Evaluation of PHR160 Spray Effect on Improvement of Lung Function, Asthma Severity and Exacerbation in Severe Asthmatic Patients

Mehrdad Dargahi-Malamir[1], Seyed Hamid Borsi[1], Zahra Mehraban[1], Fatemeh Kianizadeh[2], Hoda Mohsenikia[2]

[1] *Department of Pulmonology, Air Pollution and Respiratory Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[2] *Department of Internal Medicine, Faculty of Medicine. Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

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Abstract- Pinen Hydronoplacton Ribonucleic acid (PHR160) medicine contains compounds that can be useful in the recovery of respiratory patients. The aim of this study was to determine the effect of PHR spray on improving lung function, severity and asthma attacks in patients with severe asthma. This study is a pioneering interventional study (pilot study) conducted during the years 2021-2022 on patients with severe asthma resistant to treatment who referred to the lung clinic of Imam Khomeini, Golestan Hospitals and the private practice of lung specialists of this academic center. The study includes two groups of patients with asthma, both groups were given the usual treatment according to the stage of the disease, in addition, the intervention group was given two puffs of PHR spray every eight hours, and the control group was given a placebo spray with the same dose. Before and after the intervention, GSK 2002 questionnaire, six-minute walk distance (6MWD) and spirometry tests were completed. Among of 60 patients, 27 (45%) were male. The mean age of the patients was 44.33±6.94 years. Based on findings, the forced vital capacity (FVC) and forced expiratory flow between 25% and 75% (FEF 25-75%) were significantly better in the intervention group than the control group (P<0.001 and P=0.019, respectively), but there was a statistically remarkable difference between the two groups in terms of forced expiratory volume (FEV1) and FEV1/FVC (P=0.505, P=0.575, respectively). In addition, the GSK questionnaire score in the intervention group was higher than the control group (P<0.001), however there is no significant difference between the two intervention groups in terms of the 6MWD test and the number of exacerbation (P=0.114 and P=0.09, respectively). It is generally concluded that PHR160 spray can lead to improvement of spirometry parameters and severity of disease in severe asthma patients by affecting small airways.

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Keywords: Asthma; Sever asthma; Spirometry volume; Six-minute walk distance (6MWD)

Introduction

Asthma is a chronic inflammatory disorder of the airways leading to wheezing, cough, shortness of breath, and chest tightness(1-3). Severe asthma is defined as "asthma which requires treatment with high dose inhaled corticosteroids (ICS) in addition to a second controller (and/or systemic corticosteroids) to prevent it from becoming 'uncontrolled,' or which remains 'uncontrolled' despite this therapy (4,5). More than 10% of adults with severe asthma experience reduced quality of life, increased risk of persistent airflow limitation, exacerbations, health care resource use, hospitalization, and death (6,7).

In the process of airway inflammation of patients with asthma, immunological cells such as mast cells, eosinophils, lymphocytes and neutrophils are involved (8). Eosinophils preferentially accumulate in the sites of allergic inflammation and release a variety of inflammatory mediators, including oxygen radical species and inflammatory cytokines, which play an important role in the pathophysiology of asthma (9,10).

Corresponding Author: H. Mohsenikia
Department of Internal Medicine, Faculty of Medicine. Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
Tel: +98 9163409681, E-mail address: medmosahneh@gmail.com

In traditional medicine, some substances with anti-inflammatory properties have been used for the treatment of respiratory disorders (9).

After the COVID-19 pandemic, Jaber Ebne Hayyan pharmaceutical company presented the Pinen Hydronoplacton Ribonucleic acid (PHR160) spray, which was investigated for the effectiveness of this spray on the clinical symptoms of COVID-19.

PHR160 medicine contains the combination of cineole, menthol, saffron, safranal, crocin, Alpha Tojun imol, oleic acid, inoleic acid, and linolenic Acid. Saffron and its active components such as safranal and crocin have anti-inflammatory, antioxidant and muscle relaxing effects (11,12). Another ingredient of this spray, menthol, shows its anti-inflammatory effect by inhibiting pro-inflammatory enzymes and cytokines through the activation of nuclear factor kappa B (NF-kB) and p38 mitogen-activated protein kinase (MAPK) pathways (13). Moreover, the effect of cineole is known due to its mucolytic, spasmolytic effect in the respiratory system, and reducing airway inflammation (14). Each of the PHR160 compounds has useful properties in the treatment of respiratory diseases, which can be named as anti-inflammatory, antioxidant properties, and bronchodilator (15,16). Due to the characteristics of these compounds, it can be useful in improving the severity of respiratory diseases.

However, no studies have been conducted on the effectiveness of this spray in asthmatic patients. Therefore, this study was conducted in order to determine the effect of the PHR160 spray on improving lung function, severity and disease attacks in patients with severe asthma.

Materials and Methods

This pioneering interventional study was conducted on 60 patients suffering from treatment-resistant severe asthma who referred to the lung clinic of Imam Khomeini and Golestan Hospitals in Ahvaz, Iran in 2022. Inclusion criteria were patients with severe asthma based on GINA criteria (forced vital capacity, FEV1 <60%), and their symptoms were not controlled despite routine initial treatments (long-acting beta-agonists, high-dose inhaled corticosteroids, and inhaled anticholinergics and oral corticosteroids). Pregnant women, patients with kidney and liver failure, patients with allergies or contraindications to herbal compounds such as menthol, saffron, crocin and safranal were excluded.

Before entering the patients into the study, concomitant diseases such as reflux, sinusitis, allergic rhinitis and other diseases that can affect the course of response to asthma treatment, history and examinations were obtained and if necessary, complete treatment of concomitant diseases was carried out.

Eligible people were randomly divided into two groups of 30 patients. Patients of both groups were given treatment according to the stage of the disease, medium to high dose inhaled corticosteroid, long-acting beta-agonist, inhaled anticholinergic and oral corticosteroid 5 mg. The inhaled anticholinergic included ethidium bromide with a daily dose of 18 micrograms, and the inhaled corticosteroid was fluticasone and their beta agonist was salmeterol. The first group was given two puffs of PHR160 spray for 8 hours, and the control group was given a placebo spray with the same dose. Metabolism of PHR160 is hepatic, and it is excreted through kidneys and feces. By inhalation, it is gradually absorbed from the bronchi and part of the swallowed medicine is also absorbed from the digestive system. The systemic concentration of the PHR160 after inhalation is very low. The effect of the medicine starts after 5-15 minutes and reaches its maximum after 30 minutes after a deep inhalation. The placebo was completely similar to the PHR160 spray in terms of appearance, color, smell and size. Before the start of the study, the patients performed a spirometry test, six-minute walk distance (6MWD) test, and the GSK-2002 questionnaire were completed. Also at the end of the study (after one month), GSK-2002 questionnaire, spirometry and 6MWD tests, were checked and compared to before the start of the study.

Ethical considerations

The ethics committee of Ahvaz Jundishapur University of Medical Sciences was approved this study. Moreover, the written informed consent was obtained from each patient.

Statistical analysis

Statistical analysis was accomplished by SPSS software version 26. The quantitative and qualitative variables were indicated as mean±SD and number (percentage), respectively. Kolmogorov–Smirnov and, Shapiro-Wilk tests were used to test for the distribution. Differences were compared by using the t-test or Mann–Whitney U test as appropriate. Also, paired t-test and Wilcoxon test were used to compare the variables before and after the intervention. Covariance analysis was used to adjust the influence of confounding variables. *P* less than 0.05 was considered statistically significant.

Results

The mean age of patients in the intervention and control groups was 45.13±6.24 and 43.53±7.59 years, respectively, and no significant difference was observed between the two groups (*P*=0.376). In the intervention and control groups, 50% and 60% of the patients were female (*P*=0.346). According to the results which is provided in Table1, there are no significant differences between two groups (*P*>0.05). So, they are homogenous before the intervention in aspect of spirometry tests.

Table 1. Baseline and clinical characteristics of two groups

Variables	Intervention	Control	*P*
Age (year) (mean±SD)	45.13±6.24	43.53±7.59	0.376
Sex (Female),%	50	60	0.346
FEV1 Vol	1046.00 ± 191.10	1034.83 ± 132.06	0.79
FEV1 per	40.33 ± 12.39	41.63 ± 1.92	0.57
FVC vol	1265.43 ± 542.29	1162.26 ± 160.84	0.32
FVC per	57.00 ± 13.29	55.83 ± 3.54	0.64
FEV1/FVC	69.73 ± 7.32	67.23 ± 3.45	0.09
FEF25-75 vol	667.10 ± 110.74	670.86 ± 84.40	0.88
GSK	12.93 ± 4.20	11.66 ± 3.04	0.18
6MWD	167.31 ± 47.30	157.03 ± 11.09	0.25
Exacerbation	2.44 ± 1.96	2.40 ± 1.04	0.92

The FEV1 volume in the intervention and control groups was 1409.40±606.26 and 1258.17±411.21, respectively (*P*=0.279). The FVC volume in the intervention and control groups was 1987.93±929.65 and 1186.33±173.79, respectively, and there was a remarkable difference between the two groups (*P*<0.001). There was no difference in FEV1/FVC between the two groups (*P*=0.575). The percentage of FEF 25-75 in the intervention group was significantly higher than the control group (*P*=0.019). More detailed are provided in Table 2.

Table 2. Results of spirometry test after intervention in two groups

Variable	Group	Mean±SD	*P*
FEV1 Volume (ml)	**Intervention**	1409.40±606.26	0.279
	Control	1258.17±411.21	
FEV1 (%)	**Intervention**	46.67±14.14	0.505
	Control	48.73±9.02	
FVC Volume (ml)	**Intervention**	1987.93±929.65	<0.001
	Control	1186.33±173.79	
FVC (%)	**Intervention**	65.8±15.05	<0.001
	Control	56.1±4.01	
FEV1/FVC	**Intervention**	71.3±6.91	0.575
	Control	65.93±7.44	
FEF 25-75 (%)	**Intervention**	25.06±13.71	0.019
	Control	22.8±6.6	

FEV1: Forced expiratory volume (FEV1), FVC: forced vital capacity, FEF 25-75 (%): forced expiratory flow between 25% and 75%.

The score of the GSK questionnaire in the intervention group was 15.0±4.16, and it was significantly higher than the control group (*P*<0.001). Moreover, the 6MWD test did not show any significant difference between the two groups (*P*=0.114). The number of exacerbation attacks in the intervention group was 1.61±1.74, and at the end of the study, no considerable difference was observed between the two groups (*P*=0.09) (Table 3).

Table 3. Results of GSK questionnaire, 6MWD test and number of exacerbations of the disease after the intervention

Variable	Group	Mean±SD	*P*
GSK questionnaire	Intervention	15.0±4.16	<0.001
	Control	7.43±1.43	
6MWD test	Intervention	169.2±33.84	0.114
	Control	155.5±10.7	
Number of exacerbations of the disease	Intervention	1.74±1.61	0.09
	Control	2.2±0.76	

GSK: GlaxoSmithKline, 6MWD: six-minute walk distance.

Discussion

The present study was conducted with the aim of investigating the effect of spray on improving lung function, severity and disease attacks in patients with severe asthma. Our results showed that the effect of the spray on FVC and FEF 25-75 was significantly better in the intervention group than in the control group, but no difference was observed between the two groups in terms of FEV1 and FEV1/FVC. These findings indicate that the PHR160 spray has no impact on large airways, but it can affect small airways.

To the best of our knowledge, no study has been conducted to investigate the effectiveness of the PHR160 spray on the clinical improvement of patients with asthma. PHR160 spray contains compounds such as cineole, menthol, safranal, saffron, crocin, Alpha Tojun imol, oleic acid, inoleic acid, and linolenic Acid, and the presence of these compounds is useful for improving respiratory patients. Most of the studies conducted on the effectiveness of these compounds are animal studies.

Chemically, cineole is a terpenoid oxide that is the main component of various eucalyptus species with known clinical effects. Eucalyptus oil is known for its biological effects, including anti-inflammatory, antioxidant, mucolytic, bronchodilation, antimicrobial effects, and inhibition of free radicals, which have been suggested for the treatment of respiratory diseases (17).

Active components of saffron such as safranal and crocin also have anti-inflammatory and antioxidant effects and play a useful role in the treatment of asthmatic patients (11,18). In Xiong *et al*.,'s study, crocin significantly reduced airway inflammation, levels of interleukin 4, 5, and 13, tryptase in bronchial alveolar lavage fluid (BALF), lung eosinophil peroxidase, and serum ovalbumin-specific IgE (19). The results of the study by Yusri et al. showed that treatment with crocin reduces oxidative stress biomarkers, strengthens antioxidant defenses, and restores pro-inflammatory cytokines to normal levels (20). In study by Bukhari *et al*., treatment with saffron and its compounds, including safranal and crocin, leads to a reduction in the level of nitric oxide and inos, reduction of peroxynitrite ion production and prevention of cytochrome c secretion, and safranal treatment reduces airway hyperreactivity and cellular infiltration in the lungs (21). In addition, it was observed that saffron supplementation in guinea pigs suffering from allergic asthma also reduces the serum level of the inflammatory index of endothelin 1 (22). It has been reported that safranal reduces the level of IgE in the serum and the number of mast cells in the lung tissue, inhibits the degranulation of mast cells, and prevents the production of IL-6 and TNF-α. It also inhibits the signaling pathway of NF-kB and MAPKs and reduces the levels of histamine and leukotriene 4 in the serum (23). In study by Hosseini *et al*., the effect of saffron supplementation in comparison with placebo significantly reduced the hs-CRP and anti-HSP70 concentrations in patients with allergic asthma. Also, in spirometry test, FEV1, FVC, FEV1/FVC ratio and FEF25-75% increased significantly in saffron in comparison to placebo group (24).

Metabolic effects of conjugated linoleic acids lead to fat loss and modulation of adipokines, which is useful in reducing systemic inflammation. It also directly reduces airway inflammation by a variety of peroxisome proliferator-activated receptor gamma (PPAR-γ) dependent and non-dependent mechanisms (25), but very limited studies have been conducted in this field. In the study of Millqvist *et al.*, patients with chronic cough, inhalation of menthol had an effect on inspiratory flow, and they suggested the use of menthol as a reliever for various types of airway disorders (26).

Current study has strengths and weaknesses. Limitations of the study included small sample size and short duration of spray application. The strength was that for the first time the effect of PHR160 spray was measured in patients with asthma.

We concluded that PHR160 spray can lead to

improvement of spirometry parameters and severity of disease in severe asthma patients by affecting small airways. It is suggested to conduct more studies with higher sample size in order to investigate the effectiveness of the PHR160 spray on the severity of asthma, and also to determine the possible side effects of this spray.

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