.1. Participant

This study aimed to evaluate neuroproteins and neuropeptides. For this purpose, 20 CKD and 20 age-matched healthy people as controls were selected, and the levels of neuroproteins were determined and statistically compared to the non-CKD samples as controls. Patients who referred to the hospital in martyrdom, Dr. Firoz, Wasit Governorate (Iraq) from 2021-2022 and had the following criteria were offered enrollment in the study: (1) age 30-75 years; (2) CKD stage 3 or 4; and (3) serum creatinine >2.5 mg/dL and estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2. The following exclusion criteria were used: pregnant or nursing women; antibiotic treatment at the time of screening or within 14 days before screening; refusal to sign the informed consent form; active dependency on drugs or alcohol; HIV/AIDS/liver disease. Kidney function was defined categorically as normal (eGFR≥90 mL/min/1.73 m2), mildly decreased (eGFR:60-89 mL/min/1.73 m^2), impaired (eGFR<60 mL/min/1.73 m^2) and kidney failure (eGFR less than 30 mL/min/1.73 m^2). Control patients were included when they had an eGFR of ≥60 ml/min/1.73 m2 for ≥3 months and no sign of structural or functional kidney abnormalities. All patients underwent a standardised interview to collect socio-demographic and medical history data, followed by a physical and neuropsychological examination as well as laboratory tests. Blood samples (10 ml) were collected from peripheral veins into K-EDTA tubes, plain tubes, and Na-citrated tubes. Serum was separated immediately after clotting by centrifugation at 8000 rpm for 10 min, and the levels of neuroproteins and neuropeptides were determined by the ELISA kit.

2.2. Neuroproteins and neuropeptide measurement by using ELISA kit

Human AChE (ab138871, Abcam, UK), Human NPY ELISA Kit (LS-F25742), Pin-1 Elisa kit (LS-F39041), P-Tau-181 Elisa kit (A46739), Aβ40 Elisa kit (MBS760432) were used in this research. Also, a centrifuge instrument (KK, China) was used to obtain serum samples.

2.3. Western blotting analysis

Pin1 and AChE were assessed using the western blotting method, which explained, according to Towbin et al. [19], that after SDS-PAGE, the proteins were transferred onto the PVDF membrane actively at 140 V for 1.5–2 h in the transfer buffer. After completion of the transfer, the membrane was

washed four times in TBST (50 mM Tris, pH 7.5, 150 mM NaCl, 0.05% Tween 20) and then blocked in 5% BSA in TBST for overnight at 4 °C. The membrane was washed four times in TBST again and probed with the primary specific antibody (1:1,000) (anti-Pin1 and anti-AChE antibodies from Abcam) in blocking buffer for 2 h at room temperature. The membrane was then washed four times in TBST and incubated with a secondary related IgG (1:5000) (Thermo Scientific) in TBST for 1 h at room temperature. After this step, the membrane was washed four times again in TBST. β-Actin (1:1,000) (Cell Signalling Technology) was used as a housekeeping control. Bands containing rat hippocampus proteins were visualised using an ECL detection system, according to the manual. The densities were calculated using Image J 1.46r; Java 1.6.0_20 software.

2.4. AChE activity measurement

AChE activity (0.7 μg) was spectroscopically monitored (λ= 430 nm) in 20 mM phosphate buffer (pH 7.4) at 310 K by production of 5-thio-2-nitrobenzoate anion with a UV visible spectrometer (T-60, PG Instruments LTD., Leicestershire, UK). In this assay, acetylcholine iodide as substrate was converted to thiocholine that reacts with 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) and produces 5-thio-2-nitrobenzoate. The final concentrations of DTNB and substrate were 0.33 mM and 1.56 mM, respectively. The enzyme activity was expressed by U/mg protein as specific activity [20].

2.4. Statistical analysis

The results of this study were analyzed using SPSS 26 statistical software using Chi-square ($\chi 2$), logistic regression, t-test and analysis of variance (ANOVA) methods with a significance level of $P < 0.05$.

3. Results

3.1. Neuroactive peptides changed as a result of CKD

Fig. 1a shows the level of NPY in CKD and healthy control subjects. Based on the results, NPY was significantly lower in CKD patients compared to control subjects (14.31±6.55 versus 26.83±4.90 pg/ml in CKD and control, respectively, $P<0.001$). Fig. 1b shows the level of p-tau-181 in CKD and healthy control subjects. Based on the results, p-tau-181 level is significantly higher in CKD patients than control subjects (7.04±2.58 versus 2.50±0.70 pg/ml in CKD and control, respectively, $P<0.001$). Fig. 1c shows the level of Aβ-40 in CKD and healthy control subjects. Based on the results, Aβ-40 level is significantly higher in CKD patients than control subjects (118.64±20.71 versus 53.00±15.57 pg/ml in CKD and control, $P<0.001$, respectively).

3.2. Circular content of AChE and its catalytic function

Our results revealed AChE and its catalytic function content of blood serum related to CKD patients increased amsignificantly (Fig. 2). AChE content of blood serum was measured as 284.36±18.78 nmol/min.ml while control showed its concentration is 203.06±7.99 nmol/min.ml ($P<0.001$) (Fig. 2a). However, the obtained results of western blot analysis in Fig. 2b and c shows that there is no significant difference between AChE level in patient and control subjects ($P\geq0.05$).

3.4. Pin1 concentration in blood serum

The unique enzyme Pin1 catalyses cis-trans isomerization by forming an association with the pSer/Thr-Pro motif. In the present work, the serum level of Pin1 and its expression in CKD patients was evaluated using ELISA and western blotting method. The obtained results are shown in Fig. 3. The examination of the ELISA results showed that the CKD patients had significantly lower serum levels of Pin1 (35.41 pg/mL) than the control group (79.05 pg/mL). In addition, the outcomes of the the western blot method depicted that the expression of Pin1 in CKD patients was

significantly lower than that of control group (P<0.001).

4. Discussion

It has been reported that the neurological consequences that CKD patients suffer include anxiety, sadness, movement abnormalities, sleep problems, and cognitive impairments. Therefore, for effective disease management, it is crucial to investigate the mechanisms underlying the neurological side effects of CKD. The aim of this research was to assessment of some neurological complexities in CKD patients. The obtained results in this research show that NPY level was significantly lower and p-tau-181, AChE and Aβ-40 levels were significantly higher in CKD patients than control subjects (P<0.001). Moreover, based on western blot analysis the level of Pin1 was significantly lower in CKD patients compared to control subjects and for AchE, there is no significant difference between CKD and control subjects (P>0.05). Cognitive dysfunction increases in prevalence with CKD severity, potentially affecting up to 60% of CKD patients [21].

Consistent with current findings, a study by Nechama et al. (2009) found that Pin1 activity was decreased in CKD rats [22]. Phosphorylation of Pin1 isomerase at Ser16 and Ser71, which respectively impair the enzyme's ability to connect with target proteins and catalyse isomerase activity, controls the enzyme's activity and stability [23]. In this regard, Hasan et al. (2021) investigated Pin1 phosphorylation in CKD patients and discovered a higher phosphorylation level of Pin1 in CKD patients [24]. Therefore, it can be concluded that Ser16 and Ser71 phosphorylation can occur in CKD patients, which, as a result, reduces the activity and stability of this protein. Pin1 is a key regulator of parathyroid hormone (PTH) mRNA stability, so that when Pin1 activity is reduced, PTH mRNA stability is increased and degradation is decreased, resulting in elevated levels of iPTH [25, 26]. Secondary hyperparathyroidism (SHP) is a common complication of CKD characterized by increased PTH secretion and parathyroid gland hyperplasia resulting in deranged bone and mineral metabolism [27]. In CKD patients, levels of PTH are progressively elevated as kidney function declines, as a result of skeletal resistance to the actions of PTH [28].

Depletion in the activity of AChE is one of the early and consistent findings in Alzheimer's disease (AD) patients with dementia and cognitive impairment [10, 29]. In agreement with the previous reports, the results of our study indicated a decrease in the activity of AChE in CKD patients [30, 31]. The mechanism underlying the cognitive loss in CKD may thus be explained by the fact that dementia and cognitive impairment in AD patients have been linked to decreased AChE activity in the brain's cortex and hippocampus. In vitro study by Prall et al. further showed increased AChE activity in red blood cells (RBCs) of patients with CRF, however, the results were insignificantly different in the various density-based age subfractions of RBCs in both CRF patients and controls [31]. However, although treatment of AD has been dominated by the use of AChE inhibitors, research shows that AChE itself has been implicated in the pathogenesis of AD, so it appears that AChE may directly interact with Aβ in a manner that increases the deposition of this peptide into insoluble plaques [32]. It has been proposed that decreasing AChE may help prevent the development of Aβ plaques and so serve as a fundamental component of a disease-modifying strategy, this notion has not been supported by any solid evidence. Researches shows that the kidney is involved in peripheral clearance of Aβ, which plays a central role in the pathogenesis of AD, and dialysis might be a potential therapeutic approach of Aβ removal [33, 34]. Therefore,

it could be expected that CKD patients who were receiving dialysis had lower serum Aβ levels than patients without receiving dialysis. Furthermore, serum Aβ levels were correlated with renal functions reflected by eGFR and residual GFR (rGFR) [35]. Previous reports have suggested that CKD might influence plasma p-tau181 concentrations [36-38]. Plasma p-tau181 is specific for AD neuropathology and increased 3-fold in AD [39]. Our results clearly showed that up regulated of p-tau181 observed in CKD patients shows that reduced kidney function was associated with increased levels of dementia-related blood biomarkers therefore, kidney function might influence the accuracy of dementia-related blood biomarkers.

In accordance with previously reported results, our findings revealed that the serum levels of NPY were reduced in CKD patients [40]. However, these results are in contrast to clinical observations in patients with renal abnormalities, where serum levels of NPY are increased [41-43]. The observed discrepancy may be due to some factors. Chronic sympathetic hyperactivity, high blood pressure, and renal scarring can motivate the generation of NPY in CKD patients. On the other hand, the observed variation can potentially be caused by the source of NPY production [40].

NPY is one of the most abundant peptides of the central and peripheral nervous systems, with a significant role in energy homeostasis [44, 45]. Although its expression and release into plasma is largely associated with sympathetic nervous system (SNS) activity, several cells are now known to respond to NPY, and dysregulation of the NPY system is implicated in several pathological conditions including obesity [44], cardio-vascular disease [46], diabetes [47], and insulin resistance [48], conditions which are also often accompanied by albuminuria and CKD. Interestingly, although the pathogenic involvement of NPY in albuminuric kidney disease has not previously been explored, increases in circulating NPY are observed in both the plasma and urine of type 1 and type 2 diabetic patients with nephropathy [49], and the common Leu7pro7 polymorphism in the NPY gene, thought to be associated with increased peptide secretion [50], has been associated with proteinuria and an increased susceptibility to nephropathy in type 1 diabetic patients [51]. Recently, it has also been shown that increased plasma NPY levels are associated with proteinuria, faster progression of CKD, and a higher risk of kidney failure in two independent European cohorts of CKD [12]. However, Lay et al., in 2020 reported that NPY is down-regulated in insulin-resistant vs. insulin-sensitive mouse podocytes and in human glomeruli of patients with diabetic kidney disease [43]. This contrasts with the increased NPY levels that are commonly observed in CKD patients.

4. Conclusion

In summary, in the present work, we investigated the serum levels of different factors including NPY, Pin1, p-tau-181, Aβ-40 and AChE activity in CKD patients. We found that in the studied CKD patients NPY and Pin were significantly lower ($P<0.001$) and p-tau-181, Aβ-40 and AChE activity were significantly higher than control subjects ($P<0.001$). Moreover, based on western blot analysis the level of Pin1 was significantly lower in CKD patients compared to control subjects and for AchE, there is no significant difference between CKD and control subjects ($P>0.05$). Despite the recognized association between cognitive conditions and renal failure, direct evidence connecting brain injury to CKD is still absent. These findings suggest that although depletion in the activity of AChE is associated with dementia and cognitive impairment, however, researches show that AChE overactivity may directly cause Aβ fibrillation and plaque formation. According

to the results, the molecular events that take place in hyperuremia condition and its associated diseases are linked with initiation and development of AD so, CKD could lead to dementia later in older age. Our results also could be used to improve neurological side effects of hyperuremia in patients after additional investigation.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Figure Legends

Fig. 1. Circular concentration of neurohormones in CKD patients (case) and controls. (A) Neuropeptide Y concentration of blood serum decreased in CKD patients rather than control. (B) p-tau-181 concentration in blood samples related to CKD patients (case) remarkably increased rather than control. (C) Aβ-40 also increased in CKD patients significantly. All data were expressed as mean ± SD. P value was shown on the column in comparison of each data and $P<0.001$ is significant.

Fig. 2. AChE activity and concentration in CKD patients (case) and control. Results showed significant increase of activity of AChE in CKD patients in comparison with control (A). Western blot analysis (B) and normalized intensity of bands (C) for AChE content of blood serum in control and CKD patients. 1, 2 and 3 lans represent AChE concentration in CKD patients and 4, 5 and 6 related to control samples. Western blot results display no significant difference expression of AChE content of blood serum in control and CKD. As a loading control, β-actin was utilized. Quantification of immunoblotting bands were represented in plot after normalization. ($P> 0.05$).

Fig. 3. Evaluation of Pin1 in circulation of CKD patients and control. Results showed significant reduction of Pin1 in samples CKD group in comparison with control. (A) shows Pin 1 concentration in blood serum of experimental groups was evaluated by ELISA method. Western blot analysis of Pin1 expressions in CKD (B) and normalized intensity of bands for Pin1 content of blood serum in control and CKD patients (C). β-actin was used to control of protein concentration in lanes. Lane 1, 2 and 3 are biological replicates of CKD while lane 4, 5 and 6 related to control. Intensity of bands quantified by using ImageJ software in the right plot. Data were expressed as mean ± SD.

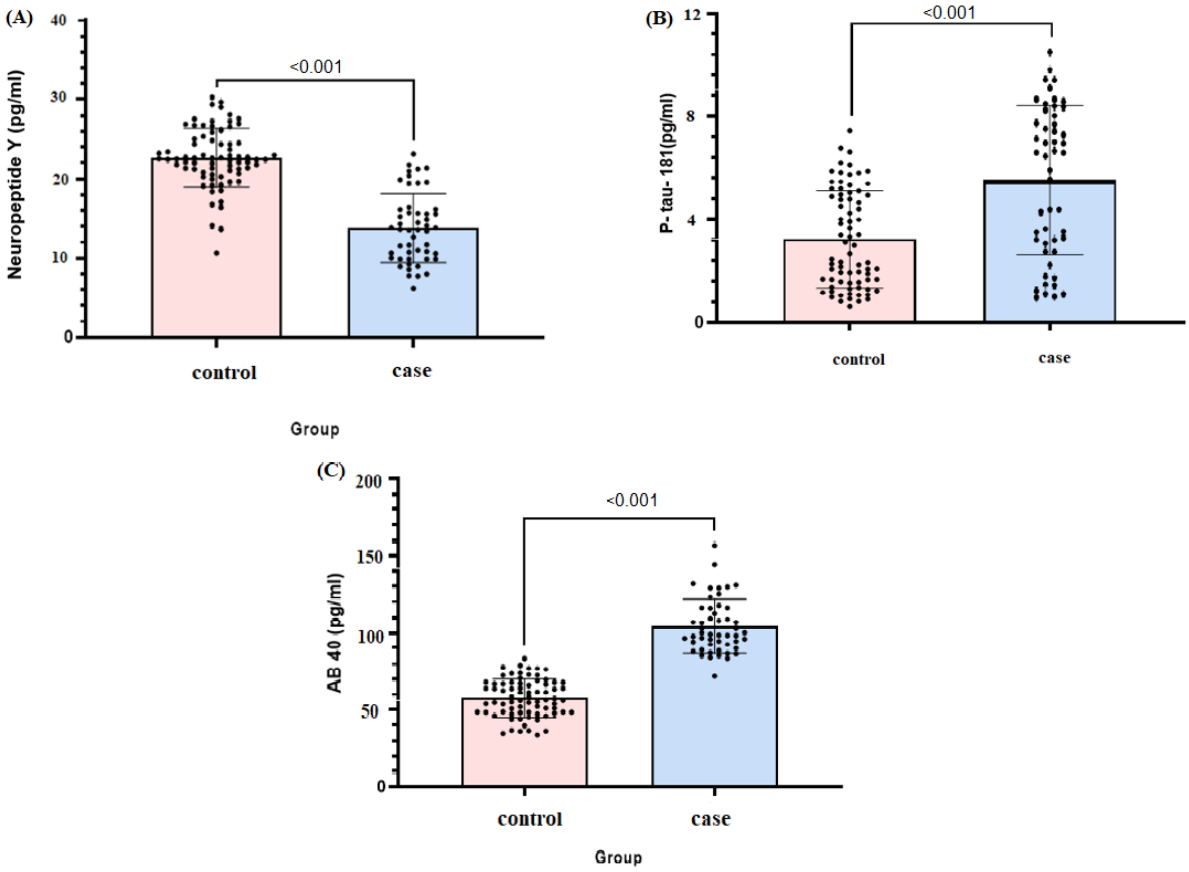
(A)
Neuropeptide Y (pg/ml)
40
30
20
10
0
<0.001
control
case
Group
(B)
P- tau- 181(pg/ml)
12
8
4
0
<0.001
control
case
(C)
AB 40 (pg/ml)
200
150
100
50
0
<0.001
control
case
Group

Fig. 1

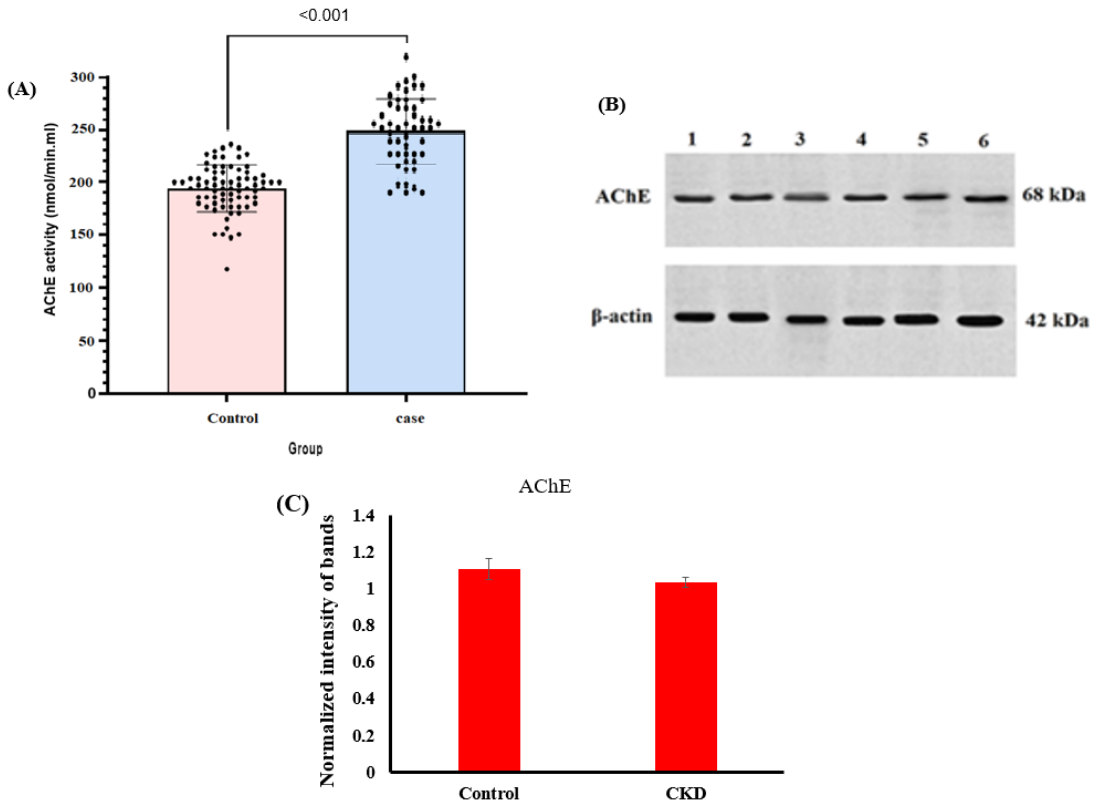
(A)
<0.001
AChE activity (nmol/min.ml)
300
250
200
150
100
50
0
Control
case
Group
(B)
1
2
3
4
5
6
AChE
68 kDa
β-actin
42 kDa
(C)
AChE
Normalized intensity of bands
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
CKD

Fig. 2

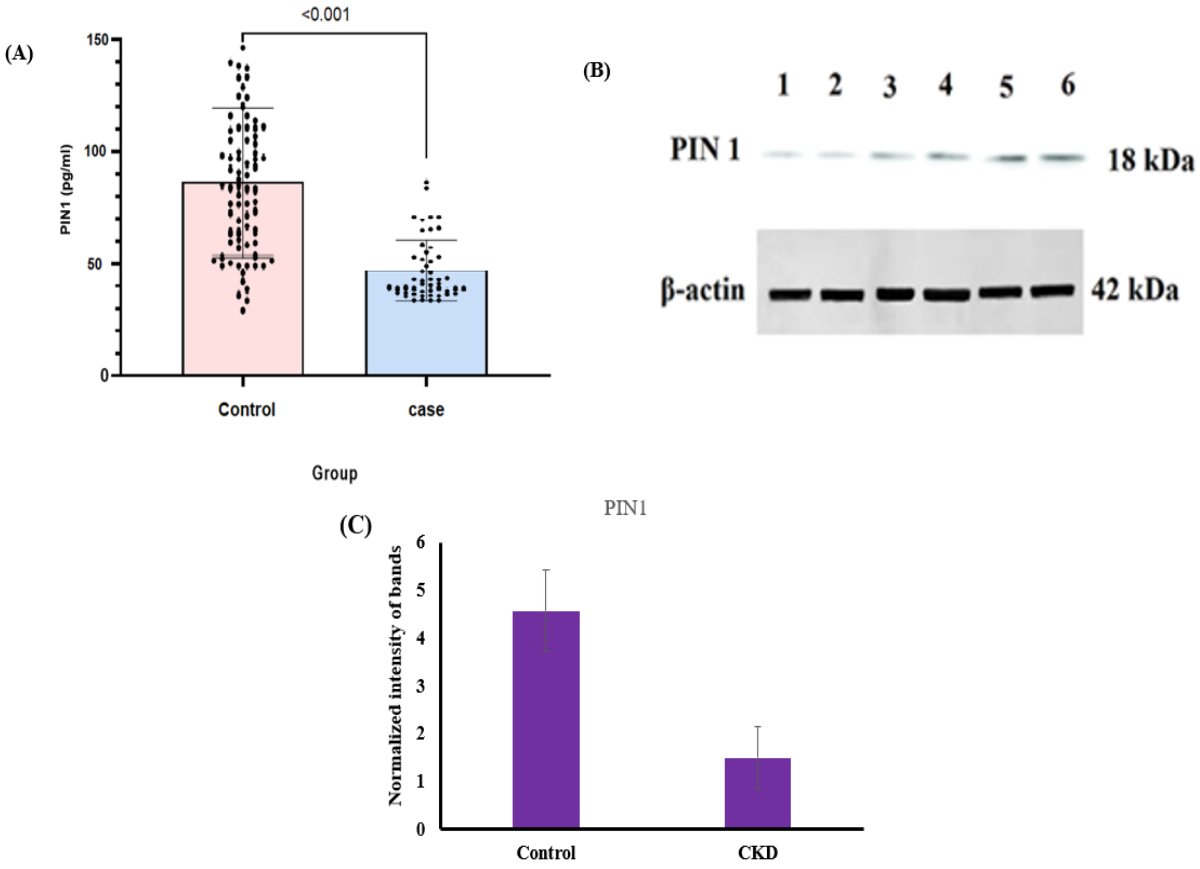
(A)
<0.001
150
100
50
0
PIN1 (pg/ml)
Control
case
Group
(B)
1 2 3 4 5 6
PIN 1
18 kDa
β-actin
42 kDa
(C)
PIN1
Normalized intensity of bands
6
5
4
3
2
1
0
Control
CKD

Fig. 3