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Original research

Association of vitamin D with cathelicidin and vitamin D binding protein in pediatric sepsis

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Background

Vitamin D is a prohormone that controls calcium and phosphorus homeostasis for bone health. Recent studies have shown that vitamin D may have extra-endocrine functions. Vitamin D receptors (VDR) have been found in cells such as macrophages, suggesting a role for vitamin D in the innate immunity [1–3]. In vitro, vitamin D has been shown to modulate levels of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and induce expression of cathelicidin, an endogenous antimicrobial peptide that that is effective against a broad spectrum of microbes [4,5].

Sepsis is a disease process with high mortality rates and associated with profound inflammation. In vitro studies have shown that treatment of septic states with vitamin D modulates levels of IL 6 and TNF-α and improves blood coagulation. [6–9] Jeng et al. in a study of septic adults in the critical care unit, found significantly lowered levels of vitamin D (25 (OH) D), D binding protein (DBP) and LL37 compared to healthy population adults with sepsis [10]. Further, there was a positive association between vitamin D and cathelicidin. No studies have explored the relationship of 25(OH)D, DBP and cathelicidin in pediatric sepsis. Our objective was to determine the association of 25(OH)D with cathelicidin and DBP in pediatric sepsis.

Methods

This was a pilot, prospective, observational study of a convenience sample of patients at a tertiary children's hospital. Patients were recruited from the Emergency Department and Intensive Care Unit for a period of 2 years from 2014 - 2016.

Children ≤18 years admitted with the diagnosis of sepsis, severe sepsis or septic shock using published definitions were enrolled [11]. Patients who were currently being treated with vitamin D were excluded. The institution's Human Investigation Committee approved the study in a full board review.

After informed consent was obtained, patient demographic and clinical data were abstracted from electronic health records. Blood samples were collected within 24 h of admission for levels of 25(OH)D and inflammatory markers. The 25(OH)D level was measured via chemiluminescence in the hospital laboratory. Additional samples were centrifuged per manufacturer's protocol, and the separated serum and plasma were stored at −80 °C until analysis. Levels of IL-4, IL-6 and TNF α were measured with MILLIPLEX® multi-analyte profiling on a Luminex FlexMap 3D system. Cathelicidin and DBP were assessed using ELISA (Hycult Biotech, Netherlands and Immundiagnostic AG, Germany) [6]. In surviving patients, an additional sample was drawn 24 h prior to discharge and all of the levels described above were measured again.

Descriptive statistics was reported using means, medians, frequencies and percentages. All tests were 2-tailed and performed at 5% level of significance. Pearson's Correlation was used to study associations while paired *t*-test or Wilcoxon were used to compare means. Regression analysis was used to study the effect of vitamin D levels on cytokines.

Results

Of 48 children enrolled, 7 were excluded from final analysis (4- for lack of sample, 2- for early discharge, and 1- for extreme lab values). Demographics and initial laboratory measurements of the 41 subjects are shown in Table 1. There was no correlation between 25(OH) D and cathelicidin, DBP, IL4 or TNF. There was a significant correlation between 25(OH) D and IL 6 levels. (Fig. 1.)

A second sample was obtained only in 19 patients due to inability to draw blood or discharge prior to obtaining blood sample. Mean time

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Table 1
Baseline Demographics and Initial Laboratory Results of Patient Group.

Category	Total (n = 41)
Age, mean, yr (sd)	9 (5)
Sex,% Male	51
Race,% African American	46
Severe Sepsis,%	17
Septic Shock,%	10
Hospital length of stay, mean, hr (sd)	99 (88)
% of children with 25(OH)D < 20 ng/ml	70
% of children with 25(OH)D < 30 ng/ml	90
Initial 25(OH)D, mean, ng/ml (sd)	17 (9)
Initial cathelicidin, mean, ng/ml (sd)	85 (47)
Initial DBP, mean, mg/dl (sd)	39 (11)
Initial IL4, mean, pg/ml (sd)	8 (6)
Initial IL6, mean, pg/ml (sd)	79 (1 3 7)
Initial TNFα, mean, pg/ml (sd)	13 (10)

between initial and discharge sample was 4 ± 2 days. Although insignificant, 25(OH) D and cathelicidin concentrations increased by an average 0.06 and 5.88 ng/ml, respectively. There was a significant increase in DBP at discharge (39 ± 11 vs 46 ± 11 mg/dl, 95% CI: 0.35–16, p = 0.04).

Discussion

In our study, 25(OH) D levels were low in children with sepsis or septic shock but did not correlated with cathelicidin or DBP. To our knowledge, this is the first study to examine the relationship between vitamin D, cathelicidin and DBP in pediatric sepsis.

The high prevalence of low 25(OH) D levels in septic children in our study is similar to previous reports suggesting a role for vitamin D in sepsis and immunity that warrants further exploration [4,6,12]. Interestingly, in our cohort, cathelicidin levels were three times that of healthy children in a previous report suggesting its importance in the initial immune response to infections [13]. However unlike adult studies, we did not find a correlation between vitamin D and cathelicidin levels. This lack of correlation could be secondary to the narrow range of vitamin D levels in our cohort. Further studies are required to confirm this finding.

Our finding of increased DBP with sepsis resolution supports the theory that DBP plays an important role in the defense against infections. The majority of 25 (OH) is tightly bound to DBP which may thus be protective by increasing the bioavailability of 25(OH) D. Hence reduced levels of DBP during sepsis has been shown to be negatively correlated with severity of sepsis [10,14].

Lastly, the strong correlation between vitamin D and IL-6 suggests that being both pro- and anti-inflammatory, IL-6 is likely involved in the vitamin D-antimicrobial peptide pathway.

Limitations

The study was a pilot and the small numbers may have affected our results. Our study protocol allowed blood and serum collections up to 24 h from presentation. Some biomarkers may have degraded during this time and this may have negatively impacted our results.

Conclusions

In our study of pediatric patients with sepsis, 25(OH)D was not associated with cathelicidin or DBP levels. Larger studies are required to further elucidate the role of vitamin D and cathelicidin in pediatric sepsis.

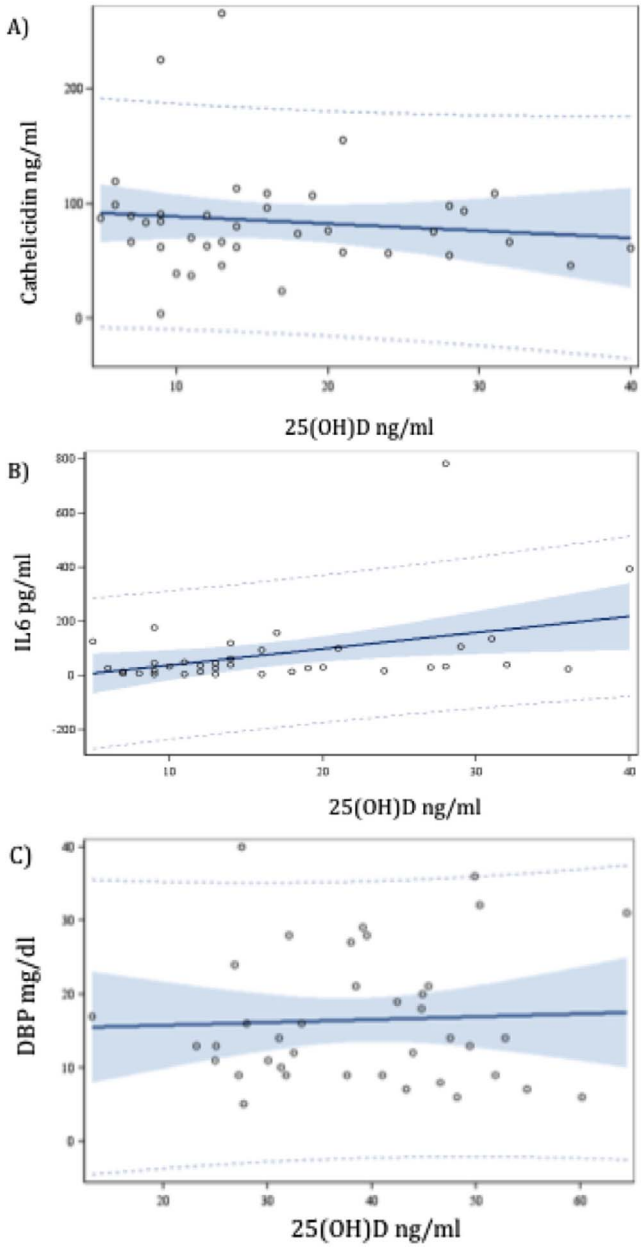

Fig. 1. A) Relationship between initial 25 hydroxyvitamin D (25(OH)D) and initial cathelicidin in pediatric subjects admitted with sepsis. There was a non-significant linear relationship between plasma 25(OH)D and cathelicidin concentrations (p = .47) B) Relationship between initial 25(OH)D and initial Interleukin-6 (IL6) in pediatric subjects admitted with sepsis. There was a significant positive relationship between 25(OH)D and IL-6 concentrations (p = .02) C) Relationship between initial 25(OH)D and initial Vitamin D Binding Protein (DBP) in pediatric subjects admitted with sepsis. There was a non-significant linear relationship between 25(OH)D and DBP concentrations (p = .77).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcte.2017.11.001.

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