

Serum 25-Hydroxyvitamin D in Children with Newly Diagnosed Type 1 Diabetes: Associations with Diabetic Ketoacidosis and Acute Kidney Injury

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Research Article

Keywords:

Posted Date: September 30th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11028987/v1>

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Serum 25-Hydroxyvitamin D in Children with Newly Diagnosed Type 1 Diabetes: Associations with Diabetic Ketoacidosis and Acute Kidney Injury

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Abstract

Background. Vitamin D deficiency (VDD) is common among children and adolescents with type 1 diabetes mellitus (T1DM), particularly at the time of diagnosis. We investigated serum 25-hydroxyvitamin D [25(OH)D] concentrations according to the presence of DKA and AKI and assessed their associations with metabolic acidosis, renal function, and inflammatory markers.

Methods. This retrospective study included 400 children with newly diagnosed T1DM (mean age 9.02 ± 4.18 years). Serum 25(OH)D was measured after resolution of DKA and severe hyperglycaemia. Patients were classified as non-DKA (n=172), DKA without AKI (n=208), or DKA with AKI (n=20). AKI was defined according to KDIGO criteria.

Results. The mean serum 25(OH)D concentration was 21.77 ± 8.87 ng/mL, and VDD was present in 44.5% of patients. VDD prevalence increased from 36.6% in non-DKA patients to 47.6% in DKA without AKI and 80.0% in DKA with AKI ($p < 0.001$). The DKA+AKI group had the lowest 25(OH)D concentration (16.52 ± 9.39 ng/mL). Serum 25(OH)D and eGFR were positively correlated with blood pH and bicarbonate and negatively correlated with leukocyte, neutrophil, and monocyte counts. eGFR was inversely correlated with corrected serum sodium. No significant association was observed between 25(OH)D and eGFR.

Conclusions. VDD is common at T1DM onset and is particularly prevalent in children with DKA complicated by AKI. Lower 25(OH)D concentrations were associated with greater preceding acidosis and inflammatory activation. AKI during DKA appears to have a multifactorial pathophysiology involving volume depletion and systemic inflammation. The association between VDD and AKI is clinically relevant but needs further investigation.

Introduction

Type 1 diabetes mellitus (T1DM) is one of the most common chronic endocrine disorders in children and adolescents and results from autoimmune destruction of pancreatic β -cells, leading to absolute insulin deficiency and chronic hyperglycaemia [1,2]. Despite advances in diabetes management, diabetic ketoacidosis (DKA) remains a frequent and potentially life-threatening manifestation of newly diagnosed T1DM, occurring in approximately 15–70% of children depending on the population studied [3]. DKA is characterised by hyperglycaemia, ketonaemia, metabolic acidosis, dehydration, and electrolyte disturbances and may be complicated by acute organ dysfunction, including acute kidney injury (AKI) [3].

AKI is increasingly recognised as an important complication of DKA in children. It has been reported in approximately 10–60% of paediatric patients with DKA and is associated with prolonged hospitalisation and, potentially, an increased risk of subsequent chronic kidney disease [4-8]. Although renal hypoperfusion resulting from severe dehydration is considered a major contributor to AKI during DKA, accumulating evidence suggests that the pathophysiology is multifactorial. Systemic inflammation, oxidative stress, endothelial dysfunction, and activation of the renin–angiotensin–aldosterone system (RAAS) may additionally contribute to renal injury [5,9,10]. Identification of factors associated with increased susceptibility to AKI during DKA may therefore improve understanding of its pathophysiology and help identify children at increased risk.

Vitamin D is a pleiotropic hormone with biological effects extending beyond calcium and phosphate homeostasis. Vitamin D signalling is involved in the regulation of immune responses, inflammation, endothelial function, and the RAAS [11-14]. The major circulating and storage form of vitamin D, 25-hydroxyvitamin D [25(OH)D,

calcidiol], is generated in the liver by 25-hydroxylation of vitamin D₃ and vitamin D₂. Subsequent hydroxylation, predominantly in the kidneys, produces 1,25-dihydroxyvitamin D (calcitriol), the biologically active metabolite that exerts its effects through the vitamin D receptor (VDR). VDR is expressed in multiple tissues, including the kidneys, pancreatic β -cells, immune cells, and vascular endothelium, supporting a potential role for vitamin D in metabolic, immune, and renal processes [15,16].

Experimental and clinical evidence suggests that vitamin D deficiency (VDD) may be associated with renal injury. Impaired vitamin D signalling has been shown to promote RAAS activation, inflammation, oxidative stress, and fibrosis, whereas VDR activation may exert renoprotective effects [17,18]. Experimental studies have also demonstrated that vitamin D deficiency can aggravate ischaemia–reperfusion-induced AKI and impair renal vascular function [19,20]. These observations raise the possibility that low vitamin D status may be relevant to renal injury occurring in the setting of severe DKA, in which dehydration, haemodynamic disturbances, and systemic inflammation coexist.

VDD is common worldwide and has also been frequently reported in children with T1DM. Several studies have demonstrated lower serum 25(OH)D concentrations in children presenting with DKA compared with those without ketoacidosis, as well as associations between vitamin D status and the severity of metabolic acidosis [21-26]. Importantly, some studies have reported an increase in serum 25(OH)D concentrations following correction of DKA, suggesting that acute metabolic disturbances may influence circulating vitamin D concentrations [21,22].

Although the association between VDD and DKA has been investigated in children with newly diagnosed T1DM, the relationship between vitamin D status and AKI during DKA remains poorly characterised. In particular, data regarding children with newly

diagnosed T1DM who develop both DKA and AKI are limited. Moreover, it remains unclear whether lower vitamin D status is associated with the severity of metabolic acidosis and systemic inflammatory activation in children who develop renal dysfunction during DKA.

Therefore, the aim of the present study was to evaluate serum 25(OH)D concentrations in children and adolescents with newly diagnosed T1DM according to the presence of diabetic ketoacidosis and acute kidney injury. Additionally, we investigated the associations between vitamin D status, the severity of metabolic acidosis, renal function, and inflammatory markers to better characterise the potential role of vitamin D in the development of acute diabetic complications.

Materials and Methods

Study Population

A retrospective observational study was conducted at the Department of Pediatric Diabetology, Auxology and Obesity, Poznan University of Medical Sciences, Poland. The study included 400 children and adolescents (189 girls and 211 boys) with newly diagnosed type 1 diabetes mellitus (T1DM). The mean age of the participants was $9,02 \pm 4,18$ years. All patients were of Caucasian ethnicity. The diagnosis of T1DM was established between 2020 and 2025, and all clinical data were extracted from electronic medical records in 2025.

The study database included demographic and clinical characteristics, including sex, date of birth, date of T1DM diagnosis, height, weight, and the results of routine laboratory investigations performed at the time of diagnosis. Detailed clinical and laboratory data were obtained from the electronic hospital records.

Laboratory Measurements

Laboratory parameters were assessed on the day of admission and included venous blood pH, bicarbonate (HCO_3^-) concentration, blood gas analysis, ketonuria, serum glucose (GLU), serum creatinine (SCr), sodium concentration, uric acid (UA), white blood cell (WBC) count with differential and glycated haemoglobin (HbA1c).

Blood pH was measured using an ABL 835 blood gas analyser (Radiometer, Denmark). Serum glucose and serum creatinine concentrations were measured using an automated biochemical analyser (Alinity c, Abbott Laboratories, USA). White blood cell counts were determined using an XN-1000 haematology analyser (Sysmex, Japan). Glycated haemoglobin (HbA1c) was determined using the Alinity c analyser (Abbott Laboratories, USA).

Blood samples for the measurement of serum 25-hydroxyvitamin D [25(OH)D] concentration were collected after resolution of diabetic ketoacidosis and hyperglycemia that is on the day following the transition to subcutaneous insulin. Serum 25(OH)D levels were determined using the Alinity i 25-OH Vitamin D Reagent Kit (Abbott, Ireland), based on chemiluminescent microparticle immunoassay (CMIA) technology.

Definitions

Vitamin D status was classified according to the Polish and European Expert Consensus Statement based on serum 25(OH)D concentration as follows:

- deficiency: <20 ng/mL;
- insufficiency: 20–29 ng/mL;
- sufficiency: 30–50 ng/mL;
- high vitamin D status: >50–100 ng/mL;
- toxicity: >100 ng/mL [27].

Diabetic ketoacidosis (DKA) was diagnosed according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) criteria as the presence of:

- hyperglycaemia (blood glucose >11 mmol/L [>200 mg/dL]);
- venous pH <7.3 and/or serum bicarbonate <18 mmol/L;
- ketonaemia or moderate-to-large ketonuria [3].

DKA severity was classified as:

- mild: venous pH <7.3 or serum bicarbonate <18 mmol/L;
- moderate: pH <7.2 or serum bicarbonate <10 mmol/L;
- severe: pH <7.1 or serum bicarbonate <5 mmol/L [3].

Acute kidney injury (AKI) was defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline as any of the following:

- an increase in serum creatinine of ≥ 0.3 mg/dL within 48 hours;
- an increase in serum creatinine to ≥ 1.5 times the baseline value, known or presumed to have occurred within the preceding 7 days;
- urine output <0.5 mL/kg/h for at least 6 hours [6].

At admission, the estimated glomerular filtration rate (eGFR) was calculated using the Full Age Spectrum (FAS) equation ($eGFR_{FAS}$),

$$eGFR_{FAS} = \frac{107.3}{(SCr/Q)}$$

where SCr represents serum creatinine concentration and Q denotes the age-specific median serum creatinine value.

Because baseline serum creatinine values before admission were unavailable for all participants, the expected baseline creatinine (EBC) concentration was estimated assuming a normal GFR of 120 mL/min/1.73 m², in accordance with previous paediatric AKI studies [28,29]. The expected baseline creatinine was calculated using the following equation:

$$EBC = \frac{107,3 \times Q}{120}$$

The serum creatinine ratio (SCr/EBC) was subsequently calculated, and AKI was classified according to the KDIGO staging system:

- Stage 1: SCr/EBC ≥ 1.5 and < 2.0 ;
- Stage 2: SCr/EBC ≥ 2.0 and < 3.0 ;
- Stage 3: SCr/EBC ≥ 3.0 [6].

Urine output criteria were not used because hourly urine output data were not consistently available in the medical records.

Corrected serum sodium concentration (cNa) during DKA was calculated using the following formula:

$$\text{CorrectedNa} = \left(\frac{\text{GLU}(\text{mg/dL})}{18} - 5.6 \right) \times 0.36 + \text{SerumNa}$$

Ethical Considerations

As this was a retrospective observational study based exclusively on anonymised medical records, the Bioethics Committee waived the requirement for formal ethical approval and informed consent from the patients (decision No. KB-875/25).

Statistical Analysis

Analyses were restricted to patients with a measured 25(OH)D concentration (n = 400; non-DKA, n = 172; DKA, n = 208; DKA+AKI, n = 20). Distributions were assessed by group using histograms, Q-Q plots, skewness, kurtosis, the Shapiro-Wilk test, and the Kolmogorov-Smirnov test with Lilliefors correction; homogeneity of variances was assessed using Levene's test (median-centred). None of the 15 continuous variables satisfied normality in all three groups, and 8 showed heterogeneous variances, so non-parametric methods were used throughout.

Continuous variables are reported as median $\pm$ IQR and were compared across the three groups with the Kruskal-Wallis test, followed by pairwise Mann-Whitney tests. 25(OH)D status was dichotomised as deficiency (<20 ng/ml) versus no deficiency and compared with a 3×2 χ^2 test, followed by pairwise Fisher exact tests with OR and 95% CI. Holm correction was applied to the three pairwise comparisons within each variable separately; pairwise results were interpreted only for variables with a significant omnibus test. Effect sizes are ϵ^2 for Kruskal-Wallis and Cramér's V for χ^2 . Exact p -values are reported. Analyses were performed in R 4.5.

Correlations between continuous variables were evaluated using Spearman's correlation coefficients, as appropriate. A two-sided p -value <0.05 was considered statistically significant.

Results

Characteristics of the Study Population (Table 1, Figure 1)

The baseline demographic and clinical characteristics of the participants are presented in Table 1. The mean serum 25(OH)D concentration in the entire cohort was 21.77 ± 8.87 ng/mL. Vitamin D deficiency was identified in 178 patients (44.5%), vitamin D insufficiency in 165 patients (41.2%), vitamin D sufficiency in 55 patients (13.8%), and high vitamin D status in only two patients (0.5%) (Figure 1).

Comparison of Clinical and Laboratory Characteristics Among the Study Subgroups (Table 2, Figure 2)

The study population was divided into three subgroups according to the presence of diabetic ketoacidosis (DKA) and acute kidney injury (AKI):

- non-DKA group: patients without diabetic ketoacidosis ($n = 172$);

- DKA group: patients with diabetic ketoacidosis but without acute kidney injury (n = 208);
- DKA + AKI group: patients with diabetic ketoacidosis complicated by acute kidney injury (n = 20; 8.8% among patients with DKA and 5% of the study population);

In all patients with DKA (228 cases) the mild DKA was diagnosed in 88 cases, moderate in 58, while severe in 62 cases.

AKI was presented in stage 1 in 12 patients, in stage 2 in 6 patients and in stage 3 in 2 patients. Resolutions of AKI was observed maksimum within 8 days depend on severity of AKI.

Among studied patients 12 of 16 variables differed significantly across subgroups (see Table 2). Age ($p = 0.145$), BMI ($p = 0.196$), HbA1c ($p = 0.121$) and lymphocytes ($p = 0.399$) did not, with negligible effect sizes ($\epsilon^2 \leq 0.011$): the groups were therefore comparable in age and nutritional status.

Vitamin D decreased progressively across subgroups (non-DKA $\rightarrow$ DKA $\rightarrow$ DKA+AKI), and all three pairwise contrasts were significant. Deficiency prevalence rose from 36.6% (63/172) to 47.6% (99/208) to 80.0% (16/20) ($\chi^2 = 15.33$, $p < 0.001$, $V = 0.196$), with odds of deficiency 1.57-fold higher in DKA vs non-DKA (95% CI 1.02-2.43), 4.38-fold in DKA+AKI vs DKA (1.35-18.60) and 6.85-fold in DKA+AKI vs non-DKA (2.09-29.42).

Acidosis severity showed the largest effects: HCO₃ 21.60 ± 2.90 / 10.05 ± 9.30 / 4.65 ± 3.23 mmol/l ($\epsilon^2 = 0.748$) and pH 7.40 ± 0.03 / 7.26 ± 0.16 / 7.08 ± 0.21 ($\epsilon^2 = 0.661$), both with all pairwise contrasts significant, confirming DKA+AKI as the most severely acidotic group.

Renal and inflammatory markers: eGFR FAS was preserved in non-DKA and DKA (129.20 ± 34.92 and 125.75 ± 34.19) and halved in DKA+AKI (65.55 ± 26.47 ; $\epsilon^2 = 0.145$); the non-DKA vs DKA contrast was not significant, i.e. the deficit is specific to the AKI group. The same pattern held for corrected sodium ($\epsilon^2 = 0.067$). Uric acid ($3.50 / 5.50 / 11.90$ mg/dl, $\epsilon^2 = 0.316$), WBC ($\epsilon^2 = 0.204$), neutrophils ($\epsilon^2 = 0.214$) and monocytes ($\epsilon^2 = 0.119$) increased stepwise with all pairwise contrasts significant.

Correlation Analysis (Table 3, Figure 3)

Correlation analyses performed in the entire study population are presented in Table 3. Serum 25(OH)D concentration and eGFR were both positively correlated with blood pH and bicarbonate concentration, whereas negative correlations were observed with total WBC count, neutrophil count, and monocyte count. No significant correlation was found between serum 25(OH)D concentration and eGFR. Estimated GFR showed a significant negative correlation with corrected serum sodium concentration.

Discussion

The present study demonstrated that vitamin D deficiency (VDD) is highly prevalent among children and adolescents with newly diagnosed type 1 diabetes mellitus (T1DM). Vitamin D deficiency was identified in 44.5% of the entire cohort, with a higher prevalence among patients presenting with diabetic ketoacidosis (DKA) and the highest prevalence among those with DKA complicated by acute kidney injury (AKI). Serum 25(OH)D concentrations decreased progressively across the non-DKA, DKA, and DKA+AKI groups, and all pairwise comparisons were statistically significant. Importantly, the prevalence of VDD reached 80% in the DKA+AKI group, compared with 47.6% in patients with DKA without AKI and 36.6% in patients without DKA. These

findings indicate that lower vitamin D status is associated with a more severe clinical presentation of newly diagnosed T1DM, particularly when DKA is complicated by AKI. Our findings are broadly consistent with previous studies reporting lower serum 25(OH)D concentrations among children presenting with DKA and associations between vitamin D status and the severity of metabolic acidosis [21-26]. Previous studies have reported positive associations between 25(OH)D concentrations and pH or bicarbonate, suggesting that lower vitamin D concentrations are observed in children with more severe metabolic acidosis. Similar associations have also been reported for total, free, and bioavailable vitamin D concentrations in paediatric patients with newly diagnosed T1DM and DKA [26]. Thus, the progressive decrease in 25(OH)D observed across the clinical groups in our study is consistent with the existing literature. However, the timing of vitamin D measurement is important when interpreting these findings. In the present study, serum 25(OH)D was measured after resolution of DKA and severe hyperglycaemia, on the day following transition to subcutaneous insulin. Therefore, our results do not directly describe vitamin D concentrations during the acute phase of metabolic decompensation. The significant positive correlations observed between 25(OH)D and pH as well as bicarbonate should consequently be interpreted as associations between vitamin D status measured after metabolic stabilisation and the severity of the preceding acidosis, rather than as evidence that vitamin D deficiency caused the metabolic acidosis. Acute metabolic disturbances may themselves influence circulating vitamin D concentrations, and previous studies have reported an increase in serum 25(OH)D following correction of DKA [21,22]. The persistence of low 25(OH)D concentrations after resolution of DKA in our cohort nevertheless suggests that the observed differences cannot be attributed solely to the acute metabolic state.

The most novel finding of the present study was the marked reduction in serum 25(OH)D concentrations among children with DKA complicated by AKI. The DKA+AKI group had both the highest prevalence of VDD and the lowest vitamin D concentrations, while 80% of these patients fulfilled the criteria for VDD. Furthermore, the odds of vitamin D deficiency were substantially higher in the DKA+AKI group than in either the DKA group or the non-DKA group. To our knowledge, data specifically addressing the relationship between vitamin D status and AKI in children with newly diagnosed T1DM and DKA remain limited. Our findings therefore provide a basis for considering vitamin D status as a potential marker associated with the severity of acute metabolic and renal complications at T1DM onset. Nevertheless, because of the retrospective observational design, these results should not be interpreted as evidence that VDD independently predisposes to AKI.

Several biological mechanisms may potentially explain the observed association between low vitamin D status and renal dysfunction. Vitamin D signalling through the vitamin D receptor is involved in regulation of inflammatory pathways, endothelial function and the renin–angiotensin–aldosterone system (RAAS), while experimental studies have suggested that impaired vitamin D signalling may promote oxidative stress, inflammation and renal injury [17-20]. These mechanisms could theoretically contribute to renal susceptibility during severe DKA, particularly in the presence of dehydration and systemic inflammation. However, the present study did not measure vitamin D metabolites, vitamin D-binding protein, VDR activity, RAAS components, oxidative stress markers, or cytokines. Therefore, these mechanisms remain hypothetical in the context of our clinical data and require confirmation in prospective studies.

The renal findings in our study support the important role of volume depletion in AKI during DKA. Estimated GFR was preserved in both the non-DKA and DKA groups but was markedly reduced in the DKA+AKI group. In parallel, corrected serum sodium concentrations differed between the groups and were inversely associated with eGFR. These findings are consistent with the contribution of dehydration and reduced effective circulating volume to renal dysfunction during DKA. Our previous paediatric studies have similarly identified increased corrected sodium as a marker associated with AKI during acute diabetic complications [5]. The present results therefore support the interpretation that pre-renal mechanisms remain an important component of AKI in children with DKA.

At the same time, the association between renal dysfunction and inflammatory markers suggests that the pathophysiology of AKI may not be explained by volume depletion alone. White blood cell, neutrophil, and monocyte counts increased progressively from the non-DKA group through DKA to DKA+AKI, and both serum 25(OH)D and eGFR were negatively correlated with these inflammatory parameters. DKA is accompanied by systemic inflammatory activation and oxidative stress, which may contribute to endothelial and tubular injury [30-34]. Thus, the combination of marked dehydration and inflammatory activation may be particularly relevant to the development of AKI in severe DKA. Our findings are compatible with a multifactorial model in which haemodynamic and inflammatory mechanisms coexist rather than with a single mechanism responsible for renal dysfunction.

The inverse association between 25(OH)D and inflammatory cell counts is also of interest. Vitamin D has recognised immunomodulatory effects, including regulation of inflammatory responses in monocytes, macrophages, dendritic cells and lymphocytes [11,32,33]. The observed association could therefore be biologically plausible.

Nevertheless, because inflammatory markers and vitamin D were assessed at different stages of the acute illness, and because the study was not designed to establish temporal relationships, it is not possible to determine whether lower vitamin D status contributed to the inflammatory response, whether systemic inflammation influenced vitamin D concentrations, or whether both findings reflect greater overall severity of DKA. The latter explanation is particularly important because 25(OH)D concentrations were measured only after resolution of the acute metabolic disturbance.

The relationship between acute hyperglycaemia and vitamin D metabolism also warrants cautious interpretation. Experimental studies suggest that diabetes and also fasting may influence enzymes involved in vitamin D metabolism. This conditions may suppress hepatic CYP2R1 and decrease concentration of 25(OH)D [35]. Moreover hyperglycemia can increase renal expression of CYP24A1 the enzyme responsible for catabolism of 25(OH)D and 1,25(OH)₂D₃ [35,36]. Consequently, acute hyperglycaemia may simultaneously reduce 25(OH)D synthesis while accelerating its degradation. Tourigny et al. demonstrated that diabetic mouse kidneys exhibit a two- to three-fold increase in CYP24A1 expression within proximal tubular cells [36]. In addition to katabolising 25(OH)D and 1,25(OH)₂D₃, CYP24A1 promoted tubular cell senescence, apoptosis, and renal injury under hyperglycaemic conditions. Because CYP24A1 expression is substrate-dependent, the authors suggested that vitamin D supplementation during severe hyperglycaemia might further increase CYP24A1 activity and potentially exacerbate tubular injury [36].

These observations provide a possible biological explanation for decrease concentration of 25(OH)D during severe metabolic decompensation in DM1. However, the present study did not measure these enzymes or serial vitamin D metabolites. Moreover, no significant association between serum glucose and 25(OH)D was

identified in our cohort. Because vitamin D was measured after correction of hyperglycaemia, the absence of such an association is not unexpected and cannot be used to determine whether hyperglycaemia directly affected vitamin D metabolism. Accordingly, the proposed involvement of altered hepatic synthesis or renal catabolism should be regarded as a possible mechanism rather than a conclusion supported directly by our data.

An additional important observation was the absence of significant differences in age, BMI, HbA1c and lymphocyte count between the three study groups. This suggests that the marked differences in vitamin D status, acid–base parameters, renal function and inflammatory markers were not simply explained by differences in age or nutritional status between the groups. Nevertheless, the study did not include a healthy control group, and therefore the prevalence of VDD observed in our cohort cannot be directly compared with that of the general paediatric population.

The present study has several limitations. First, its retrospective observational design precludes conclusions regarding causality and introduces the possibility of residual confounding. Second, 25(OH)D was measured after resolution of DKA and hyperglycaemia rather than at the time of acute metabolic decompensation. Consequently, we cannot determine the extent to which the observed vitamin D concentrations reflect pre-existing vitamin D status or changes induced by the acute illness and its treatment. Third, serial measurements of 25(OH)D were not available, preventing assessment of temporal changes during and after DKA. Fourth, AKI was classified using an estimated baseline creatinine because previous baseline serum creatinine measurements were unavailable for all participants. These methodological limitations should be considered when interpreting the association between vitamin D status and AKI. Finally, the DKA+AKI group consisted of only 20 patients, which limits

the precision of estimates and reduces the ability to perform more extensive multivariable analyses.

Despite these limitations, the study has several strengths. It included a relatively large cohort of 400 children and adolescents with newly diagnosed T1DM and used uniform laboratory measurements obtained within a single paediatric centre. The analysis also distinguished patients with DKA from those with DKA complicated by AKI, allowing the association between vitamin D status and renal complications to be examined separately. Importantly, the statistical analysis demonstrated a consistent stepwise pattern across the three clinical groups, with lower 25(OH)D concentrations accompanying greater metabolic and renal severity.

Conclusions

Vitamin D deficiency is common in children and adolescents at the onset of type 1 diabetes mellitus and is particularly prevalent among patients presenting with diabetic ketoacidosis. In the present study, the lowest serum 25(OH)D concentrations and the highest prevalence of vitamin D deficiency were observed in patients with DKA complicated by acute kidney injury. Lower 25(OH)D concentrations measured after resolution of DKA and hyperglycaemia were associated with greater severity of preceding metabolic acidosis and with increased inflammatory activity.

Renal dysfunction during DKA was associated with both markers of dehydration and systemic inflammatory activation, supporting a multifactorial pathophysiology of AKI. The observed association between low vitamin D status and AKI may reflect the severity of acute metabolic disturbance and is biologically plausible; however, the present study does not establish a causal relationship between vitamin D deficiency and renal injury.

Further prospective studies with serial assessment of vitamin D status and biomarkers of renal injury, inflammation, and vitamin D metabolism are needed to determine the temporal relationship between vitamin D deficiency, DKA severity, and AKI and to establish whether vitamin D status has prognostic or therapeutic significance in children with newly diagnosed T1DM.

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Table 1. The demographic and clinical data of the total study group .

Variable (unit)	<i>n</i> (non-DKA/DKA/DKA+AKI)
Age (ys)	±
BMI (kg/m2)	±
25(OH)D (ng/ml)	±
Deficiency 25(OH)D <20 ng/ml, n (%)	±
pH	±
HCO ₃ (mmol/l)	±
Glu (mg/dl)	±
eGFR _{FAS} (ml/min/1.73m2)	±
UA (mg/dl)	±
HbA1c (%)	±
WBC (10 ³ /ul)	±
PLT (10 ³ /ul)	±
Neutrophils (10 ³ /ul)	±
Lymphocytes (10 ³ /ul)	±
Monocytes (10 ³ /ul)	±
cNa (mmol/l)	±
Note: Values are median ± IQR; the row "Deficiency 25(OH)D <20 ng/ml" reports n (% of yes within group) ; cNa – corrected serum sodium	

Table 2. Vitamin D deficiency, acidosis severity, and systemic inflammation increase stepwise from patients without DKA to those with DKA and are most pronounced in DKA complicated by AKI.

Variable (unit)	<i>n</i> (non-DKA/DKA/DKA+AKI)	non-DKA	DKA	DKA+AKI	<i>p</i>	Effect size
Age (ys)	172/208/20	9.75 ± 6.07	8.90 ± 7.13	9.14 ± 6.66	0.145	0.01
BMI (kg/m ²)	169/206/19	15.94 ± 3.28	15.79 ± 3.30	15.06 ± 3.36	0.196	0.01
25(OH)D (ng/ml)	172/208/20	22.35 ± 10.43ac	20.80 ± 12.33ab	14.30 ± 10.22bc	<0.001	0.04
Deficiency 25(OH)D <20 ng/ml, n (%)	172/208/20	63 (36.6%)ac	99 (47.6%)ab	16 (80.0%)bc	<0.001	0.20
pH	172/208/20	7.40 ± 0.03ac	7.26 ± 0.16ab	7.08 ± 0.21bc	<0.001	0.66
HCO ₃ (mmol/l)	172/208/20	21.60 ± 2.90ac	10.05 ± 9.30ab	4.65 ± 3.23bc	<0.001	0.75
Glu (mg/dl)	172/208/20	363.00 ± 189.25ac	412.50 ± 211.75ab	721.00 ± 224.00bc	<0.001	0.11
eGFR _{FAS} (ml/min/1.73m ²)	171/206/20	129.20 ± 34.92c	125.75 ± 34.19b	65.55 ± 26.47bc	<0.001	0.14
UA (mg/dl)	169/202/19	3.50 ± 1.70ac	5.50 ± 2.80ab	11.90 ± 5.95bc	<0.001	0.32
HbA1c (%)	166/196/19	10.90 ± 3.57	11.65 ± 2.63	10.50 ± 3.50	0.121	0.01
WBC (10 ³ /ul)	172/208/20	9.07 ± 3.63ac	11.48 ± 6.19ab	26.20 ± 17.38bc	<0.001	0.20
PLT (10 ³ /ul)	172/208/20	280.50 ± 106.75ac	306.00 ± 107.25ab	393.50 ± 155.25bc	<0.001	0.03
Neutrophils (10 ³ /ul)	172/208/19	4.67 ± 2.39ac	6.83 ± 5.68ab	19.40 ± 10.50bc	<0.001	0.21
Lymphocytes (10 ³ /ul)	172/208/19	3.18 ± 1.89	3.05 ± 2.14	3.40 ± 2.77	0.399	0.00
Monocytes (10 ³ /ul)	172/208/19	0.62 ± 0.29ac	0.69 ± 0.40ab	2.36 ± 1.50bc	<0.001	0.12
cNa (mmol/l)	172/208/20	138.00 ± 3.20c	138.00 ± 5.20b	145.75 ± 9.22bc	<0.001	0.07

Note: Values are median ± IQR; the row "Deficiency 25(OH)D <20 ng/ml" reports n (% of yes within group). Global *p* values are from the Kruskal-Wallis test for continuous variables or the chi-square test for the 3×2 table (vitamin D deficiency); nonparametric tests were used because no continuous variable met the normality assumption in all three groups. Effect sizes are epsilon squared for the Kruskal-Wallis test and Cramér's V for the chi-square test. Superscript letters indicate pairs of groups that differ significantly in pairwise comparisons: a = non-DKA vs DKA, b = DKA vs DKA+AKI, c = non-DKA vs DKA+AKI; the same letter on two groups denotes a significant difference between them. Pairwise comparisons used the Mann-Whitney test for continuous variables or Fisher's exact test for vitamin D deficiency, with Holm correction across the 3 comparisons applied separately within each variable; *q* < 0.1 was considered significant.

25(OH)D concentrations fell progressively across the three groups, and 25(OH)D deficiency (<20 ng/ml) was present in 36.6% of patients without DKA, 47.6% of patients with DKA, and 80.0% of those with DKA and AKI, with the DKA+AKI group differing significantly from both other groups. Metabolic severity showed the same gradient and the largest effects (HCO₃⁻, $\epsilon^2 = 0.75$; pH, $\epsilon^2 = 0.66$), as did uric acid ($\epsilon^2 = 0.32$) and markers of

the inflammatory response (WBC and neutrophils, $\epsilon^2 = 0.20$ and 0.21), while eGFR was reduced only in the DKA+AKI group. BMI: body mass index; 25(OH)D: 25-hydroxyvitamin D; Glu: glucose; HbA1c: glycated haemoglobin; eGFR_{FAS}: estimated glomerular filtration rate (full age spectrum equation); UA: uric acid; cNa: corrected sodium; WBC: white blood cells; PLT: platelets.

Table 3. Lower 25(OH)D is associated with more severe acidosis and higher neutrophil counts, while myeloid cell counts scale inversely with pH, bicarbonate, and eGFR.

Variable	pH	HCO ₃ (mmol/l)	eGFR FAS (ml/min/1.73m ²)	25(OH)D (ng/ml)
pH	NA	0.917***	0.191***	0.180***
HCO ₃ (mmol/l)	0.917***	NA	0.155**	0.180***
eGFR _{FAS} (ml/min/1.73m ²)	0.191***	0.155**	NA	0.006
25(OH)D (ng/ml)	0.180***	0.180***	0.006	NA
cNa (mmol/l)	-0.151**	-0.126*	-0.220***	-0.061
WBC (10 ³ /ul)	-	-	-0.207***	-0.033
Lymphocytes (10 ³ /ul)	0.063	0.023	0.074	0.218***
Monocytes (10 ³ /ul)	-	-	-0.126*	-0.042
Neutrophils (10 ³ /ul)	0.371***	0.372***	-0.208***	-0.150**
HbA1c (%)	-0.137**	-0.158**	0.178***	-0.044

Note: Presented values are Spearman's rank correlation coefficients (ρ). Correlations were computed pairwise, i.e. each coefficient uses all patients with both variables available ($n = 379-400$; the lowest counts apply to pairs involving HbA1c, $n = 379-381$, all other pairs $n \geq 396$). Statistical significance is marked as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; unmarked coefficients are not statistically significant ($p \geq 0.05$).

pH and HCO₃⁻ were strongly correlated with each other ($\rho = 0.917$). Both showed moderate negative correlations with neutrophils ($\rho = -0.523$ and -0.522), total leukocytes ($\rho = -0.492$ and -0.498), and monocytes ($\rho = -0.371$ and -0.372), together with weaker negative correlations with corrected sodium and HbA1c; lymphocyte count was unrelated to acid-base status. 25(OH)D correlated positively with pH and HCO₃⁻ ($\rho = 0.180$ for both) and with lymphocyte count ($\rho = 0.218$), and negatively with neutrophils ($\rho = -0.150$), whereas its associations with monocytes, total leukocytes, corrected sodium and HbA1c did not reach significance. eGFR was essentially independent of 25(OH)D ($\rho = 0.006$) but correlated positively with pH, HCO₃⁻ and HbA1c and negatively with the myeloid cell counts and corrected sodium. HCO₃⁻: bicarbonate; eGFR_{FAS}: estimated glomerular filtration rate (Full Age Spectrum equation); 25(OH)D: 25-hydroxyvitamin D; WBC: white blood cells; cNa: corrected sodium; HbA1c: glycated haemoglobin;

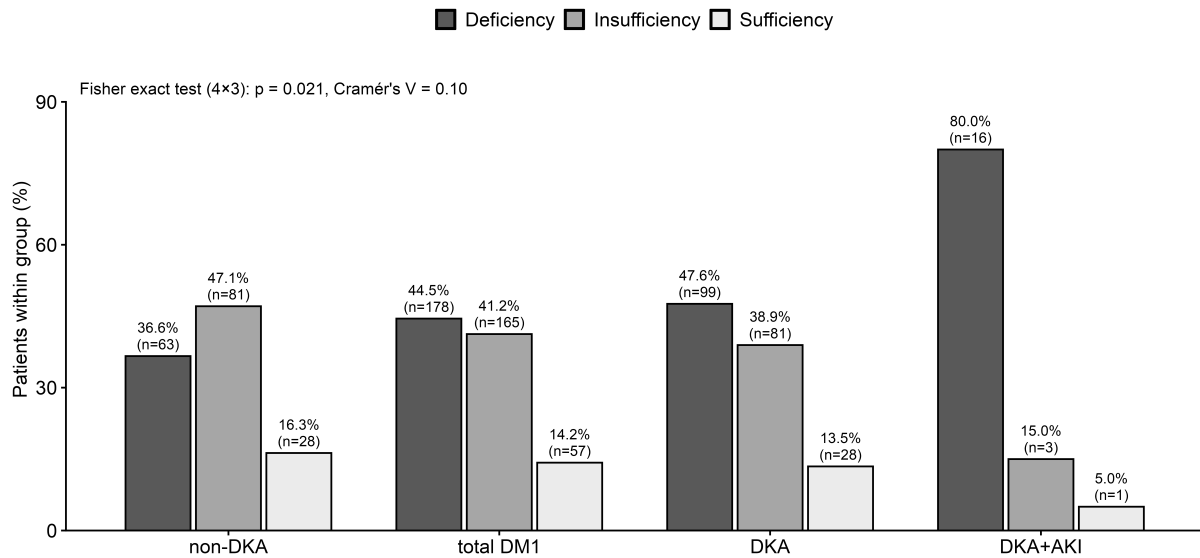


Figure 1. Vitamin D status differed significantly across the studied groups (Fisher's exact test, 3x3, Monte Carlo: p = 0.004; Cramér's V = 0.14). In the whole cohort, 44.5% (n = 178) of patients were 25(OH)D deficient, 41.2% (n = 165) insufficient, and 14.2% (n = 57) sufficient. The proportion of deficiency rose progressively across groups, from 36.6% (n = 63) in non-DKA patients to 47.6% (n = 99) in DKA and 80.0% (n = 16) in DKA+AKI, while sufficiency fell from 16.3% (n = 28) to 13.5% (n = 28) and 5.0% (n = 1), respectively. Total DM1: all diabetic patients; non-DKA: patients without diabetic ketoacidosis; DKA: patients with diabetic ketoacidosis; DKA+AKI: patients with diabetic ketoacidosis complicated by acute kidney injury.

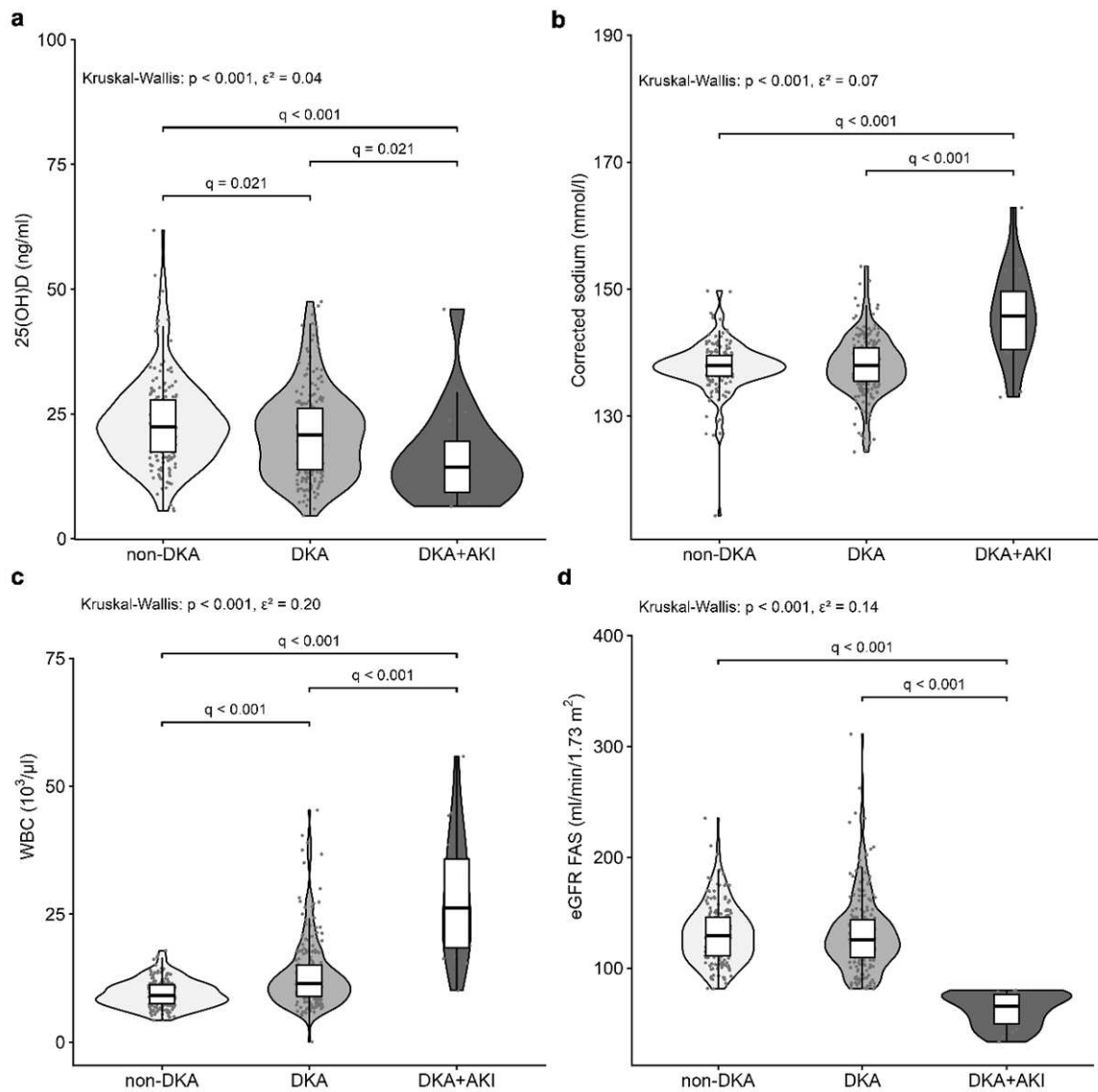


Figure 2. 25(OH)D declines and corrected sodium, leukocyte count, and renal impairment increase progressively from non-DKA to DKA and DKA complicated by AKI. (a) Distribution of 25(OH)D, (b) corrected sodium, (c) white blood cell count, and (d) eGFR in patients without DKA ($n = 172$), with DKA ($n = 208$), and with DKA complicated by AKI ($n = 20$). (a) 25(OH)D fell stepwise across the three groups, with each group differing from the others, while (b) corrected sodium and (d) eGFR separated the DKA+AKI group from both other groups without differing between non-DKA and DKA. (c) Leukocyte count showed the largest effect ($\epsilon^2 = 0.20$) and rose significantly at each step. Violins show the kernel density of the distribution, overlaid with box plots (line, median; box, interquartile range; whiskers, $1.5 \times \text{IQR}$) and individual patient values as jittered points. Global comparisons are Kruskal-Wallis tests with epsilon-squared effect sizes, shown above each panel; horizontal brackets give Holm-adjusted q values from pairwise Mann-Whitney tests, and only comparisons reaching $q < 0.1$ are annotated. DKA, diabetic ketoacidosis; AKI, acute kidney injury; 25(OH)D, 25-hydroxyvitamin D; WBC, white blood cells; eGFR_{FAS}, estimated glomerular filtration rate (Full Age Spectrum Equation).

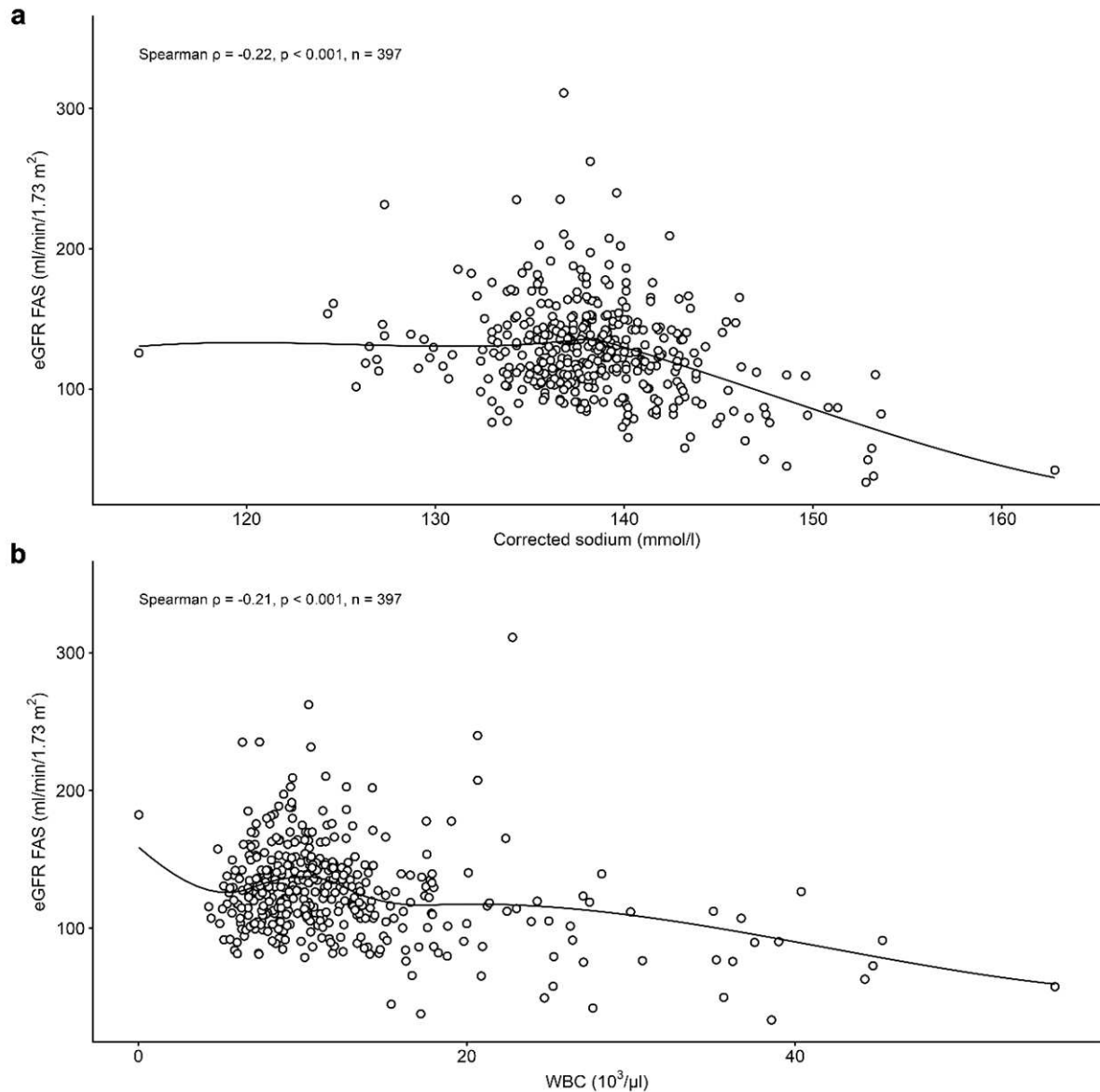


Figure 3. Renal function declines with increasing corrected sodium and leukocyte count. Relationship between (a) eGFR and corrected sodium and between (b) eGFR and white blood cell count across the whole cohort. Both associations were negative and of similar magnitude (Spearman's $\rho = -0.22$ and -0.21 , respectively; $P < 0.001$, $n = 397$), and in each case the decline in eGFR was concentrated at the upper end of the exposure range: eGFR was essentially flat for corrected sodium below approximately 140 mmol/l and for leukocyte counts below approximately $10 \times 10^3/\mu\text{l}$, falling steadily beyond those points. Each circle represents one patient; the solid line is a LOESS smoother fitted to the raw data, shown to display the shape of the association and not as a fitted statistical model. Correlation coefficients are Spearman's ρ computed on complete pairs. eGFR_{FAS}, estimated glomerular filtration rate (Full Age Spectrum equation); WBC, white blood cells.