HHS Public Access

Author manuscript

Pediatr Infect Dis J. Author manuscript; available in PMC 2016 May 01.

Published in final edited form as:

Pediatr Infect Dis J. 2015 May ; 34(5): 544–545. doi:10.1097/INF.0000000000000667.

Vitamin D Sufficiency and *Staphylococcus aureus* Infection in Children

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Abstract

Vitamin D promotes epithelial immunity by upregulating antimicrobial peptides (AMPs), including LL-37, that have bactericidal activity against *Staphylococcus aureus*. We found that children with vitamin D deficiency or insufficiency [25(OH)D <30 ng/mL] were more likely to present with recurrent, rather than primary, *S. aureus* skin or soft tissue infection (SSTI). Vitamin D sufficiency may be one of a myriad of host and environmental factors that can be directly impacted to reduce the frequency of *S. aureus* SSTI.

Keywords

Staphylococcus aureus; vitamin D; 25-hydroxyvitamin D; LL-37; cathelicidin

Introduction

Staphylococcus aureus causes a variety of infection entities ranging from skin and soft tissue infections (SSTIs) to invasive bloodstream infections, osteomyelitis, and pneumonia. Asymptomatic *S. aureus* colonization is present in approximately one-third of the United States population and is a risk factor for infection (1). Furthermore, recurrent *S. aureus* SSTIs are problematic; following treatment, up to 60% of patients with *S.* aureus SSTI will experience recurrence over 12 months (2).

The effectiveness of preventive and treatment approaches to this versatile pathogen is threatened by burgeoning bacterial resistance. Most notable is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains, but there is also increasing resistance to commonly prescribed decolonization agents, including mupirocin and chlorhexidine (3). Novel strategies for infection prevention are needed. Endogenous micronutrients, such as 25-hydroxyvitamin D [25(OH)D] or vitamin D, have potential roles as mediators of protective immunity. Though well-known for its role in bone metabolism and calcium

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Conflicts of Interest D.A.H. serves on the Scientific Advisory Board of BioVersys AG; other authors have no conflicts to declare.

homeostasis, vitamin D has recently garnered attention for its influence on host immunity, in infectious diseases and autoimmune conditions (4-7). The active form of vitamin D [1,25$(OH)_2$ D3] regulates transcription of antimicrobial peptides (AMPs) in keratinocytes, macrophages, and neutrophils (8). The human cathelicidin AMP, termed LL-37, promotes first-line defense mechanisms, including vitamin D3-induced autophagy in macrophages and *in vitro* killing of methicillin-resistant *Staphylococcus aureus* (9, 10). Therefore, it is biologically plausible that vitamin D might aid in prevention of *S. aureus* SSTI.

The high prevalence of vitamin D deficiency (7.6 million US children and adolescents; (4)) encourages further study of the antimicrobial benefits of this micronutrient. Using sera from pediatric patients enrolled in a previous observational study of host immune responses to staphylococcal toxins (11), we investigated the relationship between serum 25(OH)D concentrations and *S. aureus* clinical phenotypes – specifically primary SSTI, recurrent SSTI, and invasive disease. Our principal hypothesis was that patients with recurrent SSTI would have lower serum 25(OH)D levels than patients presenting with primary SSTI.

Patients and Methods

Subject Recruitment & Sample Collection

Healthy children >6 months of age (n=202) were recruited from St. Louis Children's Hospital (SLCH) between August 2008 and September 2011 as described previously (11). Three groups were evaluated: primary (first-time) SSTI, recurrent SSTI, and invasive disease (e.g., bacteremia, osteomyelitis, and septic arthritis).

Colonization cultures of the nares, axillae, and inguinal folds were taken from all participants at study enrollment as previously described (11). An enrollment survey collected information regarding demographics, acute infection symptoms, past medical history, household factors, and activities. Sera were drawn at the time of acute infection. Study procedures were approved by the Washington University Human Research Protection Office. Written informed consent (and assent when appropriate) was obtained for each participant.

Laboratory Testing & Vitamin D Status Definition

Serum 25(OH)D levels were measured on 300-μL samples via an electrochemiluminescence immunoassay on the Roche Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, IN), which has an analytic sensitivity of 5.0 ng/mL. Two levels of quality controls were within the specified acceptable range. Precision was reflected in between-run coefficients of variation (CVs) of 10.2% at 7.48 ng/mL and 3.2% at 53.12 ng/mL, and within-run CVs of 2.53% at 13.4 ng/mL and 3.27% at 29.21 ng/mL. Serum 25(OH)D levels were categorized as follows (5, 6): sufficient ≥30 ng/mL; insufficient 20-29 ng/mL; deficient <20 ng/mL.

Statistical Analysis

Statistical analyses utilized SPSS 22 for Windows (IBM SPSS, Chicago, IL). Pearson's chi-square tests were used to evaluate association between 25(OH)D level and variables *a priori* thought to be associated with 25(OH)D level and/or *S. aureus* infection type. These analyses

were performed twice, categorizing 25(OH)D levels using 2 approaches: 1) separating 25(OH)D levels into all 3 categories (sufficient, insufficient, and deficient) and 2) condensing 25(OH)D levels into 2 categories (sufficient and insufficient/deficient) based on existing literature (5). Two separate logistic regression analyses were run using the backwards stepwise method. The first regression sought to determine which variables were associated with the outcome 25(OH)D insufficiency/deficiency. Variables included in the multivariable regression model were those significantly associated with 25(OH)D insufficiency/deficiency in univariate analyses. The second regression determined variables associated with the outcome infection type. This model was run twice comparing different infection types: primary vs. recurrent SSTI and primary SSTI vs. invasive infection. Odds ratios (OR) and 95% confidence intervals (CI) are reported. All tests of significance were 2-tailed; *P*-values <0.05 were considered significant.

Results

The median age among 202 participants was 7.6 years (range, 0.6-22.5). The study population was 48% African-American (n=97) and 53% female (n=107). Eighty-six (43%) participants experienced primary SSTI, 62 (31%) experienced recurrent SSTI, and 54 (27%) had invasive *S. aureus* infection. When categorized by 25(OH)D levels, 74 (37%) participants were deficient, 70 (35%) were insufficient, and 58 (29%) were sufficient. The overall mean 25(OH)D level was 23.7 ± 10.2 ng/mL. Mean 25(OH)D levels for the primary SSTI, recurrent SSTI, and invasive infection cohorts were 24.3 ± 9.9 ng/mL, 23.8 ± 8.9 ng/mL, and 22.6 ± 12.0 ng/mL, respectively.

Age, race, and season of infection were significantly associated with 25(OH)D values in univariate analysis (see Table, Supplemental Digital Content 1, which includes potential associated factors by categorical 25(OH)D serum levels). Deficient 25(OH)D levels were detected most frequently in the 13-22 year (oldest) age group (63%), compared with 7-12 year olds (36%) and those <7 years of age (20%; p<0.001). African-Americans were more likely to have deficient 25(OH)D levels (56%) compared to Caucasian/other races (19%; p<0.001). Lastly, participants with infections during fall and winter (October-March) were more likely to have deficient 25(OH)D levels (50%) compared with those with infections in spring and summer (April-September) (28%; p<0.001). Other factors such as gender, BMI, weight for age, antibiotic use in the past year, hospitalization in the past year, *S. aureus* colonization, and type of infecting organism (MRSA vs. methicillin-sensitive *S. aureus*) showed no significant association with 25(OH)D level. Factors associated with vitamin D deficiency/insufficiency in multivariable analysis (including age, season, race, and study arm/infection type) were older age (increase of 1 year associated with OR 1.14; 95% CI 1.07-1.21) and fall/winter season (OR 2.89; 95% CI 1.41-5.91) when compared with vitamin D sufficiency.

Factors associated with presentation with a recurrent *S. aureus* SSTI (compared to primary SSTI) in multivariable analysis (including age, season, race, and vitamin D level) were insufficient/deficient 25(OH)D levels (OR 2.30; 95% CI 1.02-5.21) and younger age (increase of 1 year associated with OR 0.91; 95% CI 0.86-0.97). A similar multivariable regression analysis comparing patients presenting with a first-time *S. aureus* infection

(primary SSTI vs. invasive infection) did not demonstrate an association between vitamin D levels and infection type.

Discussion

Given the role of vitamin D in regulation of LL-37 expression and the bactericidal activity of LL-37 against *S. aureus*, we investigated serum vitamin D levels in patients with a range of *S. aureus* infection. The current observational study supports a plausible association between serum 25(OH)D levels and host susceptibility to *S. aureus* SSTIs. Consistent with other studies, African-American race was associated with hypovitaminosis D (4). The increased melanin content of African-American skin affects UV absorption, subsequently yielding lower vitamin D stores. Interestingly, Liu et al. also found racial differences in induction of cathelicidin mRNA, which may also impact racial susceptibility to infection (12). Infection in winter months was also associated with lower vitamin D levels, which may be explained by diminished sun exposure and greater time spent indoors (4).

Several studies have investigated the relationship of serum vitamin D levels and asymptomatic *S. aureus* colonization. Matheson and colleagues determined that individuals with vitamin D deficiency had increased risk of MRSA nasal carriage (6). On the contrary, Slow et al. found no correlation between 25(OH)D and *S. aureus* carriage risk, and vitamin D3 supplementation did not reduce persistent nasal carriage. Upon adjusting for race, age, and season of infection presentation, we found that children with deficient or insufficient vitamin D levels (< 30 ng/mL) were at a greater risk for their SSTI to be recurrent rather than primary. Perhaps AMPs, which are regulated by vitamin D, play a larger role in preventing infection rather than inhibiting colonization.

Several limitations should be noted. First, this observational study is a secondary analysis of samples used in a previous study examining humoral responses to *S. aureus* infection. As a result, we lack data regarding potential confounders such as patient sun exposure, diet, and vitamin supplementation use. Second, our cross-sectional analysis precludes determination of which occurred first: onset of infection or non-sufficient vitamin D levels. Thus, only associative rather than causative relationships can be inferred.

In a collection of observational studies, vitamin D has been shown to have a significant correlation with other clinical outcomes, including atopic dermatitis and acute otitis media (AOM) (5, 7). Vitamin D supplementation trials have been successful in reducing recurrent uncomplicated AOM episodes and severity of eczema (5, 7). With biological evidence showing vitamin D-dependent regulation of AMPs (e.g., LL-37) during a cutaneous infection episode, vitamin D supplementation trials may potentially improve clinical outcomes of *S. aureus* patients (8, 12). Because recurrent SSTIs pose a substantial burden and the prevalence of vitamin D deficiency and insufficiency is high, additional studies are needed to further support the immuno-regulatory benefits of vitamin D. As vitamin D sufficiency may be one of a myriad of host and environmental factors influencing *S. aureus* SSTI, vitamin D supplementation may serve as a safe and economical approach to reduce recurrent *S. aureus* infections.

Acknowledgements

We would like to thank the Core Laboratory for Clinical Studies [Richard E. Ostlund, MD and Licia R. Rowe, MT (ASCP)] for their help with the vitamin D electrochemiluminescence assay, as well as Michael Wallendorf, PhD for assisting with data analysis.

Source of Funding:

Funding for this project was provided by the Children's Discovery Institute of Washington University and St. Louis Children's Hospital; the National Institutes of Health (NIH) grants K23-AI091690 and UL1-TR000448; and grant R01-HS021736 from the Agency for Healthcare Research and Quality (AHRQ). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or AHRQ.

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