Clinical and Experimental Nephrology (2021) 25:522–530
https://doi.org/10.1007/s10157-021-02023-w

ORIGINAL ARTICLE

Association of serum lncRNA H19 expression with inflammatory and oxidative stress markers and routine biochemical parameters in chronic kidney disease

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Received: 6 November 2020 / Accepted: 16 January 2021 / Published online: 6 February 2021

Abstract

Background Chronic kidney disease (CKD) is a disorder that affects millions worldwide, and current treatment options aiming at inhibiting the progression of kidney damage are limited. Long noncoding RNA (lncRNA) H19 is one of the first explored lncRNAs and its deregulation is associated with renal pathologies, such as renal cell injury and nephrotic syndrome. However, there is still no research investigating the connection between serum lncRNA H19 expressions and clinical outcomes in CKD patients. Therefore, we investigated the relation of serum lncRNA H19 expressions with routine biochemical parameters, inflammatory cytokines, oxidative stress and mineralization markers in advanced CKD patients.

Methods lncRNA H19 serum levels from 56 CKD patients and 20 healthy controls were analyzed with reverse-transcription quantitative polymerase chain reaction method. Serum tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and osteocalcin (OC) levels were measured with enzyme linked-immunosorbent assay. Total antioxidant status (TAS) and total oxidative status (TOS) levels were evaluated by the routine measurement method.

Results We found that lncRNA H19 expressions were upregulated in patients with CKD compared to the controls. Furthermore, lncRNA H19 relative expression levels showed a negative relationship with glomerular filtration rate (GFR) while it was positively correlated with ferritin, phosphorus, parathyroid hormone, TNF-α, IL-6, OC, TAS and TOS levels.

Conclusion lncRNA H19 expressions were increased in CKD stage 3–5 and HD patients, and elevated lncRNA H19 expressions were associated with decreased glomerular filtration rate, inflammation, and mineralization markers in these patients.

Keywords Chronic kidney disease · lncRNA H19 · TNF-α · IL-6 · Oxidative stress

Introduction

Chronic kidney disease (CKD) is a widely seen disorder that affects millions worldwide and is characterized by the decline in renal function and glomerular filtration rate (GFR) [1]. Prevalence of CKD is globally rising, and CKD continues to contribute to adverse clinical outcomes and increased hospitalizations [2]. CKD not only causes higher morbidity and mortality but also decreases the quality of life and increases expenditure in the health care systems [3]. Therefore, a better understanding of the pathogenesis of CKD is crucial to the identification of novel therapeutic and diagnostic biomarkers. Recently, studies on RNA biology have

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revealed potential novel candidate molecules related to CKD pathogenesis [4].

Long noncoding RNAs (lncRNAs) are RNA transcripts that consist of over 200 nucleotides without protein-coding ability and can act as central regulators in various pathophysiological events in diseases [5]. lncRNAs can control gene expressions by interacting with intracellular molecules, and the dysregulation of lncRNAs expression can be associated with pathogenesis of CKD [4]. At the same time, disease-specific alterations in lncRNAs expressions make lncRNAs attractive candidates for molecular biomarkers that can be used in the early diagnosis and treatment of CKD [4]. The elucidation of lncRNAs' role in CKD pathogenic molecular mechanisms could aid us in the discovery of new diagnostic and therapeutic approaches. Although advances in RNA-sequencing technologies continue to expand our understanding of the fascinating lncRNA world, the potential role of lncRNAs in patients with advanced CKD (stage 3–5) and hemodialysis (HD) is not exactly known. lncRNA H19 is one of the first explored lncRNAs and its deregulation is associated with the renal pathologies, such as fibrosis, renal cell injury, diabetic nephropathy and nephrotic syndrome [6–9]. *Xu* et al. unveiled that circulating lncRNA H19 expressions were decreased in children with nephrotic syndrome [7]. *Fan* et al. suggested that lncRNA H19 expressions were higher in patients with diabetic kidney disease than diabetic patients without nephropathy [9]. These studies highlight the potential importance of lncRNA H19 for CKD. However, there is still no research investigating the connection between serum lncRNA H19 and health outcomes in patients with advanced CKD and HD.

We hypothesize that serum expression levels of lncRNA H19 may be associated with the pathogenesis of CKD and CKD-related complications, and thus the understanding of the role of lncRNA H19 in CKD may contribute to improving clinical outcomes of CKD. Therefore, in this paper, we investigated the relation of serum lncRNA H19 expressions with routine biochemical parameters, inflammatory cytokines, oxidative stress and mineralization markers in patients with different stages of CKD.

Materials and methods

Subjects

A total of 28 patients with CKD stage 3–5 and 28 patients receiving HD therapy who fulfilled the inclusion/ exclusion criteria were enrolled in this study. A total of 20 age-, gender-, and body mass index (BMI)-matched healthy individuals were consecutively selected from subjects who did not have any systemic disease. Subjects with acute/chronic infection, diagnosis of malignancy, any anomaly of connective tissue, inflammatory disease, using drugs, such as Cinacalcet or Coumadin, chronic liver disease, gastrointestinal hemorrhage or other acute/chronic bleeding disorders and those who are < 18 years old, pregnant, and breastfeeding were excluded from our study. HD patients were on hemodialysis (standard bicarbonate) for 4 h, three times a week. These HD patients were eligible for enrolment if they had been on dialysis for at least 3 months with a stable clinical status. All patients and healthy control group subjects provided their written informed consent before sample and data collection. Our study was approved by the Institutional Review Board of Hatay Mustafa Kemal University and performed in accordance with the Declaration of Helsinki (Approval number: 2019/26). After recruitment, we collected demographic and clinical characteristics, such as age, sex, BMI, underlying renal disease and dialysis duration (for HD patients).

Sample collection and measurements of routine biochemical parameters

Fasting venous blood samples were collected via venipuncture from healthy controls and all patients, and samples were put into regular gel-filled test tubes for serum separation. In HD patients, blood was drawn before the start of the midweek dialysis session. The samples were spun down at 1500×g for 10 min in a pre-cooled (4 °C) centrifuge to obtain serum which was then aliquoted into RNase-free tubes to store at − 80 °C until assays. The measurements of hemoglobin, hematocrit, creatinine, glucose, albumin, ferritin, parathyroid hormone (PTH), calcium, and phosphorous were performed by standard methods. To compute the estimated glomerular filtration rate (eGFR), we used the chronic kidney disease epidemiology collaboration equation (CKD-EPI).

Determination of TAS and TOS Levels

We used an automated measurement method to detect levels of total antioxidant status (TAS) and total oxidant status (TOS) in serum [10, 11]. After analyzing the absorbance of the sera spectrophotometrically using a commercial kit (Rel Assay Diagnostics), we expressed the values as TAS (mmol Trolox Eq/L) and TOS (μmol H_2O_2 Eq/L). Then, we calculated the oxidative stress index (OSI) by dividing TOS values by TAS values. OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq/L)/TAS (μmol Trolox Eq/L) × 100.

Enzyme-linked Immunosorbent Assay (ELISA)

Tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6) and osteocalcin (OC) levels were evaluated from the sera of patients with CKD and healthy controls using commercial ELISA kits. The intra-assay coefficients of variation for

TNF-α, IL-6, and OC were 4.4%, 6% and 8.3%, respectively. The inter-assay coefficients of variation for TNF-α, IL-6, and OC were 7.5%, 6.8% and 8.1%, respectively. The detection range for TNF-α, IL-6, Osteocalcin was 15.6–1000 pg/ml, 4.69–300 pg/ml and 1.2–75 ng/ml, respectively.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

We utilized the RT-qPCR method for the analysis of lncRNA expression levels in our study. We isolated total RNA from 200 µl of serum samples using RNA isolation kit with the instructions by the manufacturer (Qiagen) and then determined spectrophotometrically the concentration and purity of RNA by evaluating the A260/A280 ratio. Next, RNA samples were converted into complementary DNA (cDNA) using cDNA synthesis kit (Qiagen). Subsequently, qPCR analysis was performed on the RotorGene instrument using the SYBR Green master mix kit (Qiagen). qPCR reaction conditions were: 95 °C for 10 min (initial activation), followed by 40 cycles of 95 °C for 15 s (denaturation), 55 °C for 30 s (annealing) and 72 °C for 30 s (extension). lncRNA H19 (PPH05814B-200) and β-actin (PPH00073G-200) primers that specifically designed and experimentally verified for the target genes were purchased from Qiagen. As previously described, lncRNAs expressions were normalized to internal control β-actin, and $2^{-\Delta Ct}$ method was used to calculate relative expression levels of the lncRNAs in both patient and healthy control groups [12].

Statistical analysis

We used Kolmogorov–Smirnov and Shapiro–Wilk tests for normality of data analysis. Depending of the significance of normality tests, either *t* test or Mann–Whitney *U* test was performed to determine the differences between control and patient groups. p value < 0.05 was considered for statistical significance. To show relations between the variables, coefficients of Pearson correlation or Spearman rank correlation tests were used. Receiver operating characteristic (ROC) analysis is commonly used tool for assessment of the performance of diagnostic tests which are crucial for effective therapy in the clinical settings and especially, the area under ROC curve (AUC) presents substantial explanation to compare the healthy controls and patients [13]. We performed the ROC curve to evaluate the potential diagnostic value of lncRNA H19. We employed multiple regression analysis which tests relation between more than one independent variables and a dependent variable. lncRNA H19 was the dependent variable in our model, and we chose the predictor variables considering their significant correlations with the dependent variable among routine lab parameters. In this study, the values were expressed as mean ± SD or mean ± SEM, and we used SPSS package software (version 22) for our statistical analysis. GraphPad Prism, version 6.01 was used for plotting graphs.

Results

Baseline characteristics of patients with CKD and healthy controls

A total of 28 patients with CKD at stages 3–5, 28 HD patients, and 20 healthy controls were enrolled in our study. Table 1 presents the demographic, clinical and biochemical data of the participants. No important differences in age, gender, and BMI values were observed among the groups: healthy control, patients with CKD, patients on HD, and patients with CKD + HD ($p > 0.05$). Serum albumin and calcium levels were similar among all groups ($p > 0.05$). As expected, eGFR levels were lesser, and the mean systolic and diastolic blood pressures, serum phosphorus and C-reactive Protein (CRP) levels were higher in patients with CKD and HD compared to the healthy control group ($p < 0.05$). Also, patients with CKD and on HD had higher levels of serum ferritin and PTH compared to the healthy control group ($p < 0.05$) (Table 1). The mean dialysis duration of patients on HD was 60.81 ± 64.48 months.

Serum lncRNA H19 expression levels

We investigated relative expression levels of lncRNA H19 in CKD patients and healthy controls using the qRT-PCR method. As seen in Fig. 1a, lncRNA H19 expression levels were significantly upregulated in CKD and HD patients compared to the control group, indicating a potential relation of lncRNA H19 in the pathogenesis of CKD ($p < 0.001$). We also performed ROC curve analysis to detect the diagnostic and clinical value of lncRNA H19 for patients with CKD. We found that area under the ROC curve was 0.9170, and 95% confidence interval was 0.8499–0.9840 for lncRNA H19 indicating that lncRNA H19 may have a clinical value for the pathogenesis of CKD ($p < 0.001$) (Fig. 1b). We also compared the lncRNA H19 expression levels in CKD stage 3–5 and HD patients and our results showed that lncRNA H19 expression levels in all patients groups were significantly higher than the control group ($p < 0.01$) (Fig. 1c). We also tested the lncRNA H19 expressions in different underlying kidney diseases and we could not find any significant difference among these diseases (Fig. 1d).

Serum TNF-α, IL-6 and OC levels

We investigated the inflammatory markers TNF-α and IL-6 and bone metabolism marker OC concentrations in sera

Table 1 Clinical and biochemical characteristics of healthy control, CKD, and HD groups

Characteristics	HC ($n=20$)	CKD ($n=28$)	HD ($n=28$)	CKD + HD ($n=56$)	p value
Age (Years)	52.9 ± 7.67	54.17 ± 11.51	55.08 ± 9.36	54.6 ± 10.46	0.900
Gender *n* (%)					
Male	12 (60)	14 (50)	16 (57.1)	30 (53.6)	0.906
Female	8 (40)	14 (50)	12 (42.9)	26 (46.4)	
BMI (kg/m^2)	25.93 ± 2.32	25.25 ± 2.97	26.21 ± 4	25.77 ± 3.56	0.791
eGFR (mL/min/1.73 m^2)	113.03 ± 8.58	26.25 ± 11.46	6.21 ± 2.52	16.23 ± 13.03	**< 0.001**[a,b,c,d]
Underlying kidney disease					
Chronic glomerulonephritis		2	–	2	
Diabetes		11	13	24	
Hypertensive nephropathy		8	19	27	
Unknown		7	4	11	
SBP (mmHg)	120.5 ± 8.25	139.64 ± 24.56	144.64 ± 25.01	142.14 ± 24.69	**0.011**[a]
					0.001[b]
					1.000[c]
					< 0.0001[d]
DBP (mmHg)	75 ± 5.12	84.1 ± 11.3	84.82 ± 11.01	84.46 ± 11.06	**0.008**[a]
					0.004[b]
					1.000[c]
					< 0.0001[d]
Albumin (g/dL)	3.97 ± 0.25	3.88 ± 0.56	3.8 ± 0.31	3.84 ± 0.45	1.000[a,c]
					0.482[b]
					0.222[d]
Ferritin (ng/mL)	52.61 ± 31.81	188.98 ± 166.21	878.03 ± 531.36	533.50 ± 522.51	0.521[a]
					< 0.001[b,c,d]
Ca (mg/dL)	8.80 ± 0.48	8.76 ± 0.75	8.86 ± 0.57	8.81 ± 0.66	1.000[a,b,c,]
					0.959[d]
P (mg/dL)	3.28 ± 0.21	4.16 ± 0.97	5.19 ± 1.72	4.67 ± 1.48	**< 0.05**[a]
					< 0.001[b,d]
					0.007[c]
PTH (pg/mL)	43.82 ± 18.26	217.99 ± 225.32	551.29 ± 394.20	384.64 ± 359.84	0.104[a]
					< 0.001[b,c,d]
Glucose (mg/dL)	84.05 ± 6.66	98.92 ± 25.75	130.07 ± 74.79	114.5 ± 57.61	0.886[a]
					0.005[b]
					0.055[c]
					< 0.001[d]
CRP (mg/L)	1.5 ± 1.03	6.59 ± 5.22	8 ± 8.84	7.29 ± 7.23	**0.021**[a]
					0.002[b]
					1.000[c]
					0.001[d]
Medications *n* (%)					
Beta blockers (%)		7 (25%)	10 (35%)	17 (23%)	
Calcium channel blockers (%)		3 (11%)	11 (39%)	14 (19%)	
RAS blockers (%)		4 (14%)	6 (21%)	10 (13%)	
Diuretics (%)		3 (11%)	9 (32%)	12 (16%)	
Alpha blockers (%)		1 (3%)	4 (14%)	5 (7%)	
Statin (%)		2 (7%)	3 (11%)	5 (7%)	
İnsulin (%)		7 (25%)	5 (18%)	12 (16%)	

HC Healthy control, *CKD* Chronic kidney disease, *HD* Hemodialysis, *BMI* Body mass index, *eGFR* Estimated Glomerular Filtration Rate, *SBP* Systolic Blood Pressure, *DBP* Diastolic Blood Pressure, *Ca* Calcium, *P* Phosphorus, *PTH* Parathyroid hormone, *CRP* C-reactive Protein

The values were expressed as mean ± SD and significant statistical p values were indicated as bold

[a]Comparison between Healthy subjects and CKD patients

[b]Comparison between Healthly subjects and HD patients

[c]Comparison between CKD and HD patients

[d]Comparison between Healthy controls and CKD + HD patients

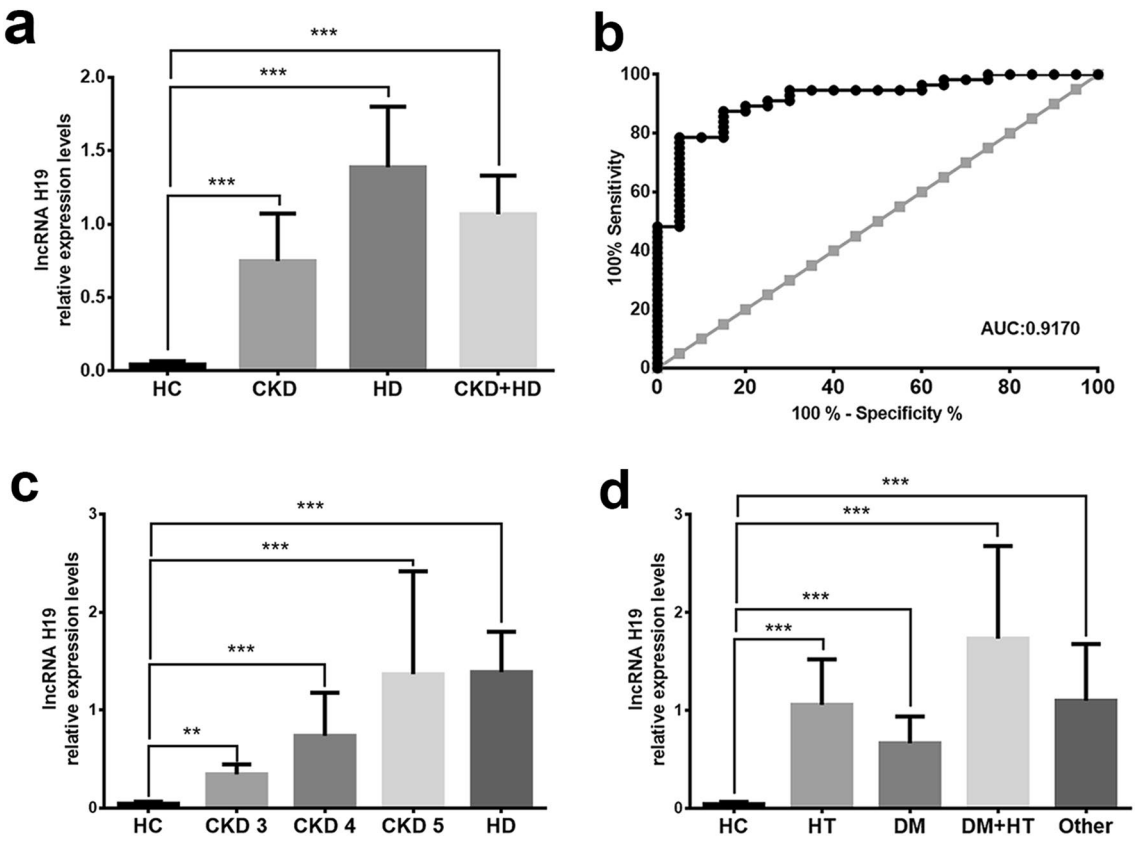

Fig. 1 The relative expression levels of lncRNA H19 (**a**) and ROC analysis depicting the diagnostic value of lncRNA H19 in CKD patients (**b**). Comparison of lncRNA H19 expression levels in CKD stages 3–5 and HD (**c**). Comparison of lncRNA H19 expression levels according to underlying kidney disease (**d**). *HC* Healthy control, *CKD* Chronic kidney disease, *HD* Hemodialysis. Data were presented as mean ± SEM. $p < 0.05$ values were considered as significant. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

of patients with CKD using the ELISA. We detected that serum TNF-α, IL-6 and OC levels were elevated significantly in all CKD groups (CKD stage 3–5 and HD), suggesting that these markers might be closely associated with CKD pathogenesis ($p < 0.01$, Fig. 2).

Serum TAS and TOS levels and calculated OSI values

We measured serum TAS and TOS levels of patients with CKD and calculated OSI levels by dividing TOS by TAS. TAS levels were significantly higher in HD and CKD + HD groups ($p < 0.05$), while serum TOS levels were significantly elevated in all patient groups ($p < 0.001$). OSI levels were significantly higher in patients with CKD and HD, indicating that increased systemic oxidative stress may be associated with CKD pathogenesis ($p < 0.05$, Fig. 3).

Association of lncRNA H19 with eGFR and biochemical and oxidative stress parameters

We analyzed the relationship of serum lncRNA H19 levels with eGFR and some routine biochemical parameters in CKD patients. As shown in Table 2, we detected that serum lncRNA H19 relative expression levels were negatively correlated with eGFR ($r = -0.581$, $p < 0.001$). In addition, we found that lncRNA H19 levels were positively related to ferritin ($r = 0.512$, $p < 0.001$), P ($r = 0.378$, $p = 0.001$) and PTH ($r = 0.450$, $p < 0.001$). Besides, we detected that lncRNA H19 expressions were positively correlated with TAS ($r = 0.325$, $p = 0.004$) and TOS ($r = 0.299$, $p = 0.009$) levels. However, we did not find any correlation between lncRNA H19 with OSI ($p = 0.859$, Table 2). Furthermore, our multiple regression analyses revealed that 20% of the alterations in lncRNA H19 levels might be explained by eGFR, ferritin, PTH, P, and CRP, and our model was statistically significant ($p = 0.001$). Also, our multivariate analysis demonstrated that ferritin, PTH, and CRP significantly

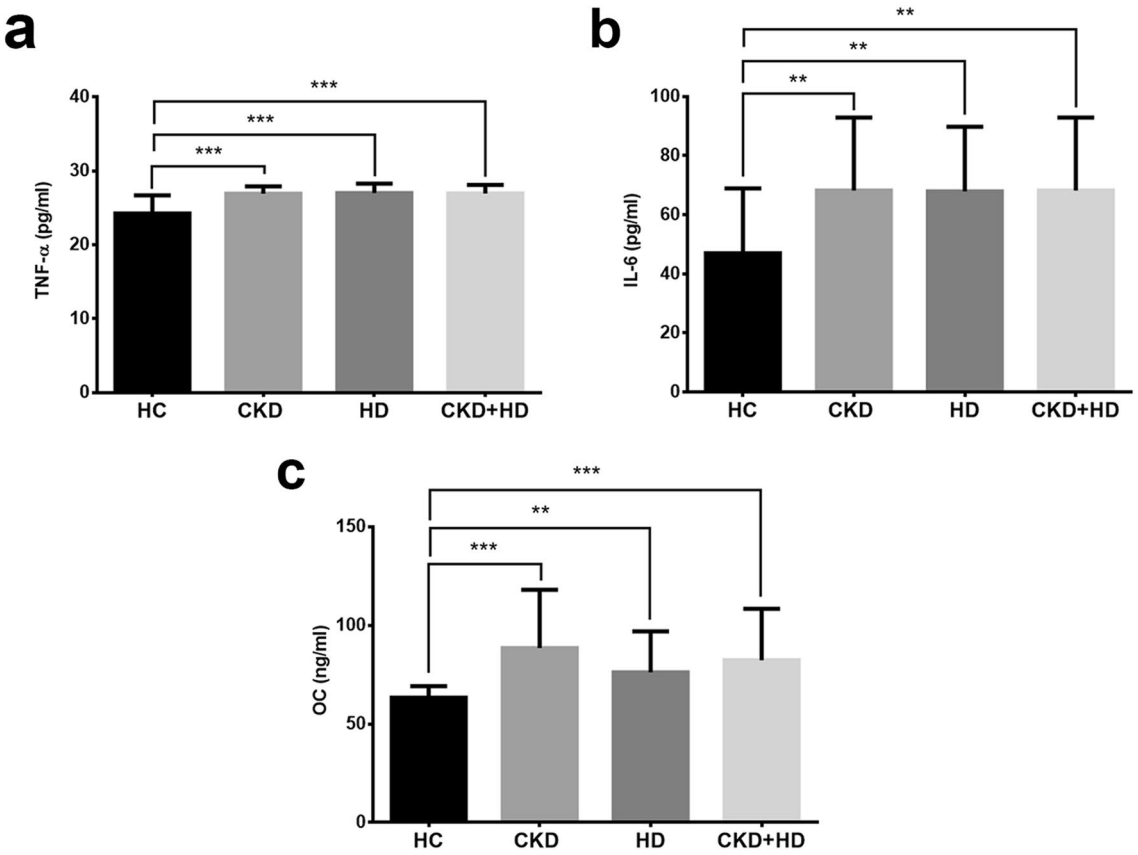

Fig. 2 Serum TNF-α (**a**), IL-6 (**b**) and OC (**c**) levels in healthy controls and patients with CKD. *HC* Healthy control, *CKD* Chronic kidney disease, *HD* Hemodialysis. Data were presented as mean ± SD. $p < 0.05$ values were considered as significant. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

contributed to the changes in the concentrations of lncRNA H19 (Table 3).

Association of lncRNA H19 with TNF-α, IL-6, and OC

We performed a correlation analysis to evaluate the relationship of lncRNA H19 with inflammatory cytokines and bone metabolism parameters. lncRNA H19 relative expression levels were positively correlated with TNF-α ($r = 0.456$, $p < 0.001$), IL-6 ($r = 0.344$, $p = 0.002$) and OC ($r = 0.355$, $p = 0.002$) levels in CKD patients, suggesting that lncRNA H19 may be associated with inflammation and disordered bone metabolism in patients with CKD (Table 2).

Association of TNF-α, IL-6 and OC Levels with eGFR and biochemical parameters

We analyzed the association of serum TNF-α, IL-6 and OC levels with eGFR and routine biochemical parameters. TNF-α ($r = -0.309$, $p = 0.007$), IL-6 ($r = -0.337$, $p = 0.003$) and OC ($r = -0.307$, $p = 0.007$) concentrations were positively correlated with eGFR levels in CKD patients (Table 2). TNF-α ($r = 0.313$, $p = 0.006$) and OC ($r = 0.251$, $p = 0.029$) levels were positively correlated with PTH levels while IL-6 ($r = 0.237$, $p = 0.039$) levels were positively correlated with phosphorus levels in patients with CKD. There were no significant correlations between TNF-α, IL-6 and OC levels and OSI (Table 2).

Discussion

CKD is a crucial health concern, and its existing therapy is challenged by undesired side effects and poor clinical outcomes. Thus, elucidating pathogenesis and defining novel diagnostic and therapeutic molecules will contribute to early diagnosis and improve the current treatment of CKD [2–4]. The deregulated lncRNAs' expressions in human blood are associated with acute kidney injury and nephrotic syndrome, suggesting that lncRNAs might have a potential role in kidney diseases [4, 7, 14]. Although several studies focused on the role of lncRNA H19 in kidney diseases, until now, there has been no study investigating the clinical value of serum lncRNA H19 in patients with advanced CKD.

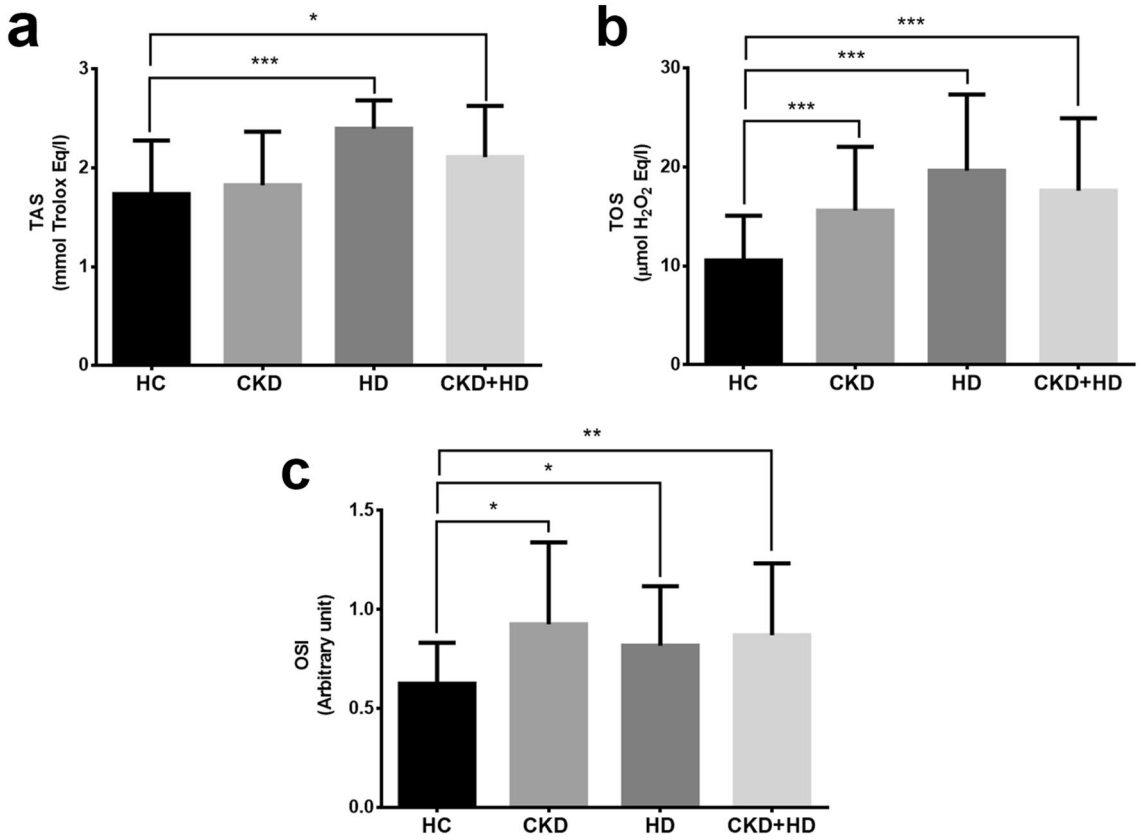

Fig. 3 Serum TAS (**a**) and TOS (**b**) levels and calculated OSI (**c**) values in healthy controls and patients with CKD. OSI values were calculated by dividing TOS by TAS levels. *HC* Healthy control, *CKD* Chronic kidney disease, *HD* Hemodialysis. Data were presented as mean ± SD. $p < 0.05$ values were considered as significant. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

To the best of our knowledge, we are the first to investigate the role of lncRNA H19 expressions in serum samples of patients with CKD stage3–5 and HD patients. Previous studies have shown that lncRNA H19 is frequently overexpressed in renal pathologies, such as fibrosis, carcinoma and cell injury [6, 8, 9, 15]. *Wang* et al. reported that the relative expression levels of lncRNA H19 were elevated in renal cell carcinoma and associated with tumor stage and metastasis [15]. Moreover, their results demonstrated that the downregulation of lncRNA H19 diminished proliferation, migration, and invasion of the tumor cells. Their results suggested that lncRNA H19 may be a biomarker or therapeutic target for renal cancer [15]. Another study by *Xie* et al. demonstrated that lncRNA H19 expressions were upregulated in in vitro and in vivo renal fibrosis models and the downregulation of lncRNA H19 ameliorated renal fibrosis [6]. These results emphasized that lncRNA H19 is associated with renal fibrosis, and its inhibition may provide anti-fibrotic therapy in kidney diseases [6]. In a study investigating the functional role of lncRNA H19, *Liu* et al. showed that lncRNA H19 expression levels were elevated in CaOx nephrocalcinosis animal model and lncRNA H19 facilitated renal tubular epithelial cell injury [8]. Their results indicated that lncRNA H19 is a functional molecule that involved in the regulation of the HMGB1/TLR4/NF-κB signalling pathway [8]. Also, *Fan* et al. reported that lncRNA H19 expression levels were higher in patients with diabetic kidney disease than diabetic patients without nephropathy, indicating that lncRNA H19 could be a novel biomarker for diabetic kidney disease [9]. Similar to previous studies mentioned above, our study demonstrated that the lncRNA H19 was overexpressed in patients with CKD stage 3–5 and HD patients, suggesting that lncRNA H19 can be released from cells at high levels or can be stable in the bloodstream. Furthermore, the major finding of our study compared to the aforementioned studies above is increased serum lncRNA H19 expression, which was not only associated with loss of kidney functions but also associated with other parameters, such as ferritin, PTH and CRP in CKD and HD patients. Furthermore, our analyses demonstrated that lncRNA H19 may have a potential clinical value for the pathogenesis of CKD and CKD-related complications.

In contrast, *Xu* et al. revealed that lncRNA H19 expressions were diminished in blood samples of children with

Table 2 Correlation analyses between lncRNA H19 and some parameters in the healthy control ($n=20$) and patients (CKD stage 3–5 and HD, $n=56$) groups

Parameters	lncRNA H19	TNF-α	IL-6	OC
Age	$p=0.963$	$p=0.475$	$p=0.905$	$p=0.697$
BMI (kg/m^2)	$p=0.553$	$p=0.329$	$p=0.527$	$p=0.835$
eGFR (mL/min/1.73 m^2)	$r=-0.581$ ***p < 0.001***	$r=-0.309$ ***p = 0.007***	$r=-0.337$ ***p = 0.003***	$r=-0.307$ ***p = 0.007***
Albumin (g/dL)	$p=0.574$	$p=0.511$	$p=0.462$	$p=0.505$
Ferritin (ng/mL)	$r=0.512$ ***p < 0.001***	$r=0.399$ ***p < 0.001***	$r=0.249$ ***p = 0.03***	$r=0.255$ ***p = 0.026***
Ca (mg/dL)	p = 0.208	$p=0.362$	$p=0.545$	$p=0.757$
P (mg/dL)	$r=0.378$ ***p = 0.001***	$p=0.093$	$r=0.237$ ***p = 0.039***	$p=0.076$
PTH (pg/mL)	$r=0.450$ ***p < 0.001***	$r=0.313$ ***p = 0.006***	$p=0.061$	$r=0.251$ ***p = 0.029***
CRP (mg/L)	$r=0.287$ ***p = 0.012***	$r=0.429$ ***p < 0.001***	$r=0.290$ ***p = 0.011***	$r=0.297$ ***p = 0.009***
TAS (mmol Trolox Eq/L)	$r=0.325$ ***p = 0.004***	$r=0.248$ ***p = 0.031***	$p=0.557$	$p=0.637$
TOS (μmol H_2O_2 Eq/L)	$r=0.299$ ***p = 0.009***	$r=0.326$ ***p = 0.004***	$p=0.071$	$p=0.170$
OSI (arbitrary unit)	$p=0.859$	$p=0.169$	$p=0.073$	$p=0.545$
TNF-α (pg/mL)	$r=0.456$ ***p < 0.001***		$r=0.264$ ***p = 0.021***	$r=0.376$ ***p = 0.001***
IL-6 (pg/mL)	$r=0.344$ ***p = 0.002***	$r=0.264$ ***p = 0.021***		$p=0.488$
OC (ng/mL)	$r=0.355$ ***p = 0.002***	$r=0.376$ ***p = 0.001***	$p=0.488$	

BMI Body mass index (kg/m^2), *eGFR* Estimated Glomerular Filtration Rate, *Ca* Calcium, *P* Phosphorus, *PTH* Parathyroid hormone, *CRP* C-reactive Protein, *TAS* Total Antioxidant Status, *TOS* Total Oxidant Status. *OSI* Oxidative Stress Index, *TNF-α* Tumor Necrosis Factor Alpha, *IL-6* Interleukin 6, *OC* Osteocalcin

Table 3 Multiple regression analysis indicating routine laboratory parameters associated with lncRNA H19 in patients with CKD

Variables	*B*	Std. error	*p* value	*F*	Adjusted R^2
eGFR	−0.007	0.006	0.218	4.87	0.20
Ferritin	0.001	0.00	0.005		
PTH	−0.001	0.001	0.04		
P	−0.119	0.160	0.458		
CRP	0.071	0.031	0.024		

eGFR Estimated Glomerular Filtration Rate, *PTH* Parathyroid hormone, *P* Phosphorus, *CRP* C-reactive Protein

Model significance: $p=0.001$

nephrotic syndrome, and lncRNA H19 acted as a regulator of ADCK4 gene expression, which is related to nephrotic syndrome [7]. However, this is in contradiction to our results and the results of other studies that described increased H19 levels in kidney pathologies [6, 8, 9, 15]. On the other hand, a previous study unveiled the prominent expression of lncRNA H19 during embryogenesis, while its expression decreased in the postnatal term [16]. So, we speculated that this discrepancy might be, in part, associated with the versatile expression pattern of lncRNAs during different life periods and patho- and normo-physiological conditions of the individuals.

We also focused on whether lncRNA H19 expression was associated with some markers pertaining to the pathogenesis of CKD and CKD-related complications. We found out that lncRNA H19 expression was significantly related with oxidative stress (TAS and TOS), mineralization (phosphorus, PTH, OC) and inflammation markers (TNF-α and IL-6) in patients with CKD. Our findings have consolidated the potential clinical value of lncRNA H19 for CKD.

Our study is not without any limitations. First, the study population size was relatively small, and only serum lncRNA levels were analyzed. For the same reason, we could not evaluate the lncRNA levels in particular patient groups, such as patients with diabetic kidney disease. Second, another limitation is that the patients recruited in the present study were on medication, and the confounder effect of this treatment on lncRNA H19 levels is unknown. Therefore, multi-center and big-scale studies are needed to clarify the implication of lncRNA H19 in CKD pathogenesis. Additionally, further experimental studies in animal and cell

culture models are required for understanding the underlying functional role of lncRNA H19 in the pathogenesis of kidney diseases.

Conclusion

Our study revealed that serum lncRNA H19 expressions were increased in CKD stage 3–5 and HD patients, and elevated lncRNA H19 expressions were associated with biochemical parameters involved in inflammation and mineralization. Furthermore, our findings suggest that lncRNA H19 may have a promising clinical value in the pathogenesis of CKD and CKD-related complications. However, more functional and large-scale studies are needed to elucidate the role of lncRNA H19 in the molecular pathways associated with inflammation and mineralization in kidney diseases.

Author contributions HMO, SD and FHT designed the study. HMO, SD, and MYT performed genetic and biochemistry laboratory analyses. HMO, SD, MYT, MAB, and FHT wrote/drafted/edited the manuscript and interpreted the results. HMO, SD, MYT, MAB, and FHT conducted analyses, prepared graphs/figures and revised the manuscript.

Funding This work is supported by the Scientific Research Project Fund of Hatay Mustafa Kemal University under project number: 19.M.013.

Compliance with ethical standards

Conflict of interest We all declare that we do not have any conflicts of interest.

Ethical approval All procedures performed in the present study were approved by the institutional review board and were in compliance with the 1964 Helsinki Declaration (Approval number: 2019/26).

Informed consent Informed consent was obtained from all individual participants included in the study.

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