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RESEARCH ARTICLE

Potential Role of *miR-484* in Modulating *VDR* Gene Expression Level in Calcium Oxalate Kidney Stone Patients

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ABSTRACT

Calcium oxalate (CaOx) kidney stones, accounting for over 70% of all types, are the most common stone type. MicroRNAs (miRNAs) are post-transcriptional epigenetic regulators, involved in disease development. One of these miRNAs is miRNA 484 (*miR-484*). Vitamin D (VD), a fat-soluble vitamin important for calcium (Ca) metabolism, is primarily mediated by the Vitamin D Receptor (VDR), a nuclear transcription factor. This study aimed to investigate *miR-484's* regulatory role in modulating *VDR* gene expression, evaluate serum VD and biochemical parameters in CaOx kidney stone patients compared to healthy controls, and their potential as diagnostic biomarkers. Blood samples were collected from 50 CaOx patients and 40 controls. Serum VD, Ca, urea, and creatinine levels were measured. To assess miR-484 and VDR gene expression, absolute quantification via RT-qPCR was performed. Comparisons, correlations, and ROC curve analyses were performed. The results showed that patients exhibited non-significantly higher serum Ca, urea, and creatinine levels than controls. While VD levels were significantly lower in patients compared to controls. Gene expression analysis revealed a significant *miR-484* downregulation and *VDR* upregulation in patients. Moderate negative correlation was observed between *miR-484* and *VDR* expression in patients. ROC analysis showed that VD, *VDR*, and *miR-484* had good diagnostic accuracy. In conclusion, the study reveals that the downregulation of *miR-484* in CaOx patients may lead to overexpression of the *VDR*, potentially altering VD metabolism and leading to VD deficiency, which can disrupt Ca handling in bone and kidneys, contributing to hypercalciuria, one of the primary risk factors for CaOx stone formation.

Keywords: Calcium oxalate kidney stone, Gene expression, Micro RNA 484, Vitamin D, Vitamin D receptor

Introduction

Kidney stone disease, known as nephrolithiasis or urolithiasis, is a globally prevalent medical condition that affects approximately 5–10% of the population worldwide, with recurrence rates reaching up to 66.9%; it can cause significant discomfort, complications, and burden on healthcare systems.[1–3] Among all types, calcium (Ca)-containing stones are the most common type, particularly **calcium oxalate (CaOX) stones,** which account for more than 70% of all cases.[4,5] The formation of these stones is influenced by a complex interplay of environmental exposures, metabolic disturbances, genetic predispositions, and epigenetic regulation.[2,6]

Epigenetic factors, particularly the regulation of gene expression, have a potential role in kidney stone formation via several mechanisms. Among these regulatory mechanisms, **microRNAs (miRNAs)** have become increasingly important in understanding disease development and progression. These small, non-coding RNAs, 19–25 nucleotides long, regulate

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gene expression at the post-transcriptional level. They function by binding to the 3'-untranslated region (3'-UTR) of target messenger RNA (mRNA), leading to translational repression or degradation.[7,8] Many miRNAs have been implicated in kidney stone development, modulating Ca and oxalate metabolism, cellular transport, and inflammation. Recent evidence indicates that microRNAs may serve as a new promising diagnostic biomarker for kidney stones.[9]

MicroRNA 484 (*miR-484*) has a vital role in metabolic regulation, inflammation, apoptosis, mitochondrial function, and cellular differentiation, all essential for healthy kidney function. Abnormal expression of *miR-484* is observed in pathological states, like cardiovascular, neurological, skin, and infection-related diseases.[10,11] However, its role in modulating gene expression in kidney stone patients remains largely unexplored.

Vitamin D (VD) (Cholecalciferol), a fat-soluble vitamin, plays an important role in various physiological processes within the human body; the active form 1,25 dihydroxyvitamin D3 (1,25(OH)2D) has been shown to affect Ca and phosphorus metabolism, bone health, and regulate immune function.[12,13] VD has a central role in intestinal Ca absorption regulation; this affects kidney stone formation indirectly and regulates the excretion of renal Ca. Also, VD is pivotal in oxalate metabolism.[14–16] The primary mediator of VD's biological effects is the **Vitamin D receptor (VDR),** a nuclear transcription factor, encoded by the *VDR* gene, located on chromosome 12q13.11.[16] Acting as a ligand-activated transcription factor, VDR binds to its ligand VD, forming a heterodimer with the retinoid X receptor (RXR).[17] VDR eventually binds to the VDR response element (VDRE), a specific DNA sequence of controlled genes, controlling the expression of target genes involved in a diverse range of cellular processes, including Ca absorption and metabolism.[17–19]

Serum concentration levels of VD are categorized as deficient at ≤ 20 ng/mL, insufficient at 20–30 ng/mL, while ≥ 30 ng/mL is considered sufficient.[19,20] VD deficiency may induce secondary hyperparathyroidism as a compensatory physiological response by the body to maintain the homeostasis of Ca blood levels. To achieve this, parathyroid hormone (PTH) secretion is increased, promoting Ca release from bone into the bloodstream and inhibiting renal reabsorption, leading to increased urinary Ca excretion and hypercalciuria. Furthermore, it may induce oxidative stress and overexpression of inflammatory mediators in renal tissue, elevating the risk of kidney stone formation.[15,21,22]

Altered expression of *VDR* has been linked to abnormal Ca handling.[16,23] However, the mechanisms regulating *VDR* expression in nephrolithiasis are still poorly understood. Recent studies suggest that miRNAs have a regulatory role in *VDR* expression, thereby influencing the molecular pathways related to kidney stone formation.[10]

In this study, we aim to shed light on the potential regulatory role of *miR-484* in modulating *VDR* gene expression and its pivotal role in Ca homeostasis. Elucidating the interaction between *miR-484* and *VDR* may enhance understanding of how genetic and epigenetic factors affect VD and Ca metabolism, leading to kidney stone formation. Understanding regulation at the molecular level may play a crucial role in identifying novel biomarkers and therapeutic targets for preventing and managing kidney stones.

Materials and methods

Study design and sample collection

This study was a case-control study. It included the collection of 90 samples: 50 patients diagnosed with CaOx kidney stones and 40 healthy controls (the control group included individuals with no history of kidney stones). Each participant underwent a renal ultrasound, general urine examination, and a urologist's evaluation to confirm the absence of kidney stones and any urological issues. Only those who met these criteria were included as controls). Patient and healthy control groups were matched for age and sex. Individuals with chronic illnesses (diabetes or hypertension) or on medications affecting VD or Ca metabolism were excluded. BMI, dietary intake, and seasonal variation were not precisely recorded; all samples were collected under consistent clinical protocols. Under sterile conditions, whole blood samples (5 mL) were collected in EDTA and serum separator gel tubes from patients and healthy control groups. The samples were collected from Al Yarmouk Teaching Hospital and Ghazi Al-Hariri Hospital for Surgical Specialties in Baghdad from October 2023 to September 2024. The study was approved by the Iraqi Ministry of Health's ethics committee, and participants signed the ethical approval.

Biochemical and stone analysis

Biochemical tests were performed to measure serum Ca, urea, and creatinine levels in both patient and control groups. Blood samples were collected in serum separator gel tubes and centrifuged at 3000 rpm for 10 minutes, then serum Ca, urea, and creatinine levels (mg/dL) were measured using a fully automated clinical chemistry analyzer (AU5800, Beckman Coulter Inc, Japan). VD serum

levels (ng/mL) were measured using a fully automated system (Access 2, Beckman Coulter Inc, Germany). The assays were performed according to the manufacturer's instructions. Moreover, to confirm that all participants did not have diabetes, glycated hemoglobin (HbA1c) levels were measured using (BR2200220, Bio-Rad D-10 Hemoglobin Testing System, Germany), less than 6.5% was used as a cut-off value to confirm non-diabetic status. Kidney stones were collected and chemically analyzed using the Stone Analysis Set (Biolabo, France) to determine the main chemical components of the urinary stones and their type; the analysis was performed according to the manufacturer's instructions.

MicroRNA target prediction

To predict potential binding sites of *miR-484* in the 3' UTR of the *VDR* gene, TargetScanHuman 8.0 (https://www.targetscan.org/vert_80/) (miRNA target prediction web-based tool) was used; *miR-484* was selected based on previous reports linking it to renal pathology, *VDR*, and miRNA regulatory roles in Ca signaling pathways. The analysis focused on conserved binding sites. Prediction scores, including the context++, context++ score percentile, and site conservation, were noted; however, no strong affinity scores were observed for the *miR-484-VDR* interaction.

RNA extraction

For isolating the miRNA from serum, the miRNeasy Serum/Plasma Kit (QIAGEN, Germany) was used, and to isolate the mRNA from whole blood, the QIAamp RNA Blood Mini Kit (QIAGEN, Germany) was used, both according to the manufacturer's protocol. To determine the purity and concentration of mRNA and miRNA samples, we evaluated the A260/A280 ratio using the NanoDrop™ OneC Microvolume UV-Vis Spectrophotometer (Thermo Scientific), with all samples showing approximately 2.0 of purity.

Reverse transcription - quantitative real-time PCR (RT-qPCR)

MiR-484 cDNA was synthesized using the TaqMan™ MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, USA) with a stem-loop RT primer specific for *hsa-miR-484*; *VDR* cDNA was synthesized using the High-Capacity RNA-to-cDNA™ Kit (Thermo Fisher Scientific, USA). Both syntheses followed the manufacturer's instructions.

MiR-484 and *VDR* expression levels were quantified separately using an Absolute Quantification approach on the QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific, USA). This method enabled precise quantification of transcript abundance in serum and blood samples as copy number (copies/μL), providing clinically interpretable results.

Synthetic single-stranded RNA oligonucleotides (5 nmol; Thermo Fisher Scientific, USA), corresponding to mature *hsa-miR-484* and *VDR* sequences, were initially resuspended in 500 μL of nuclease-free water to yield a 10 μM stock solution. The copy number per microliter was calculated using Avogadro's number and molecular weight. From this stock, tenfold serial dilutions were prepared (ranging from $\sim 10^6$ to ~ 10 copies/μL) and subjected to reverse transcription and qPCR under the same conditions as the experimental samples.

Each 20 μL qPCR reaction consisted of 10 μL TaqMan™ Universal PCR Master Mix (Thermo Fisher Scientific, USA), 1 μL of TaqMan™ Gene Expression Assay (specific for *miR-484* Assay ID: 478308_mir; specific for *VDR* Assay ID: Hs01045843_m1) (Thermo Fisher Scientific, USA), 2 μL of cDNA, and 7 μL of nuclease-free water.

Standard curves were generated by plotting Cycle threshold (Ct) values of the synthetic RNA dilutions against the logarithm of the input copy number. The QuantStudio™ software was used to generate the curves, assess the curves' efficiency, and calculate the target genes (*miR-484* and *VDR*) absolute number of copies per microliter (copies/μL) in each sample by automatically comparing Ct values of the samples with the standard curve. Unlike relative quantification methods such as the $2^{-\Delta\Delta Ct}$ approach, this absolute quantification method does not require reference or housekeeping genes and provides direct, accurate measurement of transcript abundance without requiring normalization to an internal control.

Data analysis

Data analyses were conducted using GraphPad Prism 10.3 (GraphPad Software, Boston, Massachusetts, USA) and MedCalc Statistical Software 22.025 (MedCalc Software Ltd., Ostend, Belgium). The normality of the data distribution was evaluated using the Kolmogorov-Smirnov test. Data that followed a normal distribution were analyzed using parametric tests, and non-parametric tests were used to analyze non-normally distributed data to compare differences between patients and controls. Spearman's rank correlation was used for correlation analysis. A p-value < 0.05 was considered statistically significant for all tests.

Table 1. General characteristics and biochemical analysis of the studied groups. Statistical significance was determined using the t-test for Ca and VD_3, and the Mann–Whitney U test for the other variables.

Biochemical test	Patients (n = 50)	Controls (n = 40)	*p-value*
Age (years)	35.78 ± 11.28	38.20 ± 8.95	$p = 0.173$
Sex (M/F)	2:3	2:3	-
Male	20 (40%)	16 (40%)	-
Female	30 (60%)	24 (60%)	-
Ca (mg/dL)	9.44 ± 0.530	9.33 ± 0.264	$p = 0.202$
Urea (mg/dL)	46.14 ± 30.65	41.40 ± 6.24	$p = 0.363$
Creatinine (mg/dL)	1.34 ± 1.50	0.955 ± 0.074	$p = 0.346$
HbA1c (%)	5.35 ± 0.737	5.26 ± 0.66	$p = 0.520$
VD_3 (ng/mL)	20.66 ± 7.07	33.90 ± 10.40	$p < 0.0001$
	Deficient 28 (56%)	Deficient 3 (7.5%)	$p < 0.0001$
VD_3 Status*	Insufficient 16 (32%)	Insufficient 15 (37.5%)	$p = 0.5874$
	Sufficient 6 (12%)	Sufficient 22 (55%)	$p < 0.0001$

* VD_3 classification: Deficient (≤ 20 ng/mL), Insufficient (20–29 ng/mL), Sufficient (≥ 30 ng/mL).

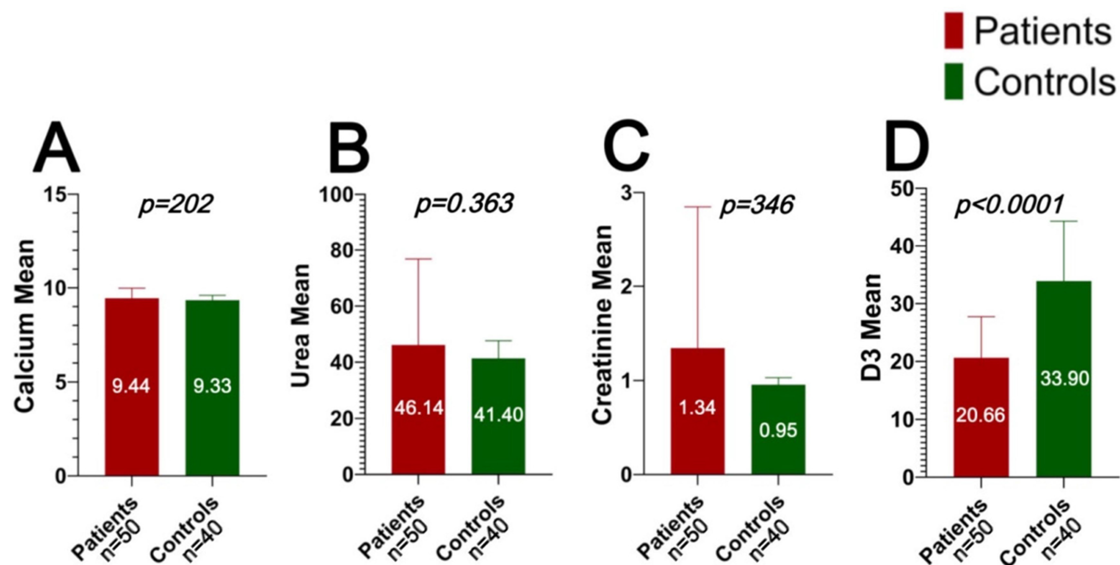

Fig. 1. Patients and controls means ± SD for A. Calcium (mg/dL) B. Urea (mg/dL) C. Creatinine (mg/dL) D. VD_3 (ng/mL). Statistical significance was determined using the t-test for A. Calcium and D. VD_3, and the Mann–Whitney U test for B. Urea and C. Creatinine.

Results

Study population characteristics

In this study, 90 participants were divided into 50 (CaOx) kidney stone patients and 40 healthy controls. The mean age of the patient group was 35.78 ± 11.28 years, and the healthy control group was 38.20 ± 8.95 years ($p = 0.173$). The male-to-female ratio was 2:3 for both the patients and the controls. All are detailed in Table 1.

Biochemical analysis

Biochemical analysis showed that serum Ca, urea, and creatinine levels were higher in CaOx kidney stone patients compared to healthy controls; however, the differences were non-significant (Ca: 9.44 ± 0.530 vs. 9.33 ± 0.264 mg/dL, $p = 0.202$; urea: 46.14 ± 30.65 vs. 41.40 ± 6.24 mg/dL, $p = 0.363$; creatinine: 1.34 ± 1.50 vs. 0.955 ± 0.074 mg/dL, $p = 0.346$), as shown in Fig. 1. The HbA1c levels were 5.35 ± 0.737 for the patients group and 5.26 ± 0.66 for the healthy controls group ($p = 0.520$). Serum VD levels were lower in patients (20.66 ± 7.07) than in healthy controls (33.90 ± 10.40) and had significant differences ($p < 0.0001$) between the two groups. VD sufficiency status showed a significant difference between patients and healthy controls on the deficient and sufficient levels. 56% of patients and 7.5% of healthy controls were VD3-deficient (p < 0.0001), while only 12% of patients and 55% of healthy controls were VD3-sufficient ($p < 0.0001$). As shown in Fig. 2. All are detailed in Table 1.

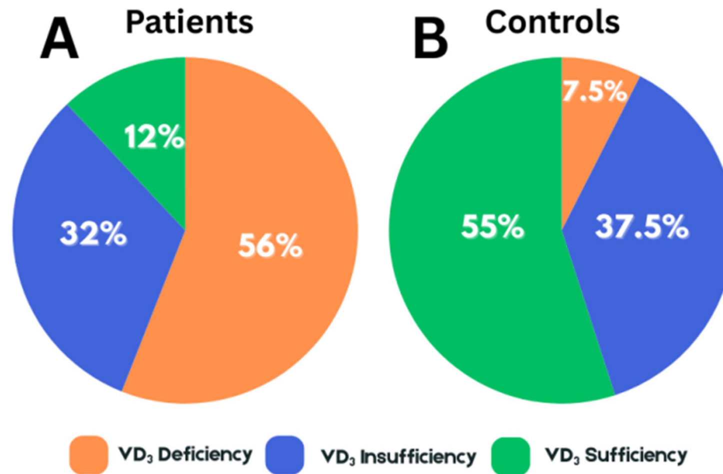

Fig. 2. VD Status A. Patients B. Controls.

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	P_{CT}	Predicted relative K_D
Position 864-870 of VDR 3' UTR hsa-miR-484	5' ...ACCGAGAGUAGCCGAGAGCCUGC... ||||||| 3' UAGCCCUCCCCUGACUCGGACU	7mer-m8	-0.07	82	-0.07	0.073	N/A	N/A

Fig. 3. TargetScanHuman 8.0 prediction of *miR-484* binding sites in the *VDR* 3' UTR.

Prediction of miR-484 binding sites in the VDR 3' UTR

Using TargetScanHuman 8.0, a conserved 7mer-m8 site for *miR-484* within the 3' UTR of the *VDR* mRNA (position 864–870) was identified. The Context + + score was (–0.07), and the Context + + score percentile was 82, suggesting a moderate likelihood of repression, Fig. 3. These results suggest a potential but not strongly supported interaction between *miR-484* and *VDR*, warranting further experimental validation.

Gene expression results

The expression level of *miR-484* was significantly downregulated in CaOx kidney stone patients (16.88 $\pm$ 11.37 copies/μL) compared to healthy controls (31.44 $\pm$ 14.19 copies/μL, $p < 0.0001$). Conversely, *VDR* mRNA expression was significantly higher in patients (37.62 $\pm$ 32.20 copies/μL) than in healthy controls (10.40 $\pm$ 5.075 copies/μL, $p < 0.0001$) as determined by absolute quantification comparing Ct values of the samples with the standard curve, as shown in Fig. 4 and Table 2.

To evaluate the diagnostic potential of serum VD levels (ng/mL), *VDR*, and *miR-484* expression levels (copies/μL), Receiver operating characteristic (ROC) curve analysis was performed Fig. 5. It revealed that serum VD levels area under curve (AUC) was 0.858 (95% CI: 0.78 – 0.93 $p < 0.0001$), *VDR* expression AUC was 0.858 (95% CI: 0.78 – 0.93 $p < 0.0001$), and *miR-484* expression AUC was 0.792 (95% CI: 0.69 – 0.89 $p < 0.0001$), indicating a good diagnostic accuracy for all three variables and could be a potential biomarker.

Correlation analysis

Spearman's rank correlation coefficient analysis examined the relationships among *miR-484* expression, *VDR* expression, and serum VD levels in patients and control groups. It revealed a moderate, negative, and significant correlation between miR-484 and VDR expressions in the patient group (ρ = –0.55, p < 0.0001), and a weak, negative, and significant correlation in the control group (ρ = –0.32, p = 0.023). Also, there was no or very weak negative correlation between *miR-484* and serum VD levels in the patient group (ρ = –0.08, p = 0.566), and a weak, positive, and significant correlation in the control group (ρ = 0.32, p = 0.044). Moreover, there was no or very weak positive correlation between *VDR* and serum VD levels in the patient group (ρ = 0.16, p = 0.282) and no or very weak negative correlation in the control

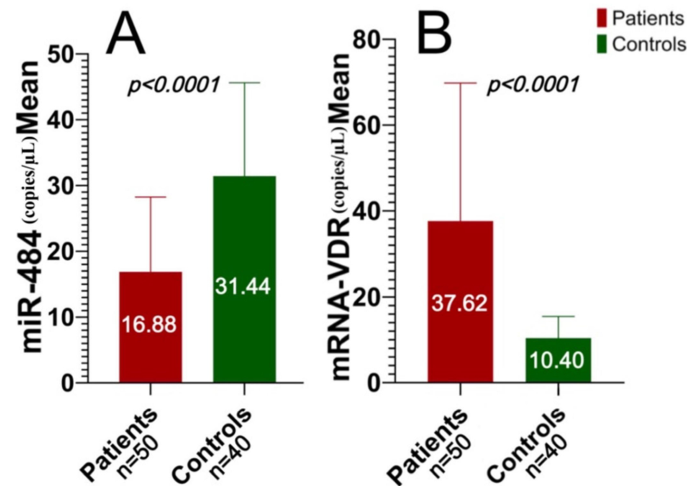

Fig. 4. Gene expression level. A. *miR-484* expression (copies/μL). B. *VDR* Expression (copies/μL). Statistical significance was determined using the Mann–Whitney U test.

Table 2. Expression levels of *miR-484* and *VDR* mRNA (copies/μL) in patients and controls determined by absolute quantification. Statistical significance was determined using the Mann–Whitney U test.

Gene	Group	Sample Size (n)	Mean ± SD (copies/μL)	Median (IQR) (copies/μL)	Min–Max (copies/μL)	*p-value*
miR-484	Patients	50	16.88 ± 11.37	13.20 (8.88–21.63)	2.60–56.70	p <0.0001
miR-484	Controls	40	31.44 ± 14.19	32.40 (20.53–40.60)	5.80–59.60	
VDR	Patients	50	37.62 ± 32.20	26.2 (11.98–53.03)	4.90–134.8	p <0.0001
VDR	Controls	40	10.40 ± 5.075	9.4 (7.6–11.68)	3.50–23.70	

*Gene expression levels were quantified using standard curves generated from known synthetic standards and are expressed as (copies/μL). A Ct value cutoff of 35 was used as the threshold for detectability.

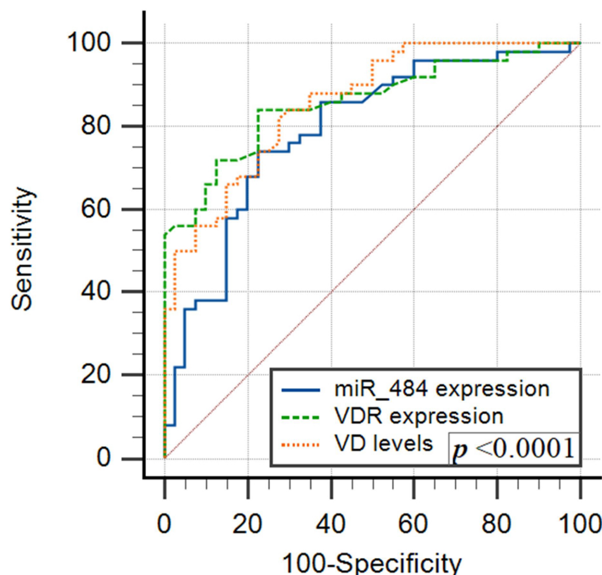

Fig. 5. ROC curves showing *miR-484* expressions (copies/μL), *VDR*, expressions (copies/μL) and serum VD levels (ng/mL) diagnostic performance in distinguishing CaOx kidney stone patients from healthy controls.

group (ρ = –0.05, p = 0.773). As shown in the scatter plot Fig. 6.

Discussion

Kidney stone prevalence is rising, with a high recurrence rate; the most common stone type, CaOx, forms due to multiple factors.[24] This study explores *miR-484's* role as an epigenetic regulator and its effects on *VDR* expression, and some biochemical variables, all influencing CaOx stone formation, along with their potential diagnostic value and correlations.

Biochemical analysis showed that serum Ca, urea, and creatinine levels were slightly higher in patients than in controls. Although the differences were insignificant, they suggest minor but potentially meaningful alterations in renal function and metabolic mechanisms among CaOx stone patients. Slightly higher serum urea and creatinine levels in patients could reflect a decline in renal clearance, indicating early or subclinical renal impairment, which is consistent with previous studies that had similar findings.[25–27] Although Ca is the main component of CaOx stones, serum Ca levels in patients (9.44 ± 0.530 mg/dL) were within the normal range (8.5 to 10.2 mg/dL), consistent with most previous studies. This stability in Ca levels is a result of Ca homeostasis processes.[26,28–30] Individuals with diabetes (HbA1c ≤ 6.5%) were excluded from this study, as diabetes

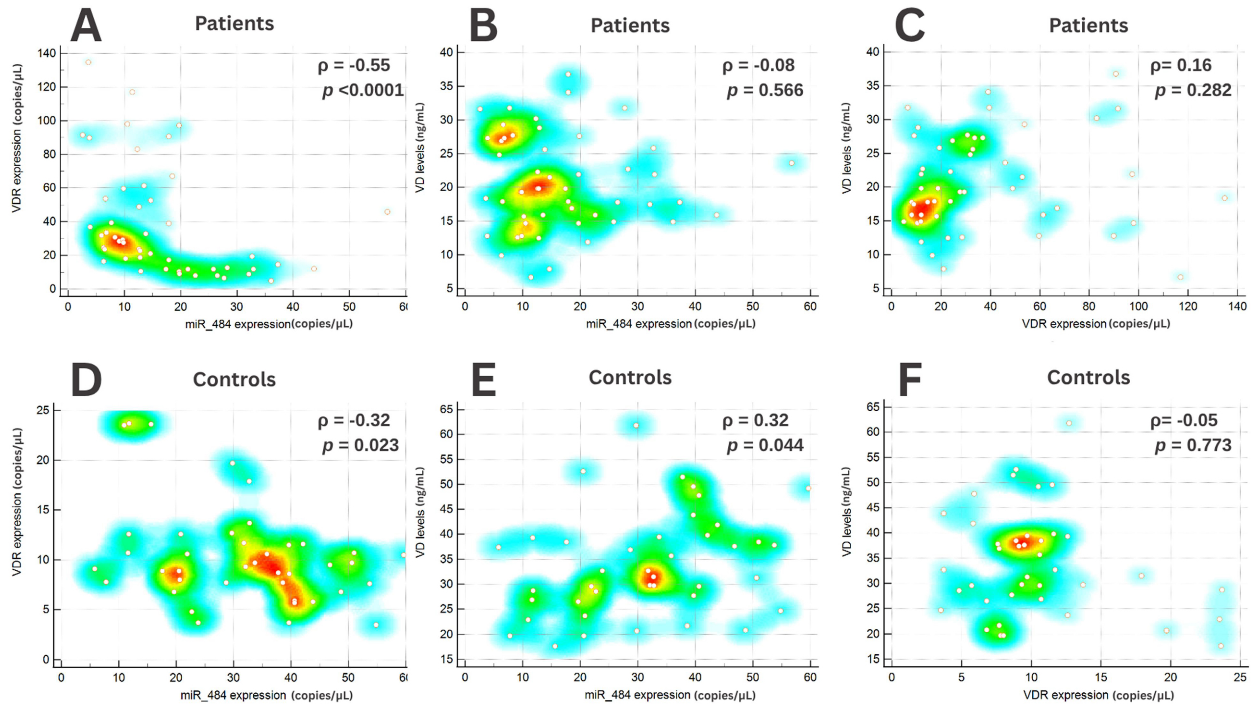

Fig. 6. Scatter diagram – correlation. A. between *miR-484* and *VDR* expressions (copies/μL) in the patients group. B. between *miR-484* (copies/μL) and serum VD levels in the patients group. C. between *VDR* (copies/μL) and serum VD levels in the patients group. D. between *miR-484* and *VDR* expressions (copies/μL) in the controls group. E. between *miR-484* (copies/μL) and serum VD levels in the controls group. F. between *VDR* (copies/μL) and serum VD levels in the controls group. Spearman's rank correlation was used.

causes a significantly lower urine pH and larger amounts of urinary oxalate excretion compared to non-diabetics; both factors increase the risk for CaOx stone formation and may affect the accuracy of the results.[24] Due to limitations in the current study, data on urinary oxalate, citrate, urinary pH, serum PTH, eGFR, BMI, dietary habits, and seasonal timing were not recorded.

In addition, our results revealed a significantly lower VD serum level among CaOx kidney stone patients compared to healthy controls (20.66 ± 7.07 vs. 33.90 ± 10.40). 56% of patients were VD deficient, and only 7.5% of controls. These findings are important given the central role of VD in Ca homeostasis and renal physiology.[31] VD facilitates intestinal Ca absorption and regulates PTH secretion, influencing urinary Ca excretion. Deficiency in VD may lead to secondary hyperparathyroidism, enhancing Ca release from bone into the bloodstream, increasing serum Ca, which can, in turn, cause hypercalciuria.[15] Hypercalciuria was proven to be a key risk factor for stone formation and is commonly observed in CaOx stone patients.[2,30,31] Furthermore, deficiency in VD levels may impair renal handling of calcium and phosphate, increasing the risk of crystal precipitation.[32] Our results align with previous studies that reported low VD status in stone formers and support its potential role as a risk factor in kidney stone pathogenesis.[33]

In this study, absolute quantification revealed a significant downregulation of *miR-484* and upregulation of *VDR* gene expression in CaOx kidney stone patients compared to healthy controls. These findings align with Fan et al. (2022), who demonstrated that *miR-484* inhibits CaOx crystallization by targeting *VDR* and affecting the VDR/FoxO1 regulatory axis, in the Sprague–Dawley rat model induced CaOx kidney stone pathological condition.[34] Also, Guo et al. (2022) observed that VDR gene expression was elevated in the GHS rat model, affecting VDR target genes that regulate VD_3 metabolism and Ca homeostasis.[35] These findings suggest a potential change in the molecular regulation of Ca and VD metabolism in stone-forming patients. Spearman's correlation analysis revealed a significant, moderate negative correlation between *miR-484* and *VDR* expression in the patient group ($\rho = -0.55$, $p < 0.0001$), supporting the hypothesis that *miR-484* may act as a post-transcriptional suppressor of *VDR*. This relationship was significant but weaker in healthy controls ($\rho = -0.32$, $p = 0.023$), indicating that this regulatory mechanism may also function under normal physiological conditions, even at a lower level.

In parallel, TargetScanHuman 8.0 predicted that *miR-484* may target the 3' UTR of the *VDR* gene through a 7mer-m8 site, indicating seed region complementarity. This prediction, supported by a modest Context + + score (–0.07) and an 82-percentile rank, suggests a potentially moderate post-transcriptional regulatory interaction. However, the lack of strong affinity or evolutionary conservation scores highlights the need for cautious interpretation.

The observed increase in *VDR* expression may initially seem compensatory to altered Ca homeostasis or VD signaling; however, an alternative interpretation involves *VDR's* role in VD catabolism. Once activated by 1,25-dihydroxyvitamin D_3, *VDR* binds to vitamin D response elements (VDREs) in the *CYP24A1* promoter, enhancing the transcription of *CYP24A1*, the primary responsible enzyme for degrading active VD into inactive metabolites. As previously described[18,36,37] this pathway suggests that elevated *VDR* expression may paradoxically promote VD deficiency through accelerated inactivation, leading to disrupted calcium–phosphate homeostasis in stone formers.

Further supporting this hypothesis, correlation analyses revealed very weak or no relationship between *VDR* expression and serum VD levels in either patients ($\rho = 0.16$, $p = 0.282$) or controls ($\rho = -0.05$, $p = 0.773$), suggesting that *VDR* may be regulated independently of circulating VD. Similarly, *miR-484* expression did not correlate significantly with VD levels in patients ($\rho = -0.08$, $p = 0.566$), while a weakly significant positive correlation was observed in controls ($\rho = 0.32$, $p = 0.044$). These patterns suggest that *miR–484–VDR* interactions and their influence on VD metabolism may be disrupted in CaOx patients, possibly due to pathological feedback loops or altered post-transcriptional control.

To assess clinical relevance, ROC curve analysis showed good diagnostic performance for serum VD levels and *VDR* expression (AUC = 0.858, $p < 0.0001$) and for *miR-484* expression (AUC = 0.792, $p < 0.0001$), indicating their potential as biomarkers for CaOx kidney stone detection.

Taken together, these findings suggest a model in which downregulation of *miR-484* leads to *VDR* overexpression, which, in turn, promotes CYP24A1-mediated VD catabolism, resulting in VD3 deficiency and alterations in Ca metabolism, thereby maintaining Ca homeostasis. This dysregulated *miR-484–VDR*–VD axis may contribute significantly to the pathogenesis of CaOx kidney stone formation.

Conclusion

In conclusion, this study revealed a significant downregulation of *miR-484* expression and upregulation of *VDR* expression in CaOx kidney stone patients, suggesting a potential post-transcriptional regulatory relationship. The significantly lower serum VD_3 levels in patients compared to healthy controls support the participation of disrupted Ca metabolism in stone formation. These findings highlight the diagnostic potential of miR-*484*, *VDR* expression, and VD_3 level patterns, providing a foundation for future research into miRNA-based therapeutic strategies for CaOx stone formation.

Acknowledgment

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- Author(s) signed on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' contribution statement

H.B.B. and L. H. A. Al. designed the study. H.B.B. Collected samples, performed the biochemical, genetic, and statistical analysis, prepared and wrote the manuscript, drew figures, and created tables. L. H. A. Al and I.S.Al. Supervised, oversaw project administration, and reviewed and edited the manuscript. I.S.Al. diagnosed and examined patients and healthy controls.

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الدور المحتمل للـ miR-484 في تنظيم مستوى تعبير جين مستقبل فيتامين د (VDR) لدى مرضى حصى الكلى من نوع أوكسالات الكالسيوم

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الملخص

تُعدّ حصى الكلى من نوع أوكسالات الكالسيوم(CaOx) ، التي تمثل أكثر من 70% من جميع أنواع الحصى، الأكثر شيوعًا. وتُعدّ الميكرو RNAs (miRNAs)منظِّمات لاجينية على مستوى ما بعد النسخ، وتشارك في تطور الأمراض. ومن بين هذه الجزيئات miRNA-484 (miR-484).يُعد فيتامين د (VD) فيتامينًا ذائبًا في الدهون ومهمًا في أيض الكالسيوم(Ca) ، ويعمل أساسًا من خلال مستقبل فيتامين د(VDR) ، وهو عامل نسخي نووي. هدفت هذه الدراسة إلى تقصي الدور التنظيمي لـ miR-484 في تعديل مستوى تعبير جينVDR ، وتقييم مستويات فيتامين د في المصل وبعض المؤشرات الكيموحيوية لدى مرضى حصى الكلى من نوع CaOxمقارنةً بالأصحاء، واستكشاف إمكانيتها كواسمات تشخيصية. تم جمع عينات دم من 50 مريضًا بحصى CaOx و40 شخصًا سليمًا كمجموعة سيطرة. وتم قياس مستويات فيتامين د، والكالسيوم، واليوريا، والكرياتينين في المصل. ولتقييم تعبير miR-484 وجينVDR ، أُجري القياس الكمي المطلق باستخدام تقنية RT-qPCR. كما أُجريت تحليلات المقارنة والارتباط ومنحنيات ROC. أظهرت النتائج أن المرضى سجّلوا ارتفاعًا غير معنوي في مستويات الكالسيوم واليوريا والكرياتينين في المصل مقارنةً بمجموعة السيطرة، في حين كانت مستويات فيتامين د منخفضة بشكل معنوي لدى المرضى. وكشف تحليل التعبير الجيني عن انخفاض معنوي في تعبير miR-484 وارتفاع معنوي في تعبير VDR لدى المرضى. ولوحظ ارتباط سلبي متوسط بين تعبير miR-484 و VDR لدى المرضى($\rho = -0.55$ ، $p < 0.0001$).كما أظهر تحليل منحنى ROC أن مستويات فيتامين د في المصل، وتعبيرVDR ، و miR-484تمتلك دقة تشخيصية جيدة. تشير الدراسة إلى أن انخفاض تعبير miR-484 لدى مرضى حصى CaOx قد يؤدي إلى فرط تعبير جين VDR، مما قد يؤثر في أيض فيتامين د ويساهم في حدوث نقصه، الأمر الذي قد يخلّ بتنظيم الكالسيوم في العظام والكلى، ويسهم في حدوث فرط كالسيوم البول، وهو أحد عوامل الخطورة الرئيسية لتكوّن حصى أوكسالات الكالسيوم.

الكلمات المفتاحية: حصى أوكسالات الكالسيوم، التعبير الجيني، الميكروRNA-484، فيتامين د، مستقبل فيتامين د.