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Resveratrol plus carboximethyl-β-glucan may affect respiratory infections in children with allergic rhinitis.

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To the Editor:

Social and economic costs of allergic rhinitis (AR) are impressive; AR may also significantly impact school attendance and performance, quality of life, and sleep (1).

The AR immunopathology is characterized by T-helper-2 (Th2)-dependent inflammation and Th1-response impairment. This imbalance is sustained by dysfunction of immune system: allergic patients are lacking in allergen-specific T regulatory cells, so T helper 2 cells may polarize the immune response to allergen and produce large quantity of some interleukins, including IL-4, IL-5, and IL-13, that in turn promote IgE synthesis and eosinophil production, recruitment, and activation at nasal level. Moreover, Th2-cytokines up-regulate the endothelial and epithelial expression of adhesion molecules (such as ICAM-1), which interact with other adhesion molecules (e.g. LFA-1 expressed by leukocytes) to induce the mucosal infiltration by eosinophil.

On the other hand, it is strengthened belief that allergic patients could present higher susceptibility to contract respiratory infections than non-allergic subjects. In this regard, it has been evidenced that allergic children have more numerous and severe respiratory infections than non-allergic children (2). The involved pathways may be: i) impaired immune defence because of Interferon-γ defective production (consequent to Th2-polarization), ii) overexpression of ICAM-1 (the main rhinovirus receptor) depending on allergen exposure, and iii) persistent mucosal inflammation as *pabulum* for microbes overgrowth (3). In addition, it is well known that AR is a main risk for asthma, both concerning the onset and its worsening. In other words, allergic reaction may be considered the promoter of a vicious circle characterized by different sequential steps: i) allergen exposure induces expression of adhesion molecules, ii) so allergic inflammation flares, iii) symptoms occur, iv) overexpression of ICAM-1 favours viral adherence, v) respiratory infections cause asthma attack. Therefore, adequate control of allergic inflammation in AR patients is

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important as it induces symptom relief and may prevent asthma worsening and infections recurrence.

The treatment of AR is generally based on medications, mainly concerning antihistamines and intranasal corticosteroids, which are usually effective, but may have adverse events that may limit their use, such as sedation, weight gain, and epistaxis. For these reasons, many parents opt for resorting to complementary medicine, such as phytotherapy. Indeed, it is settled the firm belief that natural cures, such as herbs, are effective and not detrimental in comparison with chemical compounds.

Resveratrol (*trans*-3,4,5-trihydroxystilbene) is a natural non-flavonoid polyphenol and belongs to a subclass of stilbenes. It is found in various fruits and vegetables and abundant in grape skin, it functions as a phytoalexin (a class of vegetal antibiotics) so protecting the plant from environmental stress or infections. Resveratrol is well known in Chinese and Japanese medicine, using a root of *Polygonum cuspidatum* in the preparation of Ko-jo-kon, a medication prescribed in several diseases. Resveratrol exerts a wide range of biological and pharmacological activities, including anti-infective, chemopreventive, anticarcinogenesis, cardioprotective, and anti-inflammatory ones (4). The anti-inflammatory effects of resveratrol depend on the inhibition of the transcription factor NF-kB, mainly inhibiting Ik-B kinase (5). NF-kB activation is the requisite for the expression of several pro-inflammatory proteins, such as IL-8, GM-CSF, COX2, and inducible nitric oxide synthase. In addition, resveratrol is able of inhibiting viral replication (6,7). In particular, a very recent study showed that resveratrol reduced the replication of rhinovirus (RV) on nasal epithelial cells and the RV-dependent expression of ICAM-1, the main RV receptor (8). Moreover, resveratrol provided anti-inflammatory and anti-asthmatic effects in mouse model of allergic asthma, as significantly reduced IL-4 and IL-5 in plasma and bronchoalveolar lavage fluid, and suppressed bronchial hyperreactivity, lung eosinophilia, and mucus hypersecretion (9).

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On the other hand, β-glucan is a polysaccharide, defined as biological response modifier, because has many immune-modulatory properties, including stimulation of phagocytosis by professional phagocytes, direct activation of NK cells and cytokine release. Previously, it has been reported that resveratrol combined with β-glucan exerted relevant *in vitro* synergistic effects on immune system (10).

On the other hand, no clinical study evaluated the capacity of resveratrol to prevent respiratory infections, thus the present double-blind, placebo-controlled, and randomized study aimed at investigating whether a spray containing resveratrol plus carboximethyl-β-glucan is able of: i) improving nasal symptoms, and ii) affecting respiratory infections in children with persistent allergic rhinitis (PER).

Globally, 76 children (males 45; mean age 9,5 years) were enrolled. Inclusion criteria were: i) age range between 4-17 years, ii) PER diagnosis according to ARIA criteria (1), iii) presence of allergic symptoms partially controlled by cetirizine, taken on demand, since at least one month before recruitment, iv) history of recurrent respiratory infections associated with wheezing, v) written informed consent signed by parents. Exclusion criteria were: i) chronic illness, ii) immune deficiency, iii) continuous use of medications (e.g. antihistamines, corticosteroids) in the last 4 weeks, and iv) concomitant use of immune-stimulants.

Patients were randomly (1:1 ratio) subdivided in two groups: placebo-treated (Group A) and actively-treated (Group B). Active medication was an isotonic solution in spray containing resveratrol 0.05% (extracted by *Polygonum cuspidatum*) and carboximethyl-β-glucan 0.33%. This spray is available as Class I EC Medical Device (manufactured by Noos, Rome, Italy). Placebo was an isotonic solution in spray, such as the diluent of active drug. Patients were instructed to apply 2 sprays (100 μL/spray) for nostril 3 times/day for 2 months. Cetirizine syrup (1 drop/3Kg/bw) was

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permitted as rescue medication along the treatment period.

The study was performed during the fall: season usually characterized by worsening of allergic symptoms (because of high levels of exposure to mites) and recurrence of respiratory infections (frequent in this period).

Nasal symptoms (itching, sneezing, rhinorrhea, and obstruction) were scored using a four-point scale (0=no symptom; 1=mild symptom; 2= moderate; 3= severe) recorded by parents on a daily diary card. Sum of these symptoms was calculated and expressed as Total Symptom Score (TSS). In addition, the severity of cough (assessed during the day and the night) and wheezing was assessed using the same four-point scale. The on demand use of bronchodilators (e.g. inhaled salbutamol) was recorded as number of puff. The days with fever and/or with antibiotic treatment were also considered as well as the number of school absences and emergency room visits for respiratory problems.

The Patients were visited at baseline (T0) and after the treatment (T1); all above-mentioned parameters were evaluated during both visits. Adverse events were as usually registered. The study protocol was approved by the Ethics Committee of the Second University of Naples and registered (ClinicalTrials.gov ID NCT02130440).

Seventy-three children completed the study, the treatments were well tolerated and no significant adverse event was reported.

At baseline, symptom severity (evaluated as TSS) and all considered parameters were similar in the 2 groups, so they were homogeneous (Figure 1,2 and 3). Actively-treated children reported a significant reduction of TSS ($p<0.0001$), whereas placebo group did not achieve an improvement of

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nasal complaints. However, the intergroup analysis showed that active treatment had a more evident reduction of AR symptoms in comparison with the control group.

Resveratrol plus carboximethyl-β-glucan treatment significantly reduced: cough severity, both during the day (p=0001) and the night (p=0.0001), wheezing intensity (p=0.003), short acting β2-agonist use (p=0.003), as reported in Figure 2. Placebo treatment did not change these parameters. The intergroup analysis confirmed the intragroup one as reported in Figure 2.

In addition, actively-treated children had significantly less days with fever (p=0.002) and school absences (p=0.04); whereas antibiotic use and emergency room visits had not changed (Figure 3). Placebo treatment did not modify any parameter. The intergroup analysis confirmed the intragroup findings, as shown in Figure 3.

Allergic reaction is characterized by Th2-polarized immune response and mucosal inflammation: both events predispose to infection susceptibility. Indeed, Th2-polarization determines a relative functional defect in Interferon-γ production: cytokine deputed in fighting infections. Moreover, mucosal inflammation represents a condition allowing a better catching on by microbes. In addition, allergic subjects overexpress ICAM1, an adhesion molecule involved in inflammatory events as ligand of LFA1, expressed on leukocytes. ICAM1 is also the main receptor for rhinovirus: frequently involved in asthma exacerbations. It is also to underline that the relationship between allergy and respiratory infections is of considerable interest as there are few studies over this topic and findings are conflicting.

The present study demonstrates that intranasal resveratrol plus carboximethyl-β-glucan spray was able of significantly reducing nasal symptoms in children with persistent allergic rhinitis. In addition, this treatment was also able to significantly affect some aspects concerning the outcome of respiratory infections occurring in these subjects. The possible explanations of its efficacy may

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depend on the anti-inflammatory and anti-viral mechanisms, exerted by resveratrol in experimental studies, and on the immune-modulation and osmotic activities provided by glucan.

On the other hand, it is to note that control group continued to be anyhow treated with on demand antihistamine, started before the study beginning. This fact should explain the lack of significant change of evaluated parameters observed during the study period.

The main shortcomings of this study are: the lack of data concerning immunological parameters and allergic inflammation, the limited number of enrolled patients, and the absence of an adequate follow-up. For these reasons, further studies should be designed to answer to these issues.

In conclusion, the present study provides the first evidence that a spray containing resveratrol plus carboximethyl-β-glucan is able of relieving nasal symptoms and respiratory infections *sequelae*. Thus, this outcome could suggest that one course of this compound, prescribed during the risky season, such as fall, might prevent respiratory afflictions, both allergic and infectious.

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Figures legend

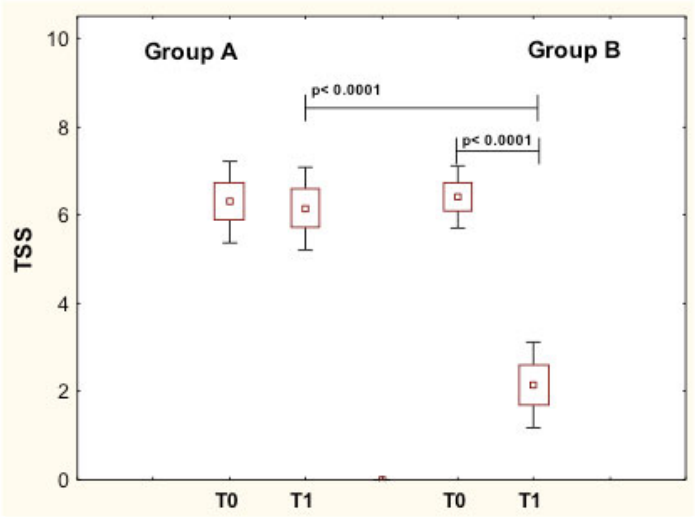
Figure 1: Total Symptoms Score (TSS) in Group A (placebo) and B (active treatment) assessed before (T0) and after (T1) treatment.

Figure 2: Respiratory complaints in Group A (placebo) and B (active treatment) assessed before (T0) and after (T1) treatment. A= cough at day; B= cough at night; C= wheezing; D= β2-agonist use.

Figure 3: Respiratory infections problems in Group A (placebo) and B (active treatment) assessed before (T0) and after (T1) treatment. A= antibiotic use; B= fever; C= school absences; D= emergency visits.

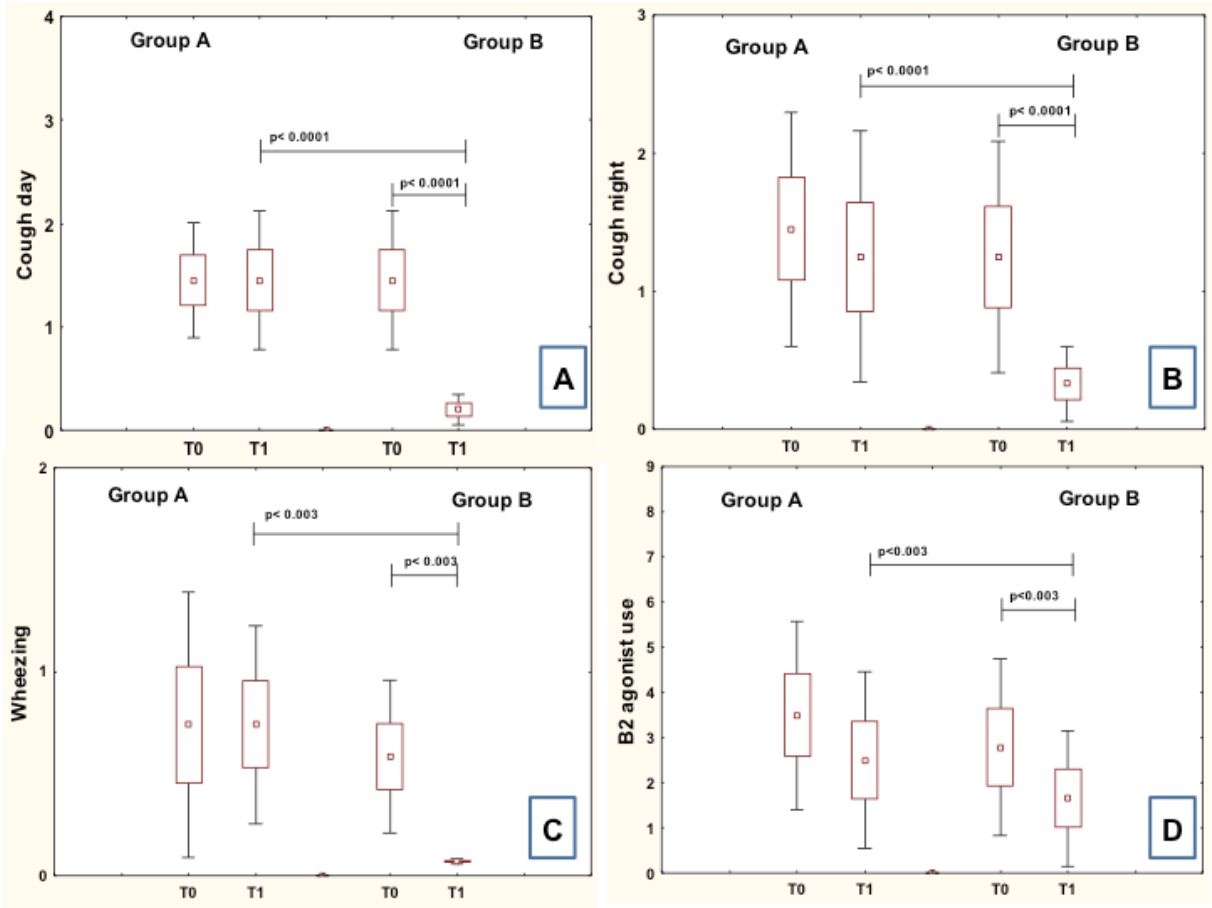
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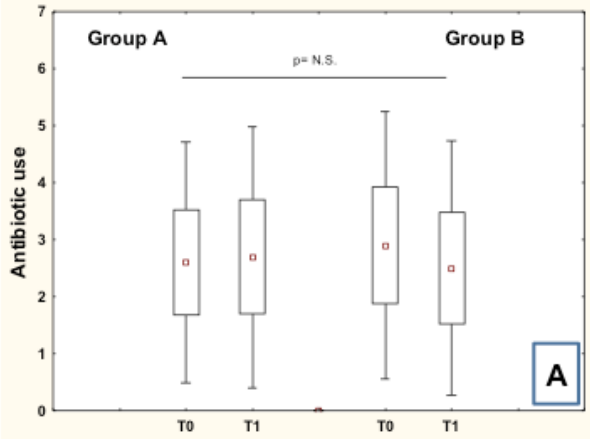
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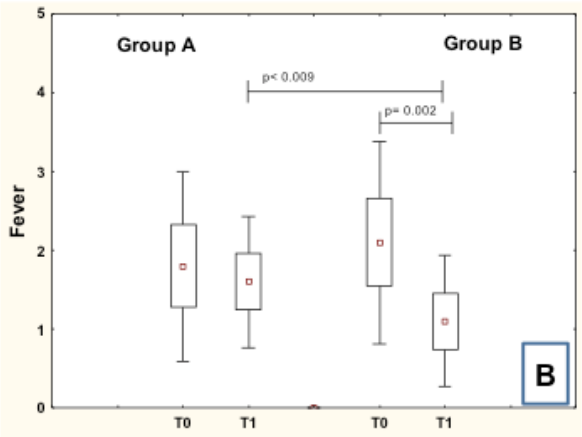
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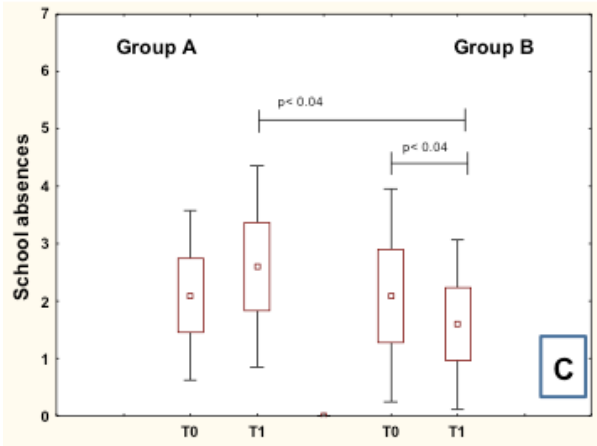


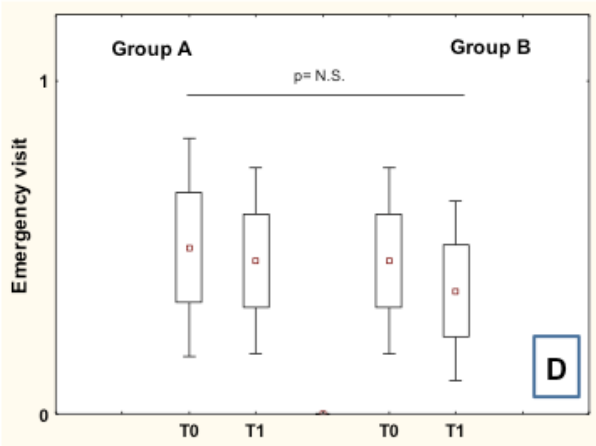
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