Meta-analysis of Zn, Cu and Fe in the hair of Chinese children with recurrent respiratory tract infection

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Abstract
Trace elements play an important role in maintaining the normal metabolic and immune function. The onset of recurrent respiratory tract infection (RRI) is associated with the immune function, genetic factors and nutritional status. However, the association between the levels of trace elements and RRI remains inconclusive. We aimed to investigate the alterations of hair levels of zinc (Zn), copper (Cu) and iron (Fe) in Chinese children with RRI by performing a meta-analysis. A predefined electronic databases search was performed to identify eligible studies for the analysis of hair Zn, Cu or Fe levels in Chinese children with RRI. Thirteen studies were included. RRI patients displayed significantly lower levels of hair Zn (13 studies, random effects SMD: 1.215, 95% CI: 1.704 to 0.725, \( p < 0.0001 \)), Cu (11 studies, random effects SMD: 0.384, 95% CI: 0.717 to 0.052, \( p = 0.023 \)) and Fe (12 studies, random effects SMD: 0.569, 95% CI: 0.827 to 0.312, \( p < 0.0001 \)) compared with controls. No evidence of publication bias was observed. Sensitivity analysis did not change the results significantly. In conclusion, the deficiency of Zn, Cu and Fe may be contributing factors for the susceptibility of RRI in Chinese children. However, more studies in different ethnicities should be performed in the future.

Key Words: Hair, zinc, copper, iron, children, recurrent respiratory tract infection

Introduction
Recurrent respiratory tract infection (RRI), an important cause of death in children, is the most prevalent disease among children, particularly for children at the age of 2–6 years [1]. RRI is prevalent both in developing and developed countries. In China, RRI incidence is nearly 20% [2]. RRI is likely to lead to asthma, myocarditis, nephritis, and/or other diseases, which may seriously affect children’s growth and development [3]. Many factors, such as immune function, genetic factors and nutritional status are associated with the susceptibility of RRI. However, the underlying cause of RRI remains poorly understood.

Trace elements are indispensable for life and play a very important role in many functions [4]. Most of the trace elements are essential for the maintenance of normal metabolic and immune function. The homeostasis of trace elements is essential for biological processes. The physiological function may be affected by the status of trace elements, many disease states may also influence the levels of trace elements [5,6]. Several studies showed that there were changes in the plasma concentrations of trace elements in various infections and conditions [7–9]. In addition, the therapeutic and prophylactic use of some trace elements, such as zinc, in respiratory infections could result in clinical improvement [10]. In terms of the above-mentioned data and the fact that the trace elements levels in hair are more stable and do not fluctuate easily and quickly compared with those in serum and plasma [11], we hypothesize that the status of hair trace elements are associated with RRI.

In the past decades, we have witnessed a number of studies in which the hair levels of trace elements including zinc (Zn), copper (Cu) and iron (Fe) were tested in Chinese children with RRI [12–24]. However, the results were not consistent. An improved understanding of this issue has important clinical implication that the hair levels of trace elements might be associated with the risk of RRI. Meta-analysis is an efficient way to provide more rational results compared with individual studies [25]. Pooled analysis regarding the serum levels of trace elements in Chinese RRI patients was performed before [26], However, meta-analysis relating to the hair levels of trace elements in Chinese RRI patients was rare. Thus, we performed this
meta-analysis to compare the differences in hair Zn, Cu and Fe levels between RRI cases and healthy controls.

Materials and methods

Search strategy

According to the recommendations of the PRISMA statement (Preferred reporting items for systematic reviews and meta-analyses) [27], attempt was made to search the published papers that reported the hair status of Zn, Cu or Fe both in RRI patients and controls from January 1990 to May 2013 using PubMed, Embase, Cochrane, and China National Knowledge Infrastructure (CNKI) databases. No restriction was imposed on search language. The used search terms were as follows: (i) Trace element, micronutrient, Zn, Cu, Fe, hair, China, Chinese; and (ii) recurrent respiratory tract infection, RRI, respiratory infection. Bibliographies of retrieved reviews and articles were also scrutinized. If the same data was enrolled in more than one study, we recruited the study with the most complete analysis.

Inclusion and exclusion criteria

Inclusion criteria. (1) Case-control study; (2) the outcome of interest was hair status of Zn, Cu or Fe; and (3) a minimum of two comparison groups (RRI group vs. control group).

Exclusion criteria. (1) Case reports, editorials and reviews; (2) the levels of other trace elements, serum and plasma levels of Zn, Cu or Fe; and (3) multiple publications of the same data.

Data extraction and quality assessment

Baseline characteristics, any reported mean and standard deviation were extracted from each study. Data were recorded as follows: First author's surname; year of publication; characteristics of study population and age at baseline; number of cases and controls; diagnosis, confounding factors. The quality of each study included was assessed by using Newcastle-Ottawa Quality Assessment Scale, which included the assessment for participants' selection, exposure and comparability. A study can be awarded a maximum of one score for each numbered item within the selection and exposure categories. A maximum of two scores can be given for comparability. Two authors performed the data extraction and quality assessment with any disagreements resolved by discussion.

Statistical analysis

Standard mean deviation (SMD) was used to measure the differences in hair Zn, Cu or Fe levels between RRI patients and controls across studies. Forest plots were presented with a line of zero and a linear scale, which was due to the facts that SMD represented the differences of status of Zn, Cu or Fe between cases and controls. The largest scale in the line indicated the largest differences. Weighting in the forest plots reflected the number of observations. Heterogeneity of SMDs across studies was tested by using the Q statistic (significance level at \( p < 0.10 \)). The I^2 statistic, a quantitative measure of inconsistency across studies, was also calculated [28]. The SMDs were calculated using either fixed-effects models or, in the presence of heterogeneity, random-effects models. Sensitivity analysis was performed when the quality of studies was low in terms of Newcastle-Ottawa Quality Assessment Scale [29]. Potential publication bias was assessed by Begg's test and Egger's test at the \( p < 0.05 \) level of significance [30,31]. All analyses were conducted using STATA version 12.0 (Stata Corp, College Station, TX, USA); \( p < 0.05 \) was considered statistically significant, except where otherwise specified.

Results

Literature search

We initially retrieved 131 relevant references from the PubMed, Embase, Cochrane and CNKI databases. Of these, 118 publications were excluded according to the inclusion and exclusion criteria. A total of 13 studies were identified for the analysis of the differences in hair levels of Zn, Cu or Fe between RRI cases and controls (Figure 1). We also contacted the authors of included studies for the use of the data.

Figure 1. Flow chart of study selection.
Table I. Baseline characteristics of studies included in this meta-analysis.

<table>
<thead>
<tr>
<th>Study</th>
<th>N case/control</th>
<th>Age (mean ± SD)</th>
<th>Zn case (mean ± SD)</th>
<th>Zn control (mean ± SD)</th>
<th>Cu case (mean ± SD)</th>
<th>Cu control (mean ± SD)</th>
<th>Fe case (mean ± SD)</th>
<th>Fe control (mean ± SD)</th>
<th>Quality score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jia, 2000</td>
<td>62/46</td>
<td>4.17/4.42</td>
<td>86.04 ± 24.02</td>
<td>118.25 ± 25.2</td>
<td>10.87 ± 2.8</td>
<td>11.21 ± 3.01</td>
<td>28.01 ± 11.5</td>
<td>46.17 ± 12.63</td>
<td>6</td>
</tr>
<tr>
<td>Li, 2000</td>
<td>100/50</td>
<td>2.6/2.8</td>
<td>98.52 ± 33.79</td>
<td>114.3 ± 27.3</td>
<td>8.41 ± 5.9</td>
<td>9.48 ± 6.62</td>
<td>26.78 ± 12.07</td>
<td>32.97 ± 11.91</td>
<td>4</td>
</tr>
<tr>
<td>Li, 2000</td>
<td>95/50</td>
<td>6.5/6.5</td>
<td>87.72 ± 40.15</td>
<td>145.64 ± 47.16</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>Wu, 2000</td>
<td>718/2886</td>
<td>4/4</td>
<td>80.58 ± 17.99</td>
<td>82.88 ± 18.89</td>
<td>10.25 ± 2.32</td>
<td>10.05 ± 2.37</td>
<td>30.19 ± 5.05</td>
<td>30 ± 5.41</td>
<td>3</td>
</tr>
<tr>
<td>Li, 2001</td>
<td>56/48</td>
<td>2.6/2.3</td>
<td>85.20 ± 20.5</td>
<td>105.29 ± 25.0</td>
<td>8.8 ± 2</td>
<td>11.3 ± 1.3</td>
<td>27.3 ± 3.6</td>
<td>30.5 ± 4.5</td>
<td>6</td>
</tr>
<tr>
<td>Wang, 2001</td>
<td>73/80</td>
<td>5/5</td>
<td>80 ± 37</td>
<td>120 ± 15</td>
<td>10 ± 4</td>
<td>11 ± 5</td>
<td>15 ± 8</td>
<td>25 ± 12</td>
<td>3</td>
</tr>
<tr>
<td>Meng, 2002</td>
<td>62/50</td>
<td>&lt;12</td>
<td>89.26 ± 20.13</td>
<td>110.51 ± 29.16</td>
<td>11.74 ± 4.31</td>
<td>11.01 ± 3.15</td>
<td>17.43 ± 11.12</td>
<td>22.32 ± 10.17</td>
<td>3</td>
</tr>
<tr>
<td>Cai, 2003</td>
<td>150/150</td>
<td>4.5/4.5</td>
<td>81.92 ± 21.24</td>
<td>112.89 ± 27.88</td>
<td>9.69 ± 2.59</td>
<td>9.69 ± 2.34</td>
<td>34.33 ± 16.99</td>
<td>42.56 ± 14.11</td>
<td>5</td>
</tr>
<tr>
<td>Zhao, 2006</td>
<td>298/100</td>
<td>3.75/3.75</td>
<td>69.47 ± 13.46</td>
<td>107.85 ± 8.82</td>
<td>9.35 ± 2.22</td>
<td>9.68 ± 2.08</td>
<td>28.28 ± 3.84</td>
<td>29.95 ± 4.15</td>
<td>3</td>
</tr>
<tr>
<td>Yang, 2006</td>
<td>170/150</td>
<td>3.2/2.9</td>
<td>85.1 ± 20.7</td>
<td>103.3 ± 29.2</td>
<td>8.7 ± 2.1</td>
<td>11.4 ± 1.2</td>
<td>27.2 ± 3.7</td>
<td>30.4 ± 4.6</td>
<td>5</td>
</tr>
<tr>
<td>Jin, 2006</td>
<td>350/150</td>
<td>3.5/3.5</td>
<td>81.2 ± 30.3</td>
<td>112 ± 30.7</td>
<td>7.4 ± 1.7</td>
<td>8.6 ± 2.6</td>
<td>29.2 ± 8</td>
<td>35 ± 10.9</td>
<td>6</td>
</tr>
<tr>
<td>Zhang, 2010</td>
<td>120/120</td>
<td>3.5/3.2</td>
<td>76.5 ± 20.2</td>
<td>128.5 ± 25.4</td>
<td>8.2 ± 2.1</td>
<td>8.72 ± 2.05</td>
<td>44.08 ± 20.4</td>
<td>44.72 ± 17.86</td>
<td>6</td>
</tr>
<tr>
<td>Jiang, 2011</td>
<td>34/30</td>
<td>4/4</td>
<td>75.2 ± 22.58</td>
<td>130.14 ± 64.5</td>
<td>8.2 ± 2.01</td>
<td>8.72 ± 2.05</td>
<td>44.08 ± 20.4</td>
<td>44.72 ± 17.86</td>
<td>6</td>
</tr>
</tbody>
</table>

Zn, zinc; Cu, copper; Fe, iron; (mean ± SD) = (mean ± SD)μg/g.

Discussion
To the best of our knowledge, this is the first meta-analysis of the alterations in levels of Zn, Cu, and Fe in RRI. Among the 12 included studies, we found that RRI patients demonstrated significantly lower levels of hair Zn, Cu, and Fe compared with controls. A total of 2288 cases and 3890 controls were included in this study. Our investigation indicated that the lower levels of hair Fe (12 studies, random effects SMD: -1.215, 95% CI: -1.825 to -0.606, p < 0.001, 97.9% of studies) were significantly altered across the studies. The sensitivity analysis and publication bias analysis showed that the results were robust and reliable. No evidence of publication bias was noted (Begg, p = 0.827; Egger, p = 0.483). Sensitivity analysis showed that the results were not influenced by any single study.

The characteristics of the 13 case-control studies (12-24) are shown in Table I. These studies were published between 2000 and 2011. The sizes of studies ranged from 64 to 563. The majority of studies included more than 100 cases, and all healthy control volunteers included were at the active stage of RRI. The controls were all healthy check-up volunteers. Twelve studies were included and provided data for the analyses.
Both acute and chronic infections may be associated with the levels of Zn, Cu, and Fe in humans. For example, Javadmoosavi et al. [32] reported that Zn level in bronchiectasis patients was significantly lower than the control group. Zn supplement can reduce progression of the infectious disease regarding its role in improving the immune system reactions. Elemraid et al. [33] reported that children with chronic suppurative otitis media were more undernourished than healthy controls with lower mean serum Zn level. Queiroz et al. [34] reported that *Helicobacter pylori* infection was a significant predictor of low iron status. Mirastchijski et al. [35] reported that Cu was mobilized to injured sites possibly to enhance host defense, which may explain the mechanism behind the decreased status of the trace elements after chronic infection.

Animal studies also supported the notion that trace elements were associated with infection. For example, Crocker et al. [36] reported that copper-deficient rats showed greater numbers of parasites than controls throughout the infection. The duration of the trypanosomal infection was longer in copper-deficient rats compared with other groups. Yong et al. [37] reported that Cu had a role in the mechanism of cell-mediated immunity in sheep infected with...
Trichostrongylus. In terms of the above-mentioned plus the fact that children themselves are more susceptible to parasites and have a lower immunity compared with adults [38], the monitoring the status of Zn, Cu and Fe will be helpful for the prevention of infections. Hair element analysis may help examine the levels of elements accumulated over a long period of time, which is not possible in serum analysis.

The decreased levels of Zn in RRI are probably attributable to malabsorption, low intake, and increased losses due to diarrhea, a common complication of respiratory infection. Zn is required for normal intestinal mucosal integrity, skeletal growth, sodium and water transport and immune function [39]. Zn deficiency is associated with impaired cell-mediated immunity with thymic atrophy resulting in increased susceptibility to infections [40]. Zn also influences the function of other micronutrients. For example, Zn affects the absorption, transport and utilization of vitamin A, which would lead to a poor nutritional status [41]. Meanwhile, the nutritional status might influence the levels of trace elements. A previous study [42] by Thakur et al. showed that serum levels of Zn and Cu were significantly lower in patients with protein energy malnutrition compared with those in healthy controls.

Cu, a transition metal, can catalyze the production of highly toxic hydroxyl radicals [43]. Cu is a constituent of the respiratory enzyme complex cytochrome c oxidase and blood pigment hemocyanin. Cu also participates in the metabolism of Fe. Hence, Cu deficiency may be one of the reasons for the
anemia, which would affect the nutritional status. In addition, although toxicity in humans can occur at high concentrations, in general exposure to Cu is safe, which is evidenced by widespread use of Cu intrauterine devices [44]. Conversely, microorganisms are extremely sensitive to its toxic effects. The exact mechanism may be that Cu ions compete with Zn or other metal ions for important binding sites on proteins, leading to the conformational change and the loss of protein function [45]. In this sense, the low levels of Cu in RRI might result from dietary Cu insufficiency and increased depletion due to the microorganisms.

Fe constitutes essential nutrient for most of the human pathogens [46]. The decreased levels of Fe are probably due to the low dietary intake and increased consumption due to the pathogens. Fe contributes to the normal human immunity. Several studies have indicated that both cell-mediated and humoral immunity were impaired in the deficiency of Fe [47,48]. For instance, the phagocytic activity of the monocytes decreased in patients with Fe deficiency and no other potential diseases [49]. Enzymes that mediate bacterial killing, such as hydroxyl radicals, are not adequately produced by the macrophages in Fe-deficient patients [50]. Lymphocyte responses, particularly interleukin-2 production, was impaired in Fe-deficient children [51]. All these data supported that the deficiency of Fe might contribute to the onset of RRI.

Although all the studies included had similar baseline characteristics, such as study region, age at baseline, measurement of parameters and relatively high scores in terms of quality assessment, several limitations should be considered in interpreting the results. First, substantial heterogeneity was observed across studies, affecting the results of our meta-analysis, although a random-effects model had been conducted.

Nevertheless, no evidence of publication bias was observed. Second, it is not ethical to make models of RRI in human subjects, the study design had to be case-control, which might result in reversal causation. Third, most studies included in this meta-analysis indicated lower levels of Zn, Cu and Fe in cases compared with controls, which indicated that results of our meta-analysis were comparatively robust. However, one study by Wu et al. [23] showed the higher level of Cu, similar level of Fe in cases compared with controls, which may be due to the facts that it is low quality in terms of Newcastle-Ottawa Quality Assessment Scale. In this study, the data regarding the age and sex was not provided, the sample of hair was only 0.2 g, which may affect the results. Further larger studies with high quality should be performed.

Finally, the diagram of Zn and Fe looks convincing, whereas in the case of the copper diagram, two studies lie to the right of the zero line and one on it, while five studies cross it, which may be due to the facts that Cu-rich foods are mainly shellfish, organ meats, nuts and grains [52]. Children, particularly low-age children, are deficient in these foods; and some of these foods are not easily available in inland areas of China, which cause the common low level of Cu in children. Hence, it resulted in the insignificant differences of Cu status between cases and controls. In addition, the stable level of Cu often requires some time. Nonetheless, our findings had important clinical significance due to the fact that hair levels of trace elements reflect the long-term nutritional status and the majority of cases included were in the active stage of RRI, which suggested that the trace elements deficiency might precede the onset of RRI. Finally, only 13 studies in Chinese were included in our meta-analysis, which limited the statistical power and made it difficult to generalize its results to other populations. Further larger number of studies in different ethnicities are needed.

In conclusion, this meta-analysis suggests that the deficiency of Zn, Cu and Fe may be associated with the susceptibility of RRI in Chinese children. It should be recommended to perform frequent monitoring and early intervention. However, more studies in different ethnicities should be performed in the future.

Declaration of interest: The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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