IMMUNE-BOOSTING EFFECT OF VITAMIN C IN COMBINATION WITH ZINC AGAINST PEDIATRIC PNEUMONIA: A RANDOMIZED CONTROLLED TRIAL

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Abstract:
Lower respiratory tract infections are a major cause of illness and hospitalization in children under 5 years old. It is usually caused by bacteria, viruses, or fungi and is spread through contact with an infected person or their respiratory droplets. Symptoms include fever, rapid breathing, chills, cough, wheezing, fast heart rate, poor appetite, and fatigue. This study aims to evaluate the Immune-boosting effect of Vitamin C and Zinc against pediatric pneumonia. A randomized control trial was done on 40 patients between 2 years and 5 years of age with a diagnosis of pneumonia who were enrolled at Raiwind RHC (rural health center). The control, group being studied included 20 children who had pneumonia and were receiving medication for it. The experimental group contains 20 children on medications and given Vitamin C (125mg) and Zinc (20mg). Pre-testing of all participants was done and immunoglobulin levels (IgG, IgA, IgM) were studied. Post-testing of all participants was done at the end of these interventions. The results show that vitamin C and zinc have significant (<0.05) effects against IgG, IgA, and IgM in pediatric pneumonia. Both combined have health-beneficial effects against immunity in pneumonia.

Keywords: Immunoglobulins, Pediatric Pneumonia, Vitamin C, Zinc.

Introduction:
Pneumonia is a respiratory infection that affects the alveoli and distal airways, causing high morbidity and mortality rates worldwide. It is a leading cause of death for children under 5 and requires urgent attention (1). According to WHO, pneumonia in children aged 2-59 months is characterized by coughing or difficulty breathing with rapid or chest indrawing. Severe pneumonia is accompanied by the inability to drink, persistent vomiting, convulsions, lethargy, stridor, or severe malnutrition. (2). The burden of pneumonia cases in 2015 was largely concentrated in India, Nigeria, Indonesia, Pakistan, and China, which accounted for over 54% of all global cases. India alone was responsible for 32% of the global burden of pneumonia (3,4). The prevalence of acute respiratory infection (ARI)

and pneumonia in 2018 was 14%. Out of those affected, 84% of children were taken to a health facility, and 46% were treated with antibiotics **(5).**

According to recent studies in Pakistan, 23% of children under five are underweight and 8% are severely underweight. Severe pneumonia is linked to underweight children **(6).** Low levels of IgA, IgG, or IgM are associated with significantly increased risks of bacterial respiratory tract infections in primary immunodeficiency syndromes **(7).**

Vitamin C (VC), a well-known antioxidant, has been reported to have beneficial effects in the prevention and treatment of pneumonia **(8).** Vitamin C is an antioxidant by limiting damage caused by oxygen and nitrogen free radicals produced during normal cell metabolism and by neutrophils activated in response to bacteria, viruses, and toxins **(9).** Multiple recent trials have been published that suggest the positive effects of treating septic patients with a combination therapy of high-dose vitamin C, thiamine, and fludrocortisone **(10).** Vitamin C may protect against acute kidney injury, shorten hospital stays for cardiac surgery patients, and reduce blood pressure. **(11).**

Zinc and other micronutrient like selenium also play an essential role in strengthening and supporting the immune system, the body's natural defense mechanism against infections and diseases **(12,23).** Zinc is critical for human immunity as it affects T cell generation, cytokine production, and metabolic toxin removal **(13,14,15).** During the early stages of an infection, the most effective antiviral antibodies are neutralizing ones. They bind to the viral envelope or capsid proteins and regulate the virus's entry into the host cell **(14,15).** Zinc ions regulate the activity of the zinc-finger antiviral protein, which helps degrade RNA viruses **(15).** Zinc salts have broad-spectrum antimicrobial properties against various viruses, including HSV, SARS-CoV, HIV, and others **(16).** Many studies have been conducted to see the relationship between vitamin C and zinc against pneumonia and many other diseases **(17,18)**, But the combo effect of vitamin C and zinc needs to be studied to reduce the severity of pneumonia and other diseases.

Methodology:

Sample Size and Study Group: The study's sample size of 40 participants was determined using the Raosoft 2020 online calculator. The participants were then divided into two groups, with 20 individuals in each group. Group 1 was the experimental group that received intervention treatment. Group 2 was the control group that received placebo treatment.

Study conducted at: The study was conducted at Raiwind RHC (Rural Health Center), Punjab.

Inclusion Criteria
- Age ranges from 2 -5 years old.
- Newly diagnosed with pneumonia.
- Willing to cooperate.
- On medication.

Exclusion Criteria
- Having other diseases.
- Have allergy.

Tools:

Anthropometric measurements:
Height and weight measurements were taken, and BMI was calculated using a percentile graph for ages (2-20) (**19,20).**

Immunoglobulins blood test:
There are five immunoglobulin (Ig) types in the human body - IgG, IgM, IgA, IgD, and IgE. In a regular serum, IgG accounts for approximately 80%, IgA for about 15%, and IgM for 5%. These tests

are beneficial in diagnosing various medical conditions and diseases that affect the levels of one or more of these immunoglobulin types **(21).**

Ethical consent
The research obtained approval from the Research and Ethics Committee of Riphah International University, Lahore (No. REC/RCR & AHS/22/0807).

STATISTICAL INVESTIGATION
The data analysis was carried out using SPSS software version 24. A T-test was performed on the data, and the results were presented as mean ± SD. Any p-value less than 0.05 was deemed to be significant.

Results:
Baseline and Contestants:
In the control group, 55% were boys and 45% were girls however in the experimental group, 38.8% were boys and 61.1% were girls. About 45.95% of children are less than <1percentile according to the BMI chart. The mean IgG, IgA, and IgM levels of participants were 2118.95, 0.20, and 213.9 mg/dl respectively.

Effect of Vitamin C and Zinc on Immunoglobulins:
It was observed that the levels of IgG and IgM significantly decreased (p<0.05), While the levels of IgA significantly improved (p<0.05) when participants were given a combination of Vitamin C and zinc. However, in the control group, there was no significant impact on these values.

Table 1: Mean and SD of Immunoglobulins level (n= 40) before intervention

Parameter	Control group	VC-Zn group
	Mean±SD	Mean±SD
IgG (mg/dl)	2118.2±9.37	2119.7±23.55
IgA (mg/dl)	0.19±0.06	0.20±0.06
IgM (mg/dl)	213.25±5.0	214.5±4.4

VC-Zn: Vitamin C and Zinc, IgG: Immunoglobulin G, IgA: Immunoglobulin A, and IgM: Immunoglobulin M.

Table 2: After intervention the effect of Vitamin C and Zinc

parameter	Groups	Before intervention	After intervention
		Mean±SD	Mean±SD
IgG (mg/dl)	Control	2118.2±9.37	2102±4.46
	VC-Zn	2119.7±23.55	2085.9±5.76
IgA (mg/dl)	Control	0.19±0.06	0.33±0.04
	VC-Zn	0.20±0.06	0.42±0.04
IgM (mg/dl)	Control	213.25±5.0	197±4.54
	VC-Zn	214.5±4.4	191.5±4.14

Table 3: boy's percentile table

Percentile			
Age	**<5th**	**<85th**	**Grand Total**
2ys	4	2	6
3y	6	-	6
4y	4	2	6
5y	2	1	3
Total	16	5	21

Table 4: girl's percentile

Percentile			
Age	**<5th**	**<85th**	**Grand Total**
2ys	5	-	5
3y	7	2	10
4y	2	2	4
5y	1	-	1
Total	15	4	19

Discussion:

A recent study demonstrated the positive effects of Vitamin C and Zinc on human subjects without causing any harm. The intervention group received 125mg of Vitamin C and 20mg of Zinc in the study. The results showed that the combined effect of Vitamin C and Zinc significantly boosted immunity in pediatric pneumonia. Pneumonia patients experience declining nutrient levels, affecting their muscles and immune response. Hospitalized patients need immediate dietary support alongside treatment. Micronutrient deficiencies lead to secondary immunodeficiency and increase the risk of infections [22]. In 2014 a study was conducted in Islamabad by Imran Mahmood showed a significant effect of vitamin C against pneumonia and reduced the severity of pneumonia [24]. In 1977 W Prinz conducted a study that showed that vitamin C has a statistically significant increase in IgA and IgM serum levels [25]. Vitamin C is an antioxidant that prevents damage from free radicals (oxygen and nitrogen) produced during normal cell metabolism and immune response to pathogens [13]. Vitamin C enhances the migration of neutrophils to the site of infection in response to chemo-attractants, which ultimately leads to killing pathogens by increasing phagocytosis and oxidant generation [13]. In 2015 Noshina Riaz conducted a study that also shows the significant effect of vitamin C against pediatric pneumonia. In this study, she evaluates the hospital stay and the vitamin C group shows less stay in the hospital [26]. In 2014, an RCT trial was conducted that showed Zn has a significant effect against pediatric pneumonia lessens the symptoms of pneumonia, and also reduces the drug resistance caused by multiple therapies [27]. In 2019 Jorge Alberto conducted a study on Zn against pneumonia and found significant improvements in antibodies, and a significant improvement in sign and symptoms and have significant improvement in morbidity rate of pneumonia in children [28].

A double-blind placebo control trial was conducted in Bangladesh on children having pneumonia. They were given Zn (20mg/day) until they were discharged. This study also showed a significant effect on pneumonia and its severity [29]. A RCT conducted in 2022 shows that Zn can improve the level of immunoglobulin G and have an effect on antibodies in our body [30]. Due to the beneficial role of VC and Zn, this study aims at the effect of both on immunoglobulin levels in pneumonia. The result of this study related to previous studies showed a significant effect against pneumonia symptoms and had a significant improvement in immunoglobulin levels. VC needs to be studied further for the improvement and treatment of pneumonia. VC and Zn both given at the same time for 3 months will improve the pneumonia severity and immunoglobulins level.

Conclusion:

Pneumonia is a respiratory illness that distresss the alveoli and distal airways. It is a health issue associated with high morbidity and mortality in all age groups worldwide. Most of the children's BMI was less than <5 percentile and were from rural areas. Their nutrition levels need to be monitored. In the future, more research about the combo of vitamin C and zinc needs to be studied to treat pneumonia and improve the immunoglobulin levels and their defense of action, and how many days it is to be given to pneumonia patients.

Reference:

1. Ebeledike, C., Ahmad, T., & Martin, S. D. (2021). Pediatric Pneumonia (Nursing).
2. Dean, P., & Florin, T. A. (2018). Factors associated with pneumonia severity in children: a systematic review. *Journal of the Pediatric Infectious Diseases Society*, *7*(4), 323-334..

3. Carr, A. C., & Rowe, S. (2020). The emerging role of vitamin C in the prevention and treatment of COVID-19. *Nutrients*, *12*(11), 3286.

4. Wintergerst, E. S., Maggini, S., & Hornig, D. H. (2006). Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. *Annals of Nutrition and Metabolism*, *50*(2), 85-94.

5. Das, J. K., Siddiqui, F., Padhani, Z. A., Khan, M. H., Jabeen, S., Mirani, M., ... & Bhutta, Z. A. (2023). Health behaviors and care seeking practices for childhood diarrhea and pneumonia in a rural district of Pakistan: A qualitative study. *Plos one*, *18*(5), e0285868.

6. Asmaa, Y., Kakalia, S., Irtza, M., Malik, R., & Malik III, R. (2024). The Diagnostic Association of Radiological and Clinicopathological Parameters in Community-Acquired Pneumonia in Children: A Cross-Sectional Study. *Cureus*, *16*(2).

7. Jafari, D., Esmaeilzadeh, A., Mohammadi-Kordkhayli, M., & Rezaei, N. (2019). Vitamin C and the immune system. *Nutrition and immunity*, 81-102.

8. Li, R., Guo, C., Li, Y., Liang, X., Yang, L., & Huang, W. (2020). Therapeutic target and molecular mechanism of vitamin C-treated pneumonia: a systematic study of network pharmacology. *Food & function*, *11*(5), 4765-4772.

9. Padhani, Z. A., Moazzam, Z., Ashraf, A., Bilal, H., Salam, R. A., Das, J. K., & Bhutta, Z. A. (2020). Vitamin C supplementation for prevention and treatment of pneumonia. *Cochrane Database of Systematic Reviews*, (4).

10. Mahmoodpoor, A., Shadvar, K., Sanaie, S., Hadipoor, M. R., Pourmoghaddam, M. A., & Saghaleini, S. H. (2021). Effect of Vitamin C on mortality of critically ill patients with severe pneumonia in intensive care unit: a preliminary study. *BMC Infectious Diseases*, *21*, 1-7.

11. Kim, W. Y., Jo, E. J., Eom, J. S., Mok, J., Kim, M. H., Kim, K. U., ... & Lee, K. (2018). Combined vitamin C, hydrocortisone, and thiamine therapy for patients with severe pneumonia who were admitted to the intensive care unit: Propensity score-based analysis of a before-after cohort study. *Journal of critical care*, *47*, 211-218.

12. Solomons, N. W. (1998). Mild human zinc deficiency produces an imbalance between cell-mediated and humoral immunity. *Nutrition Reviews*, *56*(1), 27-28.

13. Carr, A. C., & Maggini, S. (2017). Vitamin C and immune function. *Nutrients*, *9*(11), 1211.

14. Ishida, S. T. (2020). Zinc (II) Immune Virucidal Activities for 2019-nCoV Prevention and COVID-19 Respiratory Ailment and Pneumonia. *IJMRHS*, *5*, 21-33.

15. Brazel, E. B., Tan, A., Neville, S. L., Iverson, A. R., Udagedara, S. R., Cunningham, B. A., ... & McDevitt, C. A. (2022). Dysregulation of Streptococcus pneumoniae zinc homeostasis breaks ampicillin resistance in a pneumonia infection model. *Cell reports*, *38*(2).

16. Fatima, A., Khan, M. U. A., Najeeb, M., Khan, M. Y. A., & Haq, F. U. (2021). Role of high dose IV vitamin C and zinc in coronavirus. *Geriatric Care*, *7*(1).

17. Maywald, M., & Rink, L. (2022). Zinc in human health and infectious diseases. *Biomolecules*, *12*(12), 1748.

18. Chambial, S., Dwivedi, S., Shukla, K. K., John, P. J., & Sharma, P. (2013). Vitamin C in disease prevention and cure: an overview. *Indian journal of clinical biochemistry*, *28*, 314-328.

19. Zierle-Ghosh, A., & Jan, A. (2018). Physiology, body mass index.

20. World Health Organization. (2024). *Body mass index-for-age (BMI-for-age).* Retrieved March 29, 2024.

21. Loh, R. K., Vale, S., & McLean-Tooke, A. (2013). Quantitative serum immunoglobulin tests. *Australian family physician*, *42*(4), 195-198.

22. Hussain, M. I., Ahmed, W., Nasir, M., Mushtaq, M. H., Sheikh, A. A., Shaheen, A. Y., & Mahmood, A. (2019). Immune boosting role of vitamin E against pulmonary tuberculosis. *Pakistan Journal of Pharmaceutical Sciences*, *32*.

23. Hussain, M. I., Ahmed, W., Nasir, M., Mushtaq, M. H., Sheikh, A. A., Shaheen, A. Y., ... & Iqbal, S. (2019). Immune modulatory and anti-oxidative effect of selenium against pulmonary tuberculosis. *Pakistan Journal of Pharmaceutical Sciences*, *32*.

24. Khan, I. M., Shabbier, A., Naeemullah, S., Siddiqui, F. R., Rabia, M., Khan, S. N., & Chaudhary, M. T. (2014). Efficacy of vitamin C in reducing duration of severe pneumonia in children. *Journal of Rawalpindi Medical College*, *18*(1).
25. Prinz, W., Bortz, R., Bregin, B., & Hersch, M. (1977). The effect of ascorbic acid supplementation on some parameters of the human immunological defence system. *International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin-und Ernahrungsforschung. Journal international de vitaminologie et de nutrition*, *47*(3), 248-257.
26. Yaqub, A., RIAZ, N., GHANI, Z., & GUL, S. (2015). ROLE OF VITAMIN C IN CHILDREN HAVING PNEUMONIA. *ISRA Medical Journal*, *7*(4).
27. Qasemzadeh, M. J., Fathi, M., Tashvighi, M., Gharehbeglou, M., Yadollah-Damavandi, S., Parsa, Y., & Rahimi, E. (2014). The effect of adjuvant zinc therapy on recovery from pneumonia in hospitalized children: a double-blind randomized controlled trial. *Scientifica*, *2014*.
28. Acevedo-Murillo, J. A., García León, M. L., Firo-Reyes, V., Santiago-Cordova, J. L., Gonzalez-Rodriguez, A. P., & Wong-Chew, R. M. (2019). Zinc supplementation promotes a Th1 response and improves clinical symptoms in fewer hours in children with pneumonia younger than 5 years old. A randomized controlled clinical trial. *Frontiers in pediatrics*, *7*, 431.
29. Brooks, W. A., Yunus, M., Santosham, M., Wahed, M. A., Nahar, K., Yeasmin, S., & Black, R. E. (2004). Zinc for severe pneumonia in very young children: double-blind placebo-controlled trial. *The Lancet*, *363*(9422), 1683-1688.
30. Kewcharoenwong, C., Sein, M. M., Nithichanon, A., Khongmee, A., Wessells, K. R., Hinnouho, G. M., ... & Lertmemongkolchai, G. (2022). Daily preventive zinc supplementation increases the antibody response against pathogenic Escherichia coli in children with zinc insufficiency: a randomised controlled trial. *Scientific reports*, *12*(1), 16084.