Effect of Liposomal Vitamin C on Immune Function in Children Aged 4-12 Years

Dr. Yogesh Dound [1], Dr. Yogita Gaikwad [2]

Abstract

Aims: Childhood Acute Respiratory Infection (ARI) is a significant public health problem especially in developing countries like India. Humoral immunity as well as cellular immunity act to reduce infections throughout the entire respiratory tract. Literature suggests that Vitamin C possesses the anti-inflammatory properties and antioxidant properties which boost the Immune system. Unfortunately, the highly unstable nature of Vitamin C has posed technological challenges for its incorporation into different food systems. We investigate the use of Liposomal Vitamin C which provides a well-tolerated, easily absorbed and highly bioavailable form of Vitamin C without any unpleasant side-effects.

Material and Methods: In the current study we studied for the first time effect of Liposomal Vitamin C in children aged 4 to 12 years suffering from URTI. This study was approved by Ethics Committee and registered with CTRI. Out of 30 participants, 26 children completed the study. The scoring was based on 'The Wisconsin Upper Respiratory Symptom Survey (WURSS)' for Kids.

Results: Within 4-5 days of starting of treatment with Liposomal Vitamin C, the score as indicated by the WURSS scale had reduced. The symptoms also subsided and no recurrence was observed till the end of 30 days.

Conclusion: The results suggest that Liposomal Vitamin C can be useful as an adjunct in the management of URTI to improve immunity. Further study with larger sample size and suitable clinical markers is required for better understanding of the effect of Liposomal Vitamin C and its mode of action in boosting Immunity.

Study Registration: CTRI/2020/03/023825

Keywords: Liposomal Vitamin C, URTI, Immune function, WURSS-21-K, children.

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Conflict of interest: Dr. Yogesh Dound is Proprietor, Shreepad Shree Vallabh SSV Phytopharmaceuticals.

[1] Proprietor, Shreepad Shree Vallabh SSV Phytopharmaceuticals, Jogeshwari, Mumbai;
[2] Consultant Paediatrician, Vakratund Hospital Pvt Ltd, Pathardi Phata, Nashik, Maharashtra.
Corresponding author: Dr. Yogesh Dound, Shreepad Shree Vallabh SSV Phytopharmaceuticals
E mail: yogesh_dound@yahoo.com

Introduction

Respiratory Tract Infection (RTI) refers to infectious diseases involving the respiratory tract. This type of infection is further classified as an Upper Respiratory Tract Infection (URTI) or a Lower Respiratory Tract Infection (LRTI). URTI includes the common cold and influenza, is the most prevalent of human illnesses especially in children. Childhood Acute Respiratory Infection (ARI) is a significant public health problem especially in developing countries. Symptoms of URTIs can include cough, sore throat, runny nose, nasal congestion, headache, low grade fever and sneezing. URTI is the most important cause of asthma exacerbation, and often leads to otitis media, sinusitis, bronchitis or pneumonia. Humoral immunity and cellular immunity acts to reduce infections throughout the entire respiratory tract. Robust epidemiological data is not available on its incidence in India [1]. As per estimations of World Health Organization ARI causes 3.9 million deaths throughout the world every year [2]. Adults obtain a common cold around two to three times yearly whereas pediatrics can have up to eight cases yearly [3,4]. Upper respiratory tract infections are accountable for greater than 20 million missed days of school and greater than 20 million days of work lost, thus generating a large economic burden [5].

Vitamin C is a water soluble vitamin known chemically as L-ascorbic acid. Vitamin C is found in many foods, particularly citrus fruits, green vegetables, tomatoes and potatoes. Vitamin C deficiency is the cause of scurvy which is marked by fatigue, spongy gums, loss of teeth, ecchymosis, petechiae and excessive bleeding including bleeding from the gums, into joints and into internal organs. Functional significance of Vitamin C has increased as a food component in the human diet for health promotion and disease prevention owing to the developments in nutritional sciences. However, since the ascorbic acid is highly unstable it has posed various technological challenges for its incorporation into different food systems. Liposomal Vitamin C provides a well-tolerated, easily absorbed and highly bioavailable form of Vitamin C without any unpleasant side-effects.

Objective

The objective of the study is to investigate the efficacy and safety of Liposomal Vitamin C (80 mg/5ml) in the prevention of acute viral RTI in children as indicated by the change in the total Wisconsin Upper Respiratory Symptom Scale-21-Korean version (WURSS-21-K) score (symptom score + quality of life score) within thirty days after developing the symptoms of URTI.

Materials and Methods

Study participants

Eligible 30 children were 4–12 years old, in good health, and with no history of chronic lung disease. Eligibility was based on the inclusion/exclusion criteria given below. Each participant was in the study until she/he developed a cold or completed a 30-day observation period without developing cold symptoms. Study data were collected from Nov 2019 through Jan 2020. The trial was approved by an Independent Ethics Committee and registered with Clinical Trial Registry of India (CTRI/2020/03/023825). Before enrolment, an informed written consent was obtained from the parents.

Study design

At enrolment, parents completed a study form that included demographic information, use of daycare >20 hours/week, school attendance and exposure to cigarette smokers. At enrolment, the parents were

Inclusion Criteria:

- 4-12 years
- Written informed consent by parents

Exclusion Criteria:

- 13 years or older, younger than 4 years
- Participation in a clinical study during past 30 days
- Intake of antimicrobial, antiviral or immunosuppressive substances
- Surgical intervention 3 months Prior to inclusion or planned intervention during the observation period
- Known type I Diabetes mellitus
- Known and treated atopy or Asthma
- Cystic fibrosis, bronchopulmonary dysplasia, chronic obstructive pulmonary disease (COPD)
- Diseases of the immunosystem (like autoimmune disorders, degenerative illnesses (like leucosis))
- Metabolic or Resorption disorders
- Liver or kidney diseases
- Serious health Problems (e.g. neurological Problems)
- Known allergies against Vitamin C or any of the substances of the investigational product.

given a cold symptom diary and asked to notify the Principal Investigator (PI) as soon as they thought that their child was developing a cold. The PI team telephoned parents of study patients every 3-4 days to remind them of the study procedures and ask about any cold symptoms in the child. If the parent indicated that the child had developed a cold, but that he/she had not contacted the study team, the patient was considered to have a "missed" cold and excluded from further analysis. Parents notified the PI team when they believed their child was developing a cold. The PI team specifically inquired about the presence of four respiratory symptoms: runny nose, nasal congestion, cough and sneezing. If they confirmed the child had one or more symptoms, he/she was considered to have a clinical cold. Once the PI determined that a study child had met criteria for a clinical cold, the parent was asked to begin completing the daily symptom diary. Participants were recruited in the study with clinical signs and symptoms of an URTI with a duration of up to 24 hours, accompanied by fever ≥37.5°C (axillary body temperature), with at least 1 of 3 types of URTI symptoms (nasal, pharyngeal or cough) and at least 1 of 5 general symptoms (feeling tired, weakness, body aches, whiney/irritable, or less active). A detailed physical examination was conducted during enrolment, after the onset of cold and every day thereafter till day 5, thereafter on Day 10, 20 and 30.

The scoring system used in the diary was based on 'The Wisconsin Upper Respiratory Symptom Survey (WURSS)' for Kids. The Wisconsin is an evaluative illness-specific quality of life instrument, designed to assess the negative impact of acute upper respiratory infection, presumed viral (the common cold). Long (WURSS-44) and short (WURSS-21) versions have been validated [6]. Symptom and fever resolution were defined as a combination of the criteria: (a) WURSS-21 item 1 "How sick do you feel today?" being graded as 0 ("not sick") or 1 ("very mildly") in both the morning and evening and (b) mean daily axillary body temperature ≤37.2°C. URTI symptoms were assessed using WURSS-21 which includes 21-item illness-specific symptom and health related quality-of-life questionnaire containing 1 global severity item, 10 symptom-based items, 9 functional quality-of-life items, and 1 global change item, each of which are rated on a 0 to 7 (Likert-type scale). The total score is used as a measure of symptom severity, and higher scores indicate higher severity of symptoms.

Once URTI is confirmed in children, the parents were advised to give the 2.5 ml of Liposomal Vitamin C (80 mg/5ml) liquid twice per day for 30 days. Liposomal Vitamin C liquid bottles were supplied by Shreepad Shree Vallabh SSV Phytopharmaceuticals, Mumbai. Dairies of WURSS-21-K were provided to participants and they are asked to record every question from day 1 to day 30. The parents continued to keep record of the scoring of the symptoms in these subject diaries provided to them. The parents were further contacted every day till 5th day, thereafter every 10th day for follow up.

Outcome Measures

Data from the questionnaires enquiring about the symptoms of URTIs will allow for the calculation of the following parameters:

- Total number of days with the symptoms of URTI
- Infection severity expressed as a sum of scores
- The most predominant symptoms
- The severity of different symptoms.

Further outcome measures were the time to the resolution of individual symptoms; the severity and course of the infection; the absence of fever; the amount and the treatment outcome as assessed by both the investigator and the children's parents, on the Integrative Medicine Outcome Scale (IMOS; complete recovery, major improvement, slight to moderate improvement, no change, deterioration); satisfaction with treatment on the Integrative Medicine Patient Satisfaction Scale (IMPSS; very satisfied, satisfied, neutral, dissatisfied, very dissatisfied) as assessed by the parents at the termination visit on day 30. The safety assessment took place by analysing the occurrence and nature of adverse events (AEs).

Results

Out of the 30 children, 26 children completed the study. The parents of these children complained of URTI. The common symptoms reported were sneezing, runny nose, nasal congestion, cough and sore throat. Within 4-5 days of starting of treatment with Liposomal Vitamin C, the score as indicated by the WURSS-21-K scale had reduced.

The average score in the criteria (a) i.e. containing 1 global severity item was 5-6 at baseline. By the end of fourth day the score reduced to 0-1. This score continued to remain steady throughout the study duration i.e. 30 days. There was no repeated episode of feeling sick following 4th day till 30th day. The average score in the criteria (b) i.e. 10 symptom-based items was 6-7 at baseline. By the end of third day the average score reduced to 1-2. There was no repeated episode of the symptoms like runny or plunged nose, sneezing, sore

or scratchy throat, cough, hoarseness, congestion in head and/ or chest and feeling tired from 3rd day till 30th day (as confirmed by the Physician). The average score in the criteria (c) i.e. 9 functional quality-of-life items was 4-5 at baseline. By the end of fifth day, the score reduced to 0-1. There was no difficulty in the ability to perform the activities which are interfered due to cold such as clear thinking, sleep, breathing, walking or climbing stairs, accomplish other daily activities along with indoor as well as outdoor activities, interaction or playing with other children.

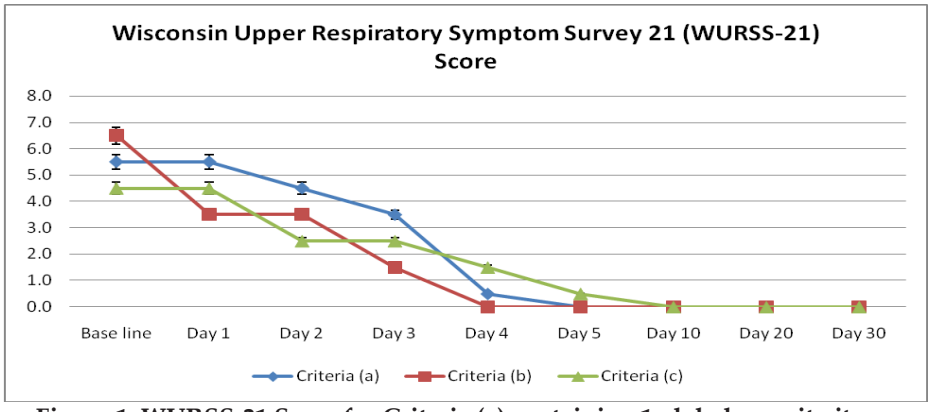

Figure 1: WURSS-21 Score for Criteria (a) containing 1 global severity item, Criteria (b) i.e. 10 symptom-based items and Criteria (c) i.e. 9 functional quality-of-life items.

The average condition in the criteria (d) i.e. change in global item was somewhat worse in 10 children, very much worse in 5 children and a little worse in 11 children. The 'somewhat worse' category reduced to 'very much better' category within 4 days in 10 children. The 'very much worse' category changed to 'very much better' within 5 days in 5 children. The 'little worse' category moved to 'very much better' category within 5 days in remaining 11 children.

Table 1 summarizes the average scoring in the criteria a to c from baseline to day 30 at various intervals in 26 children. These scores indicate that there was reduction in symptoms of URTI without any specific medication indicated for the same (Figure 1). The children continued to take the tablets for 30 days. During this period none of the children reported any symptom of URTI.

Based on the IMOS, the rating as noted by the physician was that there was major improvement in 10 children by day 4 and in remaining 16 children by day 5 (Figure 2). There was complete recovery till day 30. In the same scale as noted by the parents (Figure 3), there was major improvement in 12 children by day 4 and complete recovery in rest 14 children complete recovery was observed by day 5.

There was satisfaction with treatment as seen on the Integrative Medicine Patient Satisfaction Scale (IMPSS) assessed by the parents at the termination visit on day 30. Parents of 20 children were very satisfied and parents of 6 children were satisfied by the end of study (Table 2).

Table 1: The scoring in the criteria a to c from baseline to day 30 at various intervals in 26 children

	Base line	Day 1	Day 2	Day 3	Day 4	Day 5	Day 10	Day 20	Day 30
Criteria (a) (n=26)	5.50 ± 0.5	5.50 ± 0.6	4.50 ± 0.6	2.50 ± 0.8	0.46 ± 0.5	0	0	0	0
Criteria (b) (n=26)	6.54 ± 0.5	3.58 ± 0.5	3.46 ± 0.6	1.38 ± 0.6	0	0	0	0	0
Criteria (c) (n=26)	4.54 ± 0.5	4.42 ± 0.6	2.46 ± 0.7	2.42 ± 0.6	1.35 ± 0.6	0.50 ± 0.6	0	0	0
Criteria (d) (n=10)	Somewhat worse	a little worse	the same	Somewhat better	very much better	very much better	very much better	very much better	very much better
Criteria (d) (n=5)	very much worse	the same	Somewhat better	Somewhat better	a little better	very much better	very much better	very much better	very much better
Criteria (d) (n=11)	little worse	the same	Somewhat better	a little better	a little better	very much better	very much better	very much better	very much better

Criteria (a) containing 1 global severity item; Criteria (b) i.e. 10 symptom-based items; Criteria (c) i.e. 9 functional quality-of-life items and Criteria (d) i.e. change in global item

Values expressed as Mean ± SD; SD- Standard Deviation.

Table 2: Satisfaction assessed by IMPSS parents rating on Day 30

	(n=26)
Very satisfied	20
Satisfied	6
Neutral	0
Dissatisfied	0
Very dissatisfied	0

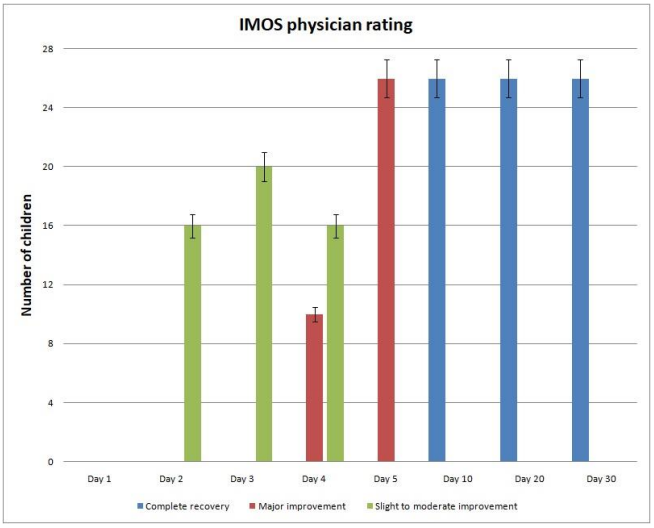

Figure 2: IMOS rating based on judgement by Physician

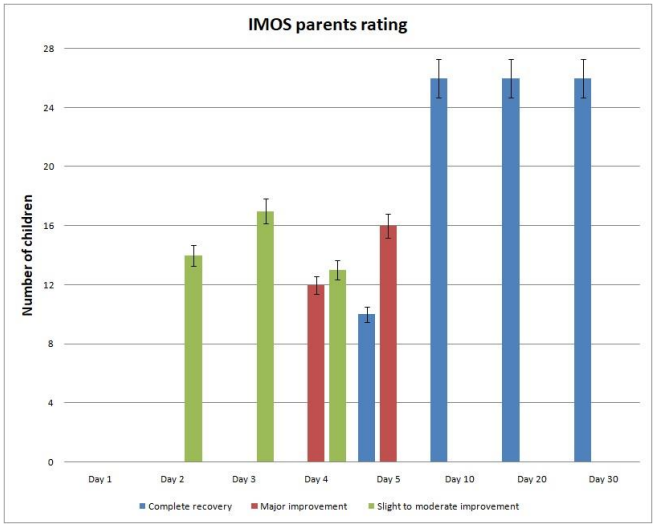

Figure 2: IMOS rating based on judgement by Parents

Discussion

Vitamin C is known to be an electron donor, and this property accounts for all its known functions. As an electron donor, vitamin C is a potent water-soluble antioxidant in humans. Antioxidant effects of vitamin C have been demonstrated in various published studies. Human diseases such as cancer and atherosclerosis may occur partially due to oxidant damage to tissues.

A high level of cytokines generally, and IL-6 particularly, is associated with decreased nitric oxide and increased reactive oxygen species, which lead to endothelial and microvascular dysfunction [7]. Accordingly, increased serum level of IL-6 stimulates the liver to synthesize and secrete the low-grade systemic inflammatory marker C-reactive protein (CRP) [8]. Through the production of reactive oxygen species, the inflammatory process may deplete stores of antioxidants, including vitamin C [9].

The literature suggests that the anti-inflammatory properties and antioxidant capacity of vitamin C can be attributed to their ability to modulate the DNA binding activity of nuclear factor-kappa B [10,11]. The activation is primarily promoted by oxidative stress and leads to cytokine-induced expression of cell adhesion molecules in the vascular endothelium, and to the TNF-α- and IL-6-induced production of CRP by the liver [12]. Jang *et al* [13] identified that vitamin C can reduce the plasma levels of the inflammatory mediators TNF-α and IL-6 via downregulation of hepatic mRNA expression. Table 3 highlights the role of Vitamin C on immune function.

In cross-sectional studies by Wannamethee *et al* [14] evaluating the relationship between inflammatory markers and blood levels of vitamin C, the plasma level of vitamin C was found to be associated with a significant reduction in risk of heart failure in men with and without pre-existing myocardial infarction. The traditional risk factors involving reduction of CRP explains the inverse association between plasma vitamin C and heart failure in men. In a study by Mohammed S Ellulu *et al* [15], it was shown that Vitamin C (500 mg

Table 3: Role of Vitamin C on various components of immune function

Sr. No.	Activity	Reference
1	Enhances antibody production	Feigen *et al*., 1982; Yamamoto et al., 1993; Azad et al., 2007
2	Improves the function of macrophages and phagocytic white blood cells	Mohammed et al., 2014; Bozonet *et al*., 2015
3	Increases interferon production	Karpinska *et al*., 1982; Kim *et a*l., 2016
4	Increases the proliferation of normal T-lymphocytes, while suppressing the proliferation of malignant T-lymphocytes	Huijskens *et al*., 2014; Molina *et al*., 2014; Uchio *et al*., 2015; Gao *et al*., 2017; Harakeh *et al*., 2017; Pangrazzi *et al*., 2017
5	Promotes the maturation of T-cells	Manning *et al*., 2013
6	Promotes the proliferation of B-lymphocytes	Schwager and Schulze, 1997
7	Promotes the proliferation of B-lymphocytes	Heuser and Vojdani, 1997

twice daily) has potential effects in alleviating inflammatory status by reducing hs-CRP, IL-6, and fasting blood sugar in hypertensive and/or diabetic obese patients.

Various epidemiological and some experimental studies, have shown that anti-scorbutic intake of vitamin C has antioxidant clinical benefit, though couldn't show any possible conclusive effects. There are many possible factors that may contribute to the failure to demonstrate the clear antioxidant benefits of vitamin C in clinical studies. A common problem is presented by the sigmoidal dose concentration curve for vitamin C. Small changes in oral intake of vitamin C produce large changes in plasma vitamin C concentrations. Various authors have suggested paying attention to the pharmacokinetics of orally administered vitamin C [16].

With increasing vitamin C intake, the plasma steady state concentration reaches a maximal level of about 70–80 μM [17,18]. From the available literature, it appears that a daily intake of about 200–400 mg of vitamin C ensures saturation of the blood in healthy individuals [19]. Due to temporary physiological needs such as pregnancy or increased turnover during disease or smoking, there is altered distribution leading to higher intakes to maintain sufficient levels of Vitamin C.

Various efforts by researchers have been attempted to bypass the maximum steady state plasma concentration of about 70–80 μM which is achieved through oral administration. An approach to increase the maximum achievable plasma concentration through oral administration has been via liposomes. Liposomal Vitamin C can be a solution to address the issue of pharmacokinetics of orally administered vitamin C. Liposomal Vitamin C provides a well-tolerated, easily absorbed and highly bioavailable form of Vitamin C without any unpleasant side-effects.

Liposomes are manufactured as microscopic, hollow spherical vesicles composed of a lipid bilayer. Liposomes are a very effective method of drug/supplement delivery when they are loaded with pharmaceuticals and/or dietary supplements. Once they are consumed orally, the pharmacokinetic properties of liposome in the intestinal absorption override the usual absorption pattern of the encapsulated drug. The delivery of the supplement with a typically slow or regulated pattern of absorption, such as vitamin C, is accelerated when encapsulated within a liposome [20,21]. Advantages of liposomal encapsulation include accelerated intestinal absorption, increased stability of the pharmaceutical, protection of the gut from potentially irritating agents, and greater bioavailability of the pharmaceutical [20].

The pharmacokinetic properties of a bolus of four grams of liposome-encapsulated Vitamin C were compared to those of plain vitamin C and placebo in eleven volunteers in a crossover trial [22]. The authors found a 35% increase in exposure (AUC_{0-4} hours) with a plasma C_{max} of about 200 μM after 3 h. In a single blind study, plasma levels were measured in two subjects, following ingestion of tablets of liposomal Vitamin C. The reported plasma levels were higher than is usually seen with oral administration of vitamin C [23].

In a study conducted by Parhizkar et al, it was shown that liposomal formulation of Vitamin C released about 90% vitamin C within 2 hours and showed higher (1.7-fold) in-vitro antioxidant activity. Ex-vivo antioxidant activity was 1.9 and 1.6 times higher in brain and liver cells, respectively [24]. In another study, after liposomal ascorbate intake, the average concentration of ascorbate in plasma was increased on 110% after 2 hours, reached maximum levels of 160% after 4 hours and decreased to the level 125% after 6 hours in comparison to non-encapsulated form of ascorbate where the maximum level of 170% increase was reached after 2 hours and then decreased to 90% and 80% at the 4 and 6 hours post-dose measurements respectively.

The experimental model of sepsis by cecal ligation and puncture of sepsis, shows that co-administration of ceftriaxone (A third generation Cephalosporin) and antioxidants (including Vitamin C) encapsulated in liposomes are the best way to reduce pulmonary damage induced by sepsis along with significant reduction the production of superoxide anions, lipid peroxidation, the formation of carbonyl groups and the mortality of animals. In addition, it attenuates the enzymatic activity of catalase and contributes to the restoration of the redox state in the lung [25].

In our study, with the intake of Liposomal Vitamin C by the children with symptoms of URTI, there was remarkable relief in the reduction of symptoms and also general well being with no recurrence of symptoms during the study duration. We however studied a smaller sample size of 30 children. Our study did not involve a control group for comparison.

Also in the current study no clinical or laboratory investigations were performed to ascertain the efficacy of liposomal Vitamin C on immune status. Instead, we used a validated scoring system like WURSS-21-K. This scale has been analysed and found that it supports the practice of using a simply summed daily global illness severity score to represent the overall symptom-

atic and functional impairments arising from URTI [26].

Conclusion

In this preliminary study, Vitamin C in liposomal form was effective in management of URTI in children. Liposomal Vitamin C was also well tolerated without any side effects. This suggests that Liposomal Vitamin C can be useful as an adjunct in the management of URTI to improve immunity. Further study with larger sample size and suitable clinical markers will be required for better understanding of the effect of Liposomal Vitamin C and its mode of action in boosting immunity in human beings.

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