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# Vitamin D receptor polymorphisms and vitamin D insufficiency are not associated with sepsis in critically ill children: a case-control study

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## Abstract

**Background:** Vitamin D is a fat-soluble vitamin that regulates calcium and phosphorous homeostasis to maintain a healthy mineralized skeleton. It can also influence immune responses and has immunomodulatory properties. Vitamin D receptor (VDR) is a nuclear receptor that mediates the activities of the hormonal form of vitamin D. VDR polymorphisms can alter immunity and susceptibility to infections by modulating VDR expression and vitamin D activity. This study aimed to investigate the levels of serum vitamin D as well as four VDR polymorphisms: FokI, BsmI, ApaI, and TaqI in fifty children admitted to intensive care unit (ICU) with a diagnosis of sepsis and one-hundred age- and sex-matched healthy children.

**Methods:** Vitamin D levels were measured in serum, in both patients and controls, using an enzyme-linked immunosorbent assay (ELISA) approach. VDR polymorphisms were also studied in both groups using specific restriction enzymes.

**Results:** Vitamin D levels were low in both patients and controls. Moreover, serum levels were unaffected by VDR polymorphisms, and their distribution was similar in both groups. Neither the need for mechanical ventilation or inotropic treatment nor the sepsis outcome was impacted by serum vitamin D levels or VDR polymorphisms.

**Conclusion:** In children admitted to pediatric ICU, neither vitamin D levels nor VDR polymorphisms were associated with sepsis. Further larger studies including different types of sepsis are recommended.

## Highlights

- Vitamin D level was low in both pediatric septic ICU patients and age- and sex-matched healthy controls.
- Vitamin D levels were unaffected by VDR polymorphisms, and their distribution was similar in both groups.
- Neither the need for mechanical ventilation or inotropic treatment nor the sepsis outcome was impacted by vitamin D or VDR polymorphisms.

**Keywords:** Pediatric, Polymorphisms, Sepsis, VDR, Vitamin D

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## Background

Vitamin D is a fat-soluble steroid that is necessary for higher mammals to survive. It is a prohormone, found in a small number of foods, and is synthesized

endogenously in the skin via a photochemical process [1]. Vitamin D is best known for its ability to maintain calcium and phosphorus balance and thus a healthy mineralized skeleton. Additionally, it exhibits immunomodulatory properties [2]. It has the ability to modulate both acquired and innate immune responses [3].

Vitamin D receptor (VDR) is a member of the nuclear receptor superfamily [4] that is involved in the activity of 1,25-dihydroxy vitamin D [1,25(OH) D], the hormonal form of vitamin D. Practically, vitamin D receptors are found in all tissues and cells in the human body [5]. Vitamin D receptor polymorphisms can affect the level of VDR expression, which has a significant effect on immune function and susceptibility to microbial infections [6].

Sepsis remains the leading cause of hospitalization and intensive care unit (ICU) admission. Sepsis is a term that refers to a complex clinical state that occurs as a result of toxic or adverse host response to infection. Despite breakthroughs in sepsis management, sepsis continues to increase in prevalence and is considered the primary cause of mortality in critically ill patients. A substantial proportion of individuals diagnosed with sepsis progress to severe sepsis or septic shock [7].

Numerous observational studies have established a link between low serum 25(OH) D levels and sepsis development, as well as an increase in morbidity, mortality, and ICU stay in septic and critically sick patients [8]. Vitamin D deficiency has been linked to a tendency to sepsis in multiple adult studies [9]. Similarly, multiple studies in children have demonstrated a substantial link between vitamin D insufficiency and respiratory tract infections and sepsis [10–12]. Several other investigations have discovered an association between low serum vitamin D levels and increased risk of sepsis in neonates [13, 14].

This association could be attributed to the effects of 1,25(OH) D, which inhibits the overexpression of inflammatory cytokines and promotes antibacterial responses in innate immunity [2, 9, 15]. Additionally, vitamin D3 and its metabolites have nongenomic effects on endothelial cells, preventing vascular leakage, which may be lifesaving in septic shock [16]. Low serum 25(OH) D levels in sepsis and critical illness may also be induced by extravascular vitamin D-binding protein leakage and enhanced 25-hydroxyvitamin D-24-hydroxylase activity caused by systemic inflammation [17].

The aim of the current study was to investigate the effect of serum vitamin D level and the nucleotide variants of four genetic VDR polymorphisms: FokI (rs2228570), BsmI (rs1544410), ApaI (rs7975232), and TaqI (rs731236), separately, on the clinical characteristics as well as the outcome of critically ill pediatric patients with sepsis in comparison with age- and sex-matched healthy controls.

## Methods

This study involved fifty children who were admitted to the pediatric ICU of the Cairo University Children's Hospital with a diagnosis of sepsis. We included patients with sepsis diagnosis aged from 1 month to 13 years old with community-acquired infections within the first 48 h of admission to the ICU, and we excluded patients with malnutrition, chronic kidney, and liver diseases. Patients admitted to ICU for reasons other than sepsis were also excluded. The diagnosis of sepsis was based on the criteria proposed by Goldstein et al., in 2006 [18], which included any of the following:

- *Systemic inflammatory response syndrome (SIRS)*: At least two of the four criteria are met, including an abnormal temperature or leukocyte count: first, core body temperature more than 38.5 °C or below 36 °C; second, tachycardia; third, two standard deviations above the age-normal mean respiratory rate or mechanical ventilation for an acute situation unrelated to neuromuscular disease or general anesthesia; and forth, an abnormal leukocyte count for age.
- *Sepsis*: SIRS caused by or occurring concurrently with an infection (suspected or confirmed)
- *Severe sepsis*: Sepsis with cardiovascular organ dysfunction OR with acute respiratory distress syndrome OR with two or more organ dysfunctions
- *Septic shock*: Sepsis and cardiovascular organ dysfunction

As controls, one-hundred age- and sex-matched children were recruited from outpatient clinics. They were presenting for elective surgical procedures or for assessment of noninfectious conditions. They were not receiving vitamin D supplementations.

Both cases and controls were recruited in the period from September 2019 to January 2020. Informed consents were obtained from their guardians. The study was approved by the ethical committee of each of the Clinical and Chemical Pathology and the Pediatrics Departments, at the Kasr Al-Ainy Faculty of Medicine, Cairo University, and was conducted in concordance with the Declaration of Helsinki October 2013 (ethical principles for medical research involving human subjects).

Vitamin D levels and vitamin D polymorphisms (BsmI, Fok1, TaqI, or ApaI genotypes) were tested for all patients and controls.

### Measurement of serum 25(OH) D concentrations

The levels of 25(OH) D in the serum were determined using an enzyme-linked immunosorbent assay (ELISA) approach (*25-OH-vitamin D ELISA (RE53041), Catalog number: MG59061; IBL International, Hamburg, Germany*). According to the kit used and considering the patient's age, vitamin D deficiency was defined as 25(OH) D concentrations less than 10 ng/mL and insufficient concentrations were considered for levels between 10 and 30 ng/mL and sufficient concentrations for levels between 30 and 100 ng/mL.

### Genotyping of the candidate genes

Total DNA was isolated from peripheral mononuclear cells collected on ethylenediaminetetraacetic acid (*EDTA*) using (*GeneJET Whole Blood Genomic DNA Purification Mini Kit. Catalog number: Ko781; Thermo Fisher Scientific*). Polymorphisms in the investigated genes were tested using the polymerase chain reaction-restriction fragment length polymorphism approach (PCR-RFLP) employing specific restriction endonuclease enzymes for each polymorphic site [*BsmI*, *FokI*, *TaqI*, and *ApaI* for BsmI (rs1544410), FokI (rs2228570), TaqI (rs731236), and ApaI (rs7975232) polymorphic sites, respectively] [19]. These enzymes were used to digest the amplified polymerase chain reaction products. Specific primers, supplied by (Thermo Fisher Scientific), the summary of the amplification conditions, the restriction enzymes used (Thermo Fisher Scientific), and the lengths of the restriction fragments are all shown in Supplementary Table 1. The fragments were visualized on ethidium bromide 2% stained agarose gel. For quality control, 25 randomly chosen patients and control samples were genotyped and analyzed blindly by two independent observers, who confirmed that the results were identical to those obtained first.

The resulting nucleotide variants were as follows: for BsmI (rs1544410): bb (homo mutant), Bb (hetero mutant), and BB (wild); for FokI (rs2228570): ff (homo mutant), Ff (hetero mutant), and FF (wild); for TaqI (rs731236): tt (homo mutant), Tt (hetero mutant), and TT (wild); and for ApaI (rs7975232): aa (homo mutant), Aa (hetero mutant), and AA (wild).

### Statistical analysis

The statistical package for the social sciences (SPSS) version 26 was used to code and enter data (IBM Corp., Armonk, NY, USA). The mean, standard deviation, median, minimum, and maximum values were used to represent quantitative data, while frequency (count) and relative frequency (%) were used to summarize categorical data. The Kruskal-Wallis and Mann-Whitney tests were applied to make nonparametric comparisons between quantitative variables. The chi-square (2) test was used to compare categorical data. When anticipated frequency is less than 5, the exact test was utilized instead. The Spearman correlation coefficient was used to determine correlations between quantitative variables. Statistical significance was defined as *P*-values less than 0.05.

## Results

Thirty patients (60%) were males, and 20 patients (40%) were females. Sixty-two (62%) of the controls were males, and 38 (38%) were females. The median age of patients was 12 months [1–156 months], and the median age of the control subjects was 15 months [1–140 months]. Patient characteristics are shown in Table 1.

**Table 1** Clinical characteristics of the studied patients (no. = 50)

| | | Mean | Standard deviation | Median | Minimum | Maximum |
|---|---|---|---|---|---|---|
| **Age (months)** | | **37.54** | **42.87** | **12.00** | **1.00** | **156.00** |
| **Length of stay (days)** | | **16.10** | **18.42** | **11.00** | **1.00** | **90.00** |
| | | | | | **Patients** | |
| | | | | | **Count** | **%** |
| **Central nervous system (CNS) infection** | | | | | **3/50** | **6%** |
| **Cholangitis** | | | | | **1/50** | **2%** |
| **Pneumonia** | | | | | **24/50** | **48** |
| **Bloodstream infection** | | | | | **26/50** | **52%** |
| **Mechanical ventilation** | **Yes** | | | | **39/50** | **78%** |
| | **No** | | | | **11/50** | **22%** |
| **Inotropic support** | **Yes** | | | | **31/50** | **62%** |
| | **No** | | | | **19/50** | **38%** |
| **Outcome** | **Survival** | | | | **30/50** | **60%** |
| | **Death** | | | | **20/50** | **40%** |

Vitamin D levels and the distribution of VDR polymorphisms did not significantly differ between patients and controls (Table 2). No significant association was found between vitamin D levels and the patients' gender or between vitamin D levels and ICU characteristics (need for mechanical ventilation, inotropic support, and sepsis outcome, whether survival or death) (Table 3).

In the studied patients, no significant correlation was observed between vitamin D level on one hand and each of age ($p = 0.932$, $r = -0.12$) and length of ICU stay days ($p = 0.9$, $r = 0.002$) on the other hand. Comparison between different VDR genotypes (BB, bb, Bd, FF, ff, Ff, AA, aa, Aa, TT, tt, Tt) and each of age, length of ICU stays, and vitamin D levels did not show any significant difference (Table 4). There was no significant difference between different genotypes and any of the ICU parameters, including the need for mechanical ventilation, inotropic support, and sepsis outcome, whether survival or death (Table 5).

**Table 2** Vitamin D levels and VDR polymorphisms among cases and controls

| Vitamin D (ng/ml) | Patients (No.=50) | | | | Controls (No.=100) | | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD[a] | Median | Range | Mean | SD[a] | Median | Range | |
| | 29.23 | 31.47 | 19.05 | 9.8-158.6 | 20.72 | 9.65 | 20.9 | 9.6-51 | 0.673 |
| | Patients (No.=50) | | | | Controls (No.=100) | | | | *P*-value |
| | Count | | % | | Count | | % | | |
| **Bsml** | | | | | | | | | |
| GG | 10/50 | | 20% | | 14/100 | | 14% | | 0.63 |
| AG | 17/50 | | 34% | | 38/100 | | 38% | | |
| AA | 23/50 | | 46% | | 48/100 | | 48% | | |
| **Fok I** | | | | | | | | | |
| TT | 5/50 | | 10% | | 12/100 | | 12% | | 0.512 |
| CT | 18/50 | | 36% | | 44/100 | | 44% | | |
| CC | 27/50 | | 54% | | 44/100 | | 44% | | |
| **ApaI** | | | | | | | | | |
| CC | 6/50 | | 12% | | 6/100 | | 6% | | 0.168 |
| AC | 13/50 | | 26% | | 40/100 | | 40% | | |
| AA | 31/50 | | 62% | | 54/100 | | 54% | | |
| **TaqI** | | | | | | | | | |
| TT | 18/50 | | 36% | | 24/100 | | 24% | | 0.203 |
| CT | 18/50 | | 36% | | 50/100 | | 50% | | |
| CC | 14/50 | | 28% | | 26/100 | | 26% | | |

[a] Standard deviation

**Table 3** Vitamin D levels and main ICU parameters of patients (no. = 50)

| | | Vitamin D (ng/ml) | | | | |
|---|---|---|---|---|---|---|
| | | Mean | SD[a] | Median | Range | *p*-value |
| **Sex** | **Male** | 22.33 | 13.88 | 19.05 | 9.80–65.40 | 0.866 |
| | **Female** | 39.59 | 45.52 | 18.55 | 9.80–158.60 | |
| **Mechanical ventilation need** | **Needed** | 26.21 | 26.81 | 18.80 | 9.80–158.60 | 0.355 |
| | **Not needed** | 39.95 | 44.26 | 19.70 | 11.20–156.80 | |
| **Inotropic support need** | **Needed** | 26.57 | 28.25 | 19.30 | 9.80–158.60 | 0.749 |
| | **Not needed** | 33.57 | 36.52 | 18.80 | 9.80–156.80 | |
| **Outcome** | **Survival** | 30.45 | 30.22 | 20.30 | 9.80–156.80 | 0.151 |
| | **Death** | 27.40 | 33.97 | 14.35 | 9.80–158.60 | |

[a] Standard deviation

**Table 4** VDR polymorphisms distribution among the studied patients in relation to age, length of ICU stay, and vitamin D levels

| | | BsmI | | | *p*-value | Fok I | | | *p*-value | ApaI | | | *p*-value | TaqI | | | *p*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GG | AG | AA | | TT | CT | CC | | CC | AC | AA | | TT | CT | CC | |
| **Age (months)** | **Mean** | 23.8 | 41 | 40.96 | 0.508 | 16.20 | 34.50 | 43.52 | 0.113 | 23.50 | 35.54 | 41.10 | 0.722 | 22.61 | 46.72 | 44.93 | 0.071 |
| | **SD[a]** | 35.75 | 44.32 | 45.11 | | 20.12 | 49.48 | 40.87 | | 27.46 | 44.02 | 45.24 | | 33.2 | 48.54 | 43.84 | |
| | **Median** | 12 | 24 | 12 | | 7.00 | 9.00 | 24.00 | | 14.00 | 12.00 | 12.00 | | 7.5 | 24 | 30 | |
| | **Range** | 1–120 | 3–144 | 1–156 | | 1–48 | 2–156 | 1–144 | | 2–72 | 2–156 | 1–144 | | 1–108 | 2–156 | 1–144 | |
| **Length of stay (days)** | **Mean** | 8.8 | 22 | 14.91 | 0.08 | 11.40 | 15.78 | 17.19 | 0.762 | 23.00 | 12.77 | 16.16 | 0.4 | 17.17 | 14.28 | 17.07 | 0.421 |
| | **SD[a]** | 7.16 | 22.21 | 17.98 | | 6.50 | 20.49 | 18.78 | | 20.53 | 7.36 | 21.16 | | 19.78 | 20.05 | 15.3 | |
| | **Median** | 6.5 | 15 | 9 | | 12.00 | 9.00 | 11.00 | | 17.50 | 12.00 | 9.00 | | 11.5 | 8.5 | 13.5 | |
| | **Range** | 1–26 | 4–90 | 1–90 | | 4–21 | 1–90 | 1–90 | | 1–60 | 3–22 | 1–90 | | 1–90 | 3–90 | 1–60 | |
| **Vitamin D (ng/ml)** | **Mean** | 25.20 | 33.35 | 27.95 | 0.74 | 34.92 | 26.56 | 29.97 | 0.219 | 24.75 | 35.45 | 27.50 | 0.095 | 31.48 | 35.03 | 18.90 | 0.475 |
| | **SD[a]** | 15.89 | 37.66 | 32.38 | | 24.22 | 36.05 | 30.24 | | 21.75 | 23.23 | 36.04 | | 34.99 | 37.95 | 8.94 | |
| | **Median** | 23.15 | 18.10 | 18.80 | | 20.90 | 13.25 | 21.00 | | 14.75 | 27.60 | 17.00 | | 20.4 | 17.2 | 16.3 | |
| | **Range** | 11.6–65.4 | 9.8–156.8 | 9.8–158.6 | | 12.3–64.8 | 9.8–158.6 | 9.8–156.8 | | 9.8–64.8 | 10.6–74.1 | 9.8–158.6 | | 9.8–158.6 | 10.3–156.8 | 9.8–34.3 | |

[a] Standard deviation

**Table 5** VDR polymorphisms distribution among the studied patients in relation to sex, ICU parameters, and outcome

| | | Sex | | *p*-value | Mechanical ventilation | | *p*-value | Inotropic support | | *p*-value | Outcome | | *p*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Male | Female | | Done | Not done | | Done | Not done | | Survival | Death | |
| | | Count (%) | Count (%) | | Count (%) | Count (%) | | Count (%) | Count (%) | | Count (%) | Count (%) | |
| BsmI | GG | 7/30 (23.3) | 3/20 (15) | 0.866 | 6/39 (15.4) | 4/11 (36.4) | 0.329 | 5/31 (16.1) | 5/19 (26.3) | 0.247 | 7/30 (23.3) | 3/20 (15) | 0.095 |
| | AG | 10/30 (33.3) | 7/20 (35) | | 14/39 (35.9) | 3/11 (27.3) | | 9/31 (29) | 8/19 (42.1) | | 13/30 (43.3) | 4/20 (20) | |
| | AA | 13/30 (43.3) | 10/20 (50) | | 19/39 (48.7) | 4/11 (36.4) | | 17/31 (54.8) | 6/19 (31.6) | | 10/30 (33.3) | 13/20 (65.0) | |
| Fok I | TT | 4/30 (13.3) | 1/20 (5) | 0.765 | 4/39 (10.3) | 1/11 (9.1) | 0.99 | 5/31 (16.1) | 0 (0) | 0.243 | 2/30 (6.7) | 3/20 (15) | 0.489 |
| | CT | 10/30 (33.3) | 8/20 (40) | | 14/39 (35.9) | 4/11 (36.4) | | 10/31 (32.3) | 8/19 (42.1) | | 10/30 (33.3) | 8/20 (40) | |
| | CC | 16/30 (53.3) | 11/20 (55) | | 21/39 (53.8) | 6/11 (54.5) | | 16/31 (51.6) | 11/19 (57.9) | | 18/30 (60) | 9/20 (45) | |
| ApaI | CC | 6/30 (20) | 0 (0) | 0.098 | 4/39 (10.3) | 2/11 (18.2) | 0.285 | 2/31 (6.5) | 4/19 (21.1) | 0.197 | 5/30 (16.7) | 1/20 (5) | 0.485 |
| | AC | 8/3 (26.7) | 5/20 (25) | | 12/39 (30.8) | 1/11 (9.1) | | 7/31 (22.6) | 6/19 (31.6) | | 8/30 (26.7) | 5/20 (25) | |
| | AA | 16/30 (53.3) | 15/20 (75) | | 23/39 (59) | 8/11 (72.7) | | 22/31 (71) | 9/19 (47.4) | | 17/30 (56.7) | 14/20 (70) | |
| TaqI | TT | 10/30 (33.3) | 8/20 (40) | 0.054 | 15/39 (38.5) | 3/11 (27.3) | 0.909 | 13/31 (41.9) | 5/19 (26.3) | 0.159 | 8/30 (26.7) | 10/20 (50) | 0.147 |
| | CT | 8/30 (26.7) | 10/20 (50) | | 13/39 (33.3) | 5/11 (45.5) | | 5/31 (16.1) | 5/19 (26.3) | | 7/30 (23.3) | 3/20 (15) | |
| | CC | 12/30 (40) | 2/20 (10) | | 11/39 (28.2) | 3/11 (27.3) | | 9/31 (29) | 8/19 (42.1) | | 13/30 (43.3) | 4/20 (20) | |

## Discussion

Sepsis accounts for 19% of all deaths worldwide, with the highest age-specific incidence in children under the age of 5 [20]. Due to the fact that vitamin D-related pathways are engaged in a variety of endocrine, immunological, and endothelial activities, multiple studies have demonstrated a link between vitamin D deficiency and sepsis and septic shock [21].

The median serum vitamin D level in our cohort of critically ill children with sepsis was 19 ng/ml. No statistically significant differences between cases and healthy controls were observed; both groups had vitamin D insufficiency. Vitamin D deficiency is widespread in healthy children, with recent data indicating that almost 40% of Egyptian children had vitamin D levels ranging between 10 and 20 ng/ml [19]. Vitamin D insufficiency is prevalent in acute and critically ill children and is related to higher mortality, as Cariolou et al. observed in a meta-analysis published in 2019 [22].

The reaction of humans to infections has been shown to be very variable among individuals. While the majority of patients recover and function well, a small but significant proportion develops severe sepsis and dies as a result of multiple organ system failure, refractory hypotension, and other sequelae. This variation in susceptibility to and outcome from sepsis has been ascribed to a number of factors, including the virulence of the etiologic agent, the time interval between the onset of symptoms and the initiation of treatment, and the host's genetic makeup [23].

The current study examined serum vitamin D levels in relation to severe sepsis in pediatric ICU patients, as well as the frequency and distribution of four VDR gene polymorphisms (BsmI, Fok1, TaqI, and ApaI genotypes).

Vitamin D levels did not differ substantially among critically ill septic patients and had no effect on the features of ICU patients (need for mechanical ventilation, inotropic support, ICU stay, and sepsis outcome, whether survival or death). Similarly, Ponnarmeni et al. [24] and Ayulo et al. [25] observed no link between vitamin D and sickness severity. Additionally, Prasas et al. [26] and Ray et al. [27] reported no association between vitamin D deficiency and mortality.

Contrary to our findings, El Gendy et al. [28] discovered a statistically significant association between vitamin D levels and the requirement for mechanical ventilation. Additionally, they discovered a significant correlation between vitamin D level and length of stay in the pediatric ICU, which was shorter in non-deficient patients. They also discovered a link between vitamin D and mortality.

There was no correlation between any of these VDR gene variants and sepsis in pediatric ICU patients. There was no difference in the genotype distributions (BsmI, Fok1, TaqI, or ApaI) between critical children with sepsis and controls.

Similarly, Das et al. found no association between genotypes of the Apa1 polymorphism and neonatal sepsis or 25(OH) D serum levels [29]. Another study by Zeljic et al. suggested the possible role of VDR FokI as a molecular biomarker of increased sepsis risk [30].

There is controversy around VDR polymorphism and respiratory illnesses. According to a study conducted in Saudi children having acute lower respiratory tract infection (ALRTI), neither vitamin D status nor VDR gene polymorphisms such as ApaI or TaqI were associated with higher susceptibility to ALRTI [31]. However, according to a study on community-acquired pneumonia in Egyptian children, the VDR gene FokI polymorphism confers susceptibility to community-acquired pneumonia in Egyptian children [32]. A similar observation was made in children from India [33].

Additional investigations established a link between VDR gene polymorphisms and viral infection [34], pertussis [35], severe respiratory syncytial virus bronchiolitis [36], risk of symptomatic dengue requiring hospitalization [37], and tuberculosis infections in children [38].

## Conclusions

In conclusion, neither vitamin D levels nor VDR gene variations were shown to be linked with sepsis in pediatric ICU admissions. This is the first study that we are aware of that evaluates VDR polymorphisms in critically ill children with sepsis. In addition, other strengths include the following: the measurements of vitamin D levels in addition to VDR polymorphisms in a relatively adequate sample size of patients and controls and samples were collected from cases and controls in the same enrollment duration, i.e., same time of the year to avoid season variations in vitamin D levels.

One of our study limitations was being a single-center study of critically unwell children with sepsis from all pediatric age groups. Further multicenter studies with age stratification and different septic conditions could provide more elaborative results. To fully understand the role of vitamin D therapy in various types of sepsis, additional research on vitamin D and VDR gene variants is required. Moreover, examining VDR expression levels along with VDBP (vitamin D binding protein), the vitamin D transporter protein, may be beneficial.

#### Abbreviations

ALRTI: Acute lower respiratory tract infection; CNS: Central nervous system; EDTA: Ethylenediaminetetraacetic acid; ELISA: Enzyme-linked immunosorbent assay; ICU: Intensive care unit; SIRS: Systemic inflammatory response syndrome; SPSS: Statistical Package for the Social Sciences; VDBP: Vitamin D-binding protein; VDR: Vitamin D receptor.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43054-022-00130-1.

**Additional file 1: Supplementary Table 1.** Primer sequences, PCR conditions, product size, restriction enzymes, and length of the restriction fragments for the four studied polymorphisms.

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**Authors' contributions**
All authors have contributed to the manuscript in significant ways and have reviewed and agreed upon the manuscript content. IS, overall supervision in every step. RA, patient recruitment, follow-up, manuscript writing, and revision. NA, data analysis and manuscript revision. RA, data analysis and manuscript revision. BM, sample collection and manuscript revision. HA, patient recruitment, follow-up, and manuscript writing. OA, corresponding author, laboratory work, and manuscript writing. The authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
The study was approved by the ethical committee of each of the Clinical and Chemical Pathology and the Pediatric Departments, at the Kasr Al-Ainy Faculty of Medicine — Cairo University, and was conducted in concordance with the Declaration of Helsinki October 2013 (ethical principles for medical research involving human subjects). Informed consents were obtained from the guardians of all participants.

**Consent for publication**
Not applicable

**Competing interests**
The authors declare that they have no competing interests.

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