Systematic Review with Meta Analysis

Vitamin D supplementation for the prevention of childhood acute respiratory infections: a systematic review of randomised controlled trials

Limin Xiao1†, Chao Xing2†, Zhongrong Yang3†, Shaojun Xu4, Min Wang5, Huarong Du6, Kai Liu6 and Zhaohui Huang6*

1Second Affiliated Hospital of Anhui Medical University, Hefei 230601, Anhui Province, People’s Republic of China
2Shaoxing Center for Disease Control and Prevention, Shaoxing 312000, Zhejiang Province, People’s Republic of China
3Huzhou Center for Disease Control and Prevention, Huzhou 313000, Zhejiang Province, People’s Republic of China
4Department of Maternal and Child Health, School of Public Health, Anhui Medical University, Hefei 230032, Anhui, People’s Republic of China
5Anhui Institute of Schistosomiasis Control, Hefei 230061, Anhui Province, People’s Republic of China
6Anhui Provincial Family Planning Institute of Science and Technology, Hefei 230031, Anhui Province, People’s Republic of China

(Submitted 13 November 2014 – Final revision received 6 May 2015 – Accepted 11 May 2015 – First published online 27 August 2015)

Abstract

Results from recent trials assessing the effect of vitamin D supplementation on the prevention of childhood acute respiratory infections (ARI) have been inconsistent. In the present study, we determined whether vitamin D supplementation prevents ARI in healthy children and repeated infections in children with previous ARI. We conducted a systematic literature search using MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials. The search included only randomised controlled clinical trials (RCT) comparing vitamin D supplementation with either placebo or no intervention in children younger than 18 years of age. We identified seven RCT and found that the summary estimates were not statistically significantly associated with a reduction in the risk of ARI (relative risk (RR) 0·79, 95% CI 0·55, 1·13), all-cause mortality (RR 1·18, 95% CI 0·71, 1·94), or the rate of hospital admission due to respiratory infection in healthy children (RR 0·95, 95% CI 0·72, 1·26). However, in children previously diagnosed with asthma, vitamin D supplementation resulted in a 74% reduction in the risk of asthma exacerbation (RR 0·26, 95% CI 0·11, 0·59; test of heterogeneity, I² = 0%). Our findings indicate a lack of evidence supporting the routine use of vitamin D supplementation for the prevention of ARI in healthy children; however, they suggest that such supplementation may benefit children previously diagnosed with asthma. Due to the heterogeneity of the included studies and possible publication biases related to this field, these results should be interpreted with caution.

Key words: Vitamin D; Acute respiratory infections; Randomised controlled trials

Acute respiratory infections (ARI) are the most important global cause of morbidity and mortality in young children. In 2010, an estimated 11·9 million episodes of severe ARI and 3·0 million episodes of very severe ARI in young children resulted in hospital admissions globally(1). Of the 7·6 million children worldwide who died within the first 5 years of life, almost two-thirds died of infectious diseases, among which pneumonia was the leading cause for a total of 1·396 million deaths(2).

Although vitamin D is widely recognised for its importance in Ca metabolism and bone health, researchers have spent several years focusing on its growing number of possible non-calcemic health effects(3). One of the more promising areas of study is the relationship between vitamin D status and respiratory infection. Recent research has indicated that vitamin D may play a role in protecting against ARI by increasing the body’s production of naturally acting antibiotics(4). In some observational studies, low plasma levels of calcidiol,
the accepted marker of vitamin D status, have been observed to be associated with both an increased risk and a greater severity of infection, particularly the infection of the respiratory tract\(^5\)\(^–\)\(^7\). Although the exact mechanisms by which vitamin D may improve immune responses to infection remain unknown, vitamin D supplementation trials on the prevention and adjunct therapy for infection are ongoing. Given its influence on the immune system and inflammatory cascades, vitamin D may play an important role in both the prevention and treatment of infection\(^8\).

Several recent trials investigating the effect of vitamin D supplementation on the prevention of childhood ARI have reported inconsistent results. Therefore, we conducted a study to determine whether vitamin D supplementation prevents ARI in healthy children and repeated episodes of ARI in children previously diagnosed with either asthma or pneumonia.

**Materials and methods**

**Search strategy and selection criteria**


The inclusion criteria were as follows: (1) randomised controlled trials (RCT) exploring the effect of vitamin D supplementation on the prevention of ARI, primarily focused on individuals younger than 18 years of age; (2) studies reporting the dosages, modes and durations of vitamin D supplementation; (3) studies reporting the definitions of ARI and the methods used to diagnose ARI or to determine the primary outcomes associated with ARI; (4) studies reporting either the prevalence or the relative risk (RR) estimates, along with either corresponding 95\% CI or raw data enabling the calculation of 95\% CI.

We collected data only from complete published papers, and excluded data from meetings, literature reviews, letters and news articles. We also excluded studies that contained overlapping data. When there were multiple publications involving the same population, the study containing the largest sample size was included. When a study reported results from different subpopulations, we treated the results for each subpopulation independently. We excluded trials that did not include a control group, observational studies and animal studies. We also excluded trials reporting a follow-up rate of <50\% or not reporting a follow-up rate. Trials in which vitamin D was used in combination with other interventions were not included in the final analysis.

Both the titles and the abstracts of all identified studies were screened independently by two researchers (L. X. and Z. Y.). Studies that appeared to be relevant were selected, and full-text versions were subsequently assessed by the same two reviewers. Disagreements were resolved by either consensus or the involvement of a third reviewer (M. W.). Fig. 1 depicts a flow chart for the selection of the articles.

**Data collection and methodological quality assessment**

We developed and modified a data abstraction form following a training exercise for investigators. We extracted the following data from the eligible studies: (1) general characteristics (title, first author, journal and year of publication); (2) methodology (type of study, country of origin, sequence generation, allocation concealment, masking or blinding, incomplete outcome data, selective reporting and other sources of bias, follow-up duration, and loss to follow-up); (3) characteristics of participants (recruitment site, enrolment periods, mean age and proportion of male participants); (4) trial and control groups (number of eligible trial groups, vitamin D doses and routes of administration, trial duration, frequency, and details of comparison); (5) primary outcomes. We contacted the authors of the included studies to obtain additional information regarding data items that required clarification.

The methodological quality of sequence generation, allocation concealment, blinding, missing outcome data, selective reporting, and other biases was subsequently assessed using the Cochrane Collaboration’s risk of bias method. Each domain was rated as low, high or unclear regarding the risk of bias, and the overall risk of bias was rated as low (low risk of bias for all domains), high (high risk of bias for one or more domains) or unclear (unclear risk of bias for one or more domains)\(^9\).

Discrepancies were resolved either through discussion with other team members (S. X., K. L. and H. D.) or contact with the original investigators, all of whom received data extraction sheets with requests for correction.

**Outcomes**

The primary outcome measures of the present study were the prevention of ARI (including influenza, pneumonia and asthma) in healthy children, asthma exacerbation in children with previous ARI, and recurrent pneumonia in children with a prior history of pneumonia. The secondary outcomes of the present study were the prevention of influenza and pneumonia in healthy children, all-cause mortality, the rate of hospital admission due to ARI, and changes in mean serum vitamin D levels.
Statistical analysis

For binary outcomes, we calculated risk ratios (RR); for continuous outcomes, we calculated weighted mean differences. A random-effects model was used to estimate the pooled RR and weighted mean differences to account for the heterogeneity of the estimates, and to provide more conservative estimates compared with those generated using the fixed-effects model\(^{(10)}\). The heterogeneity of the outcome measures among the studies was assessed using the \(\chi^2\) test-based \(Q\) statistic\(^{(11)}\). A significant \(Q\) statistic (\(P<0.10\)) indicated heterogeneity in the outcome measures between the studies. We quantified statistical heterogeneity with the \(I^2\) statistic, and used ‘small’, ‘moderate’ and ‘large’ heterogeneity to correspond to \(I^2\) values of 25, 50 and 75\%, respectively\(^{(12,13)}\). We did not conduct subgroup analyses or meta-regression analyses because of the small number of included RCT. We performed sensitivity analyses to establish the robustness of the primary outcomes. To establish the robustness of the primary outcomes via sensitivity analyses, we utilised a fixed-effects model and excluded studies containing small sample sizes. Publication bias was investigated using a funnel plot, in which the standard error of log RR of each study was plotted against its RR, and plot asymmetry was subsequently assessed using Egger’s linear regression test\(^{(14)}\). If the regression line did not pass through the origin, asymmetry was assigned; the intercept \(\alpha\) provided a measure of asymmetry. The larger the deviation of \(\alpha\) from zero, the more pronounced the asymmetry. The analyses were performed using Stata (version 11.0). All \(P\) values were two-sided. A \(P\) value of <0.05 was considered to be statistically significant.

Fig. 1. Flow chart for the selection of studies for the present meta-analysis.
Results

Characteristics of the eligible studies

Fig. 1 shows the study profile. A total of 1329 papers were identified via the database search, and fifteen papers were identified via reference lists and hand searches. Following screening, 1276 papers were excluded, and the sixty-eight remaining full-text articles were assessed for eligibility. We excluded an ongoing trial that did not provide any usable data. A total of seven RCT were ultimately included in the meta-analysis (Table 1) (16–22). Most of the trials (n 6/7) were conducted in Asian countries, and all study participants were younger than 18 years of age (Table 1). Of these included trials, six provided data regarding the incidence of ARI; four provided data regarding all-cause mortality; two provided data regarding the incidence of pneumonia; two provided data regarding the incidence of repeated episodes of pneumonia; two reported data regarding the rate of hospital admission; and one reported data regarding the incidences of influenza A and influenza-like illnesses.

The risk of bias was assessed using a risk-of-bias graph (Fig. 2). All of the RCT exhibited adequate sequence generation, and most of the RCT exhibited good allocation concealment, reporting of blinding methods, and incomplete outcome data; however, the extent of selective reporting in three of the seven studies was unclear.

Table 1. Characteristics of clinical trials included in the meta-analysis*

<table>
<thead>
<tr>
<th>Study, year, location</th>
<th>Participants</th>
<th>Age (years)</th>
<th>Health status</th>
<th>Follow-up</th>
<th>Adherence (100 %)</th>
<th>Dose of vitamin D†</th>
<th>Outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urashima, 2010, Japan</td>
<td>Healthy school children</td>
<td>10·2</td>
<td>2·2</td>
<td>4 months</td>
<td>77·67</td>
<td>1200 IU/d</td>
<td>Incidence of FLU (A and B), ILI, PNE, ARI, asthma exacerbation and admission to hospital</td>
</tr>
<tr>
<td>Manaseki-Holland, 2010, Afganistan</td>
<td>Children with pneumonia</td>
<td>13·19</td>
<td>9·14</td>
<td>3 months</td>
<td>91·61</td>
<td>Single bolus dose of 100 000 IU</td>
<td>Incidence of repeated episodes of PNE during the 90 d post-treatment period, and all-cause mortality</td>
</tr>
<tr>
<td>Kumar, 2011, India</td>
<td>Low-birth weight infants</td>
<td>&lt;0·5</td>
<td>3·9</td>
<td>6 months</td>
<td>72</td>
<td>1400 IU/week</td>
<td>Incidence of pneumonia, or all-cause hospital admission or death</td>
</tr>
<tr>
<td>Majak, 2011, Poland</td>
<td>Children with newly diagnosed asthma</td>
<td>11·5</td>
<td>3·3</td>
<td>6 months</td>
<td>100</td>
<td>500 IU/d</td>
<td>Incidence of asthma exacerbation triggered by ARI</td>
</tr>
<tr>
<td>Manaseki-Holland, 2012, Afganistan</td>
<td>Healthy infants</td>
<td>&lt;1</td>
<td>3·3</td>
<td>18 months</td>
<td>85·88</td>
<td>3-Monthly bolus dose of 100 000 IU</td>
<td>Incidence of PNE, repeated episodes of PNE, rate of hospital admission and all-cause mortality</td>
</tr>
<tr>
<td>Camargo, 2012, Mongolia</td>
<td>Healthy school children</td>
<td>9·97</td>
<td>0·95</td>
<td>7 weeks</td>
<td>98·79</td>
<td>300 IU/d</td>
<td>Rate of ARI</td>
</tr>
<tr>
<td>Choudhary, 2012, India</td>
<td>Children with severe pneumonia</td>
<td>1·16</td>
<td>0·98</td>
<td>5 d</td>
<td>84·50</td>
<td>1000 IU for &lt;1 year and 2000 IU for &gt;1 year</td>
<td>All-cause mortality</td>
</tr>
</tbody>
</table>

FLU, influenza; ILI, influenza-like illness; PNE, pneumonia; ARI, acute respiratory infection.

*The quality of each included trial was evaluated using the modified Jadad score.

†1 µg vitamin D = 40 IU.
Data regarding asthma exacerbations triggered by respiratory infections among children with newly diagnosed asthma were reported by two trials. The summarised results indicated that treatment with vitamin D significantly reduces the risk of asthma exacerbation (RR 0.28, 95% CI 0.12, 0.64). No significant heterogeneity was observed between the studies ($I^2 = 0\%$, $P=0.355$) (Fig. 3). Data regarding the rate of hospital admission due to respiratory infections were reported by two trials. The summary estimates for the vitamin D supplementation group were not statistically significantly associated with a reduction in the risk of hospital admission (RR 0.95, 95% CI 0.71, 1.25). No significant heterogeneity was observed between the studies ($I^2 = 0\%$, $P=0.358$; Fig. 3).

Data regarding influenza A were reported by only one study. Influenza A occurred in eighteen (10.8%) of the 167 children in the vitamin D supplementation group compared with thirty-one (18.6%) of the 167 children in the placebo group (RR 0.58, 95% CI 0.34, 0.99; Fig. 3).

Fig. 4 shows the changes in plasma 25-hydroxyvitamin D (25(OH)D) levels for both the intervention and control groups over a period of 3 months for the three trials from which such data were available. The plasma levels
of 25(OH)D in the vitamin D supplementation group were significantly higher than those in either the control or placebo group after 3 months (weighted mean difference = 9·06, 95% CI 5·43, 12·70). There was evidence of significant heterogeneity between the studies ($I^2 = 87·9\%$, $P<0·001$).

**Sensitivity analyses**

The sensitivity analyses of ARI are included in Table 2. Using a fixed-effects model and excluding trials containing fewer than thirty participants per group yielded results similar to those of the primary analysis. The other sensitivity analyses demonstrated that the effect size was significantly reduced (RR 0·76, 95% CI 0·54, 1·07 v. RR 1·02, 95% CI 0·92, 1·13).

**Evaluation of publication bias**

We assessed funnel plot asymmetry using Egger's linear regression test. There was no evidence of publication bias regarding either ARI (intercept $a = −2·12$, 95% CI $−9·09$, 4·66) or all-cause mortality (intercept $a = 0·37$, 95% CI $−1·39$, 2·13). We also generated funnel plots and visually examined these plots for signs of asymmetry. We generated two funnel plots to assess the publication bias of the RR of both ARI and all-cause mortality. For all-cause mortality, the asymmetry observed in the funnel plots was minimal; in contrast, the asymmetry was significant for ARI, which may indicate either the absence of trials with negative results or publication bias (Fig. 5). We did not complete funnel plots for the other outcomes, as only a few studies contributed to the outcome measures.

**Discussion**

We included and examined seven RCT to assess the effect of vitamin D supplementation on the prevention of ARI in children. Our findings revealed that vitamin D supplementation regarding either ARI (intercept $a = −2·12$, 95% CI $−9·09$, 4·66) or all-cause mortality (intercept $a = 0·37$, 95% CI $−1·39$, 2·13). We also generated funnel plots and visually examined these plots for signs of asymmetry. We generated two funnel plots to assess the publication bias of the RR of both ARI and all-cause mortality. For all-cause mortality, the asymmetry observed in the funnel plots was minimal; in contrast, the asymmetry was significant for ARI, which may indicate either the absence of trials with negative results or publication bias (Fig. 5). We did not complete funnel plots for the other outcomes, as only a few studies contributed to the outcome measures.
did not significantly reduce the risk of ARI, all-cause mortality, or the rate of hospital admission in healthy children. Due to the small number of included studies, there was a lack of evidence supporting the routine use of vitamin D supplementation for ARI prevention in healthy children. However, in children previously diagnosed with asthma, vitamin D supplementation may reduce the risk of asthma exacerbation triggered by a respiratory infection. Based on the primary analysis, the I² value was 50%, primarily because the primary analysis consisted of a very heterogeneous mixture of endpoints. These results should be interpreted with caution due to the small number of included trials and the significant heterogeneity between them.

The observed lack of an effect of vitamin D supplementation on the prevention of ARI in the present study may have resulted from differences in the dosing regimens used in the included trials. Bergman et al. (23) reported that the protective effect of vitamin D was larger in studies using once-daily dosing compared with bolus dosing; all studies using bolus dosing reported a null effect. Unfortunately, we did not perform subgroup analyses based on dosing regimens due to the small number of included trials. In all observational studies, the relationship between inadequate vitamin D status and susceptibility to an ARI was significant. For example, Jolliffe et al. (24) revealed evidence of a relationship between inadequate vitamin D status and susceptibility to an ARI based on observational studies involving large numbers of participants of all age groups in diverse geographical settings with wide distributions of serum 25(OH)D concentrations. Quraishi et al. (25) also observed a nearly linear relationship between vitamin D status and the cumulative frequency of childhood community-acquired pneumonia. Additional trials regarding vitamin D supplementation for the prevention of ARI should be conducted in populations with a high prevalence of vitamin D deficiency at baseline, using doses sufficient to induce sustained elevations in serum 25(OH)D concentrations and studies powered to detect clinically important subgroup effects (24).

Regarding pneumonia, one of the two studies (Urashima et al. (16)) had no discernible impact on the results. Thus, the present meta-analysis merely presented the results obtained by Manaseki-Holland et al. (18) in 2012, providing no further insight into the matter. Regarding pneumonia recurrence, heterogeneity was too large to meaningfully pool estimates (I² = 95%). Both included RCT were conducted by Semira Manaseki-Holland and utilised the same study population, inclusion criteria, and definitions of outcomes (17,18). We believe that there was no clinical heterogeneity or methodological heterogeneity, and that statistical heterogeneity primarily accounted for the high level of heterogeneity observed between these studies. Additionally, Manaseki-Holland et al. (17,18) used a single dose of 100 000 units of oral vitamin D₃ in their trials; the results of other studies indicated that a daily dose schedule exerts superior therapeutic

Table 2. Sensitivity analyses of acute respiratory infections after vitamin D supplementation
(Risk ratios and 95% confidence intervals)

<table>
<thead>
<tr>
<th>Number of trials</th>
<th>Total number of participants</th>
<th>Risk ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed-effects model</td>
<td>6</td>
<td>8673</td>
<td>0·89</td>
<td>0·67, 1·19</td>
</tr>
<tr>
<td>Using trim-and-fill analysis</td>
<td>6</td>
<td>8673</td>
<td>0·89</td>
<td>0·67, 1·18</td>
</tr>
<tr>
<td>Excluding a trial with fewer than thirty participants per group</td>
<td>5</td>
<td>8625</td>
<td>0·94</td>
<td>0·71, 1·26</td>
</tr>
<tr>
<td>Excluding two trials regarding asthma exacerbation</td>
<td>4</td>
<td>8291</td>
<td>0·97</td>
<td>0·67, 1·39</td>
</tr>
<tr>
<td>Excluding two trials regarding repeated episodes of pneumonia</td>
<td>4</td>
<td>4190</td>
<td>0·76</td>
<td>0·54, 1·07</td>
</tr>
</tbody>
</table>

Fig. 4. Effects of vitamin D supplementation on plasma 25-hydroxyvitamin D levels more than 3 months later (z = 1·98, P = 0·048). To convert nmol/l to ng/ml for 25-hydroxyvitamin D concentrations, divide by 2·496. WMD, weighted mean difference. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).
Vitamin D and acute respiratory infection

Fig. 5. Funnel plots for (a) acute respiratory infections and (b) all-cause mortality. RR, risk ratio. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

As reported, vitamin D supplementation simultaneously enhances the effectiveness of the antimicrobial response of the innate immune system and diminishes the natural consequences of inflammation, which appear to exert an adverse effect on asthma pathogenesis. A study conducted by Damera et al. demonstrated that vitamin D activity decreased the growth of airway smooth muscle cells stimulated by platelet-derived growth factor in vitro, and their result was not contradicted by our findings.

Each of the trials included in the present study was randomised, double-blinded and placebo-controlled. These criteria eliminated the possibility of reverse causation and minimised both recall and selection bias. The risk of bias was very low as a result of the use of a modified Jadad scale. However, the observational studies were characterised by a larger number of confounding factors; the incidence of ARI may have been influenced by recall bias, which may have exaggerated the effects of vitamin D supplementation.

The present meta-analysis has limitations. First, the serum concentrations of 25(OH)D were reported by only three trials; therefore, we do not know whether the baseline serum concentrations of 25(OH)D modified the effects of vitamin D supplementation. Second, we did not perform either subgroup analyses or meta-regression analyses investigating the influence of the characteristics of the trials on the observed effects of vitamin D because of the small number of trials included in the meta-analysis.

Conclusion

In summary, the present meta-analysis did not reveal sufficient evidence to support a beneficial effect of vitamin D supplementation on the prevention of ARI, the reduction of all-cause mortality, or the rate of hospital admission due to respiratory infections in healthy children. Among children previously diagnosed with asthma, vitamin D supplementation appears to significantly reduce the risk of asthma exacerbation triggered by a respiratory infection. However, these results should be interpreted with caution due to the small number of included trials and the significant heterogeneity observed between them.

Acknowledgements

The authors would like to thank each of their colleagues for their valuable contributions to this article; S. H. Xu and Huang Yong for kindly reviewing and correcting the manuscript; Y. Zhou and Y. R. Fang for their helpful comments and assistance in the literature search.

The present study was supported by the National Natural Science Foundation of China (S. X., grant number no. 81402700). The National Natural Science Foundation of China (S. X., grant number no. 81402700) had no role in the design and analysis of the study or in the writing of this article, but contributed to the interpretation of the findings or the preparation of the manuscript. The present review received no specific grant from any funding agency, commercial or not-for-profit sectors.
The authors’ responsibilities were as follows: L. X. and Z. H. reviewed the literature and selected the eligible studies; C. X., Z. Y. and S. X. extracted the data; M. W. and H. D. performed the statistical analysis; L. X., Z. H. and K. L. wrote the manuscript. All authors reviewed and approved the final manuscript.

The authors declare that they have no conflicts of interest.

References