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Effectiveness of Combined Therapy with Pirfenidone and Selenium for Idiopathic Pulmonary Fibrosis: An Observational Case–Control Study

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Abstract: **Background and objectives**: Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease (ILD) that is very fatal due to its unpredictability, various symptoms, irreversible complications, and poor prognosis. Oxidative stress is an important factor in the pathogenesis of IPF. Therefore, the use of antioxidant compounds can be a good option for the treatment of pulmonary fibrosis. Since selenium has antioxidant effects, the aim of this study was to evaluate the effectiveness of combination therapy with pirfenidone and selenium supplementation in patients with idiopathic pulmonary fibrosis.

Methods: The study was an observational study. Ten patients who were referred to Hazrat-e-Rasoul Hospital were recruited. According to the inclusion and exclusion criteria, patients were randomly divided into two equal groups (5 patients in each group) including treatment group who in addition to pirfenidone (200 mg three times a day), also received selenium supplement (200 mg once a day) orally, and the control group, who received just pirfenidone (200 mg three times a day) orally. The duration of this study was 24 weeks. At the beginning of the study, at 12 weeks and 24 weeks, respiratory indices including FVC, FEV, PO2 and FEF25 were measured.

Results: Ten patients were able to complete the experimental procedures. The results showed that treatment with selenium supplementation did not cause a significant change in any of the mentioned variables.

Conclusion: It seems that more studies are needed to reveal the different aspects of the role of selenium supplementation in patients with fibrosis. It is recommended that future studies be performed with a larger sample size. Also lung imaging and evaluating the inflammatory factors are recommended as well.

Keyword: Pirfenidone; Idiopathic Pulmonary Fibrosis; Selenium, Interstitial Lung Diseases

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1. Introduction

Idiopathic pulmonary fibrosis as an interstitial lung disease (ILD) is fatal due to unpredictability, various symptoms, irreversible complications, and poor prognosis. It accounts for about 20 to 30 percent of lung interstitial tissue diseases. It is usually a progressive disease, leading to respiratory failure and eventual death. The patients usually die 3 to 5 years after the initiation of the symptoms of the disease. The disease mainly affects men

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(13 per 100,000 for women and 20 per 100,000 for men) (1,2).

Known factors in the incidence of this disease include smoking, inhalation of environmental toxins (3), radiotherapy (1), gastroesophageal reflex (4), autoimmune diseases, connective tissue diseases such as rheumatoid arthritis (5), viral infections due to some herpes viruses and bacteria, damage caused by some mineral compounds such as asbestos and silica, as well as side effects of some chemical drugs such as bleomycin and methotrexate (1, 6) and finally the genetic background (3). If the cause of the disease is known, it is called pulmonary fibrosis (PF), and if no known cause is identified, it is called idiopathic pulmonary fibrosis (IPF).

Although, the exact molecular and cellular mechanisms involved in IPF remain unknown, various pathways such as inflammation, oxidative stress, apoptosis and imbalance between proteolytic and anti-proteolytic enzymes in the pathogenesis of pulmonary fibrosis have been proposed (7).

Nintendanib is currently the approved drug for the treatment of pulmonary fibrosis in the United States and Europe (8, 9) which probably affects the growth factor of platelet and reduces the disease progression (10). Another drug approved by the FDA is pirfenidone, which its anticystic fibrosis properties has been showed in *in vitro* studies. Pirfenidone has an anti-fibrotic effect through inhibition of the TGF-dependent collagen synthesis, reduction of the extracellular matrix production and inhibition of the fibroblasts. It also reduces the accumulation of hydroxyproline in the lungs. It has been shown that pirfenidone reduces the activity of superoxide dismutase and which indicates the anti-inflammatory and antioxidant effects of the drug (11). The results of phase 3 clinical studies show that pirfenidone significantly reduces disease progression and also improves patient survival (12, 13).

Because pirfenidone does not completely cure the disease, research continues to find drugs with fewer side effects. One of the possibilities in this regard is antioxidants such as selenium, which have been used in the treatment of various diseases. Selenium is a rare and essential element for humans and animals (14). It plays an important role in regulating the function of thyroid hormones, metabolism, growth, fertility, immune system health (15, 16) and the body's antioxidant defense system.

Selenium supplements are effective in reducing the incidence of lung, liver and colon cancers (17). The enzyme didinase, which plays a role in the synthesis of thyroid hormones in the thyroid gland, is a selenium-dependent enzyme. In addition, the enzyme GPx3 in the thyroid gland protects thyroid cells against hydrogen peroxide (18). Numerous studies showed that selenium supplementation is effective against autoimmune thyroid disease (Hashimoto's thyroiditis) (19). Valenta et al. In a study showed that taking a high dose of selenium supplementation for 14 years in patients with systemic inflammatory response syndrome (SIRS) reduces C reactive protein levels (20).

Since selenium has antioxidant effects and the use of antioxidant compounds to increase the activity of antioxidant enzymes could be a good treatment option for the treatment of pulmonary fibrosis, therefore, the aim of this study was to evaluate the effectiveness of combination therapy with pirfenidone and selenium supplementation in patients with idiopathic pulmonary fibrosis.

2. Material and method

The present study was an observational study. The aim of the study was to evaluate the effect of combined use of selenium supplement with pirfenidone on the progress of pulmonary fibrosis in IPF patients. Traditional sample size determination was used to ensure specific power to detect targeted treatment effects. Moreover, no clinical data existed on effects of combination therapy of selenium and pirfenidone in humans or patients with IPF. Therefore, pilot trial sample size estimation ($n = 5$) was based on medium standardized effect sizes (0.5) for a main trial designed with 90% power and two-sided 5% significance.

The study was conducted at the clinical research units of Hazrat-e-Rasoul Hospital. Therefore, 10 patients with stable IPF were primarily recruited. All study procedures complied with the declaration of Helsinki and informed consent and study documents were approved by the institutional review boards and were reviewed by research ethic committee (IR.IUMS.REC 1395.95-03-30-29436). After selecting the individuals according to the inclusion and exclusion criteria and justifying them from different aspects of the plan, the nature, benefits, and risks of the study were explained to the patient volunteers and written informed consent was obtained prior to participation. The age range of the patients was 40 to 70 years and had BMI within the range 19-35 Kg/m2. Eligible participants had no lung transplant, pulmonary hypertension confirmed by echocardiography or heart catheterization, myocardial infarction, angina, hospitalization for cardiac etiology, stroke or transient ischemic attack in the past 6 months, chronic heart failure, current or chronic history of liver disease, neurologic condition, drug abuse, kidney diseases, Vascular, hepatic, parathyroid, autoimmune and infectious diseases, and cancer in previous 5 years. Participants were not taking anti-arrhythmic medications known to cause QTc prolongation or Coumadin or other anti-platelet or anti-coagulant medication. They used no treatment substances other than pirfenidone to treat idiopathic fibrosis. Smokers and pregnant women and women during breastfeeding were also excluded from the study. All subjects entered the experiment based on personal consent and were excluded from the experiment if they did not wish to continue during the experiment.

Participants were randomly divided into two equal groups

(5 in each) including:

1- Treatment group: The group that in addition to pirfenidone (200 mg three times a day), also received selenium supplement (200 mg once a day) orally.

2. Control group: The group that received just pirfenidone (200 mg three times a day) orally.

The duration of the study was 24 weeks. At the beginning of the study, at 12 weeks and 24 weeks after the onset of the study, respiratory parameters including FVC, FEV, FEF25 and PO2 were measured.

Pirfenidone was prescribed by pulmonary specialist. Selenium was also recommended to take once daily by the specialist.

Pirfenidone (CIPLA company, India) and selenium (Nature Made brand) were purchased from a pharmacy and delivered to the subjects. One selenium tablet and three pirfenidone tablets were taken daily in the treatment group and three pirfenidone tablets daily in control group.

In order to maximize the level of follow-up of the treatment, the participants received reminders by phone call. In addition, reminders were done through cyberspace. Individuals were also asked to return unused drugs. The experiment lasted for 24 weeks.

2.1. The ethical points

In order to comply with the ethical principles, a written consent form was obtained from the eligible volunteers participating in the study. The subjects were excluded from the study at any stage of the study if they did not wish to cooperate. The test results were provided to patients free of charge.

2.2. Statistical analysis of data

Data are presented as mean ± SD. Statistical analyses were performed through SPSS software v.24, IBM. Prior to primary analysis, normality of each variable was assessed with the Kolmogorov-Smirnov test and by visual analysis of histograms and plots for skew/kurtosis. In order to compare the lung capacity, parametric / non-parametric inferential statistical methods were used to compare the means of each independent group (t test). The α-level for significance was 0.05.

3. Result

As shown in the graphs, administration of selenium did not cause a significant change in any of the mentioned variables (Figures 1,2,3,4 and 5).

4. Discussion

Idiopathic pulmonary fibrosis is a interstitial lung tissue disease that is fatal due to its unpredictability, variation in symptoms, irreversible complications, and poor prognosis. Due to the lack of uniform definitions of the disease in different studies and different methods of diagnosis, the exact prevalence of the disease is not known (1, 2). Clinical characteristics of individuals, radiographs and biopsies are used to diagnose the disease (21-23). Unfortunately, despite all the advances in the definitive diagnosis of this disease, it usually occurs 6 to 24 months after the onset of symptoms, when it affects the lungs (24, 25).

Although the exact molecular and cellular mechanisms involved in IPF remain unknown, the role of pathways such as oxidative stress is an important factor in the pathogenesis of IPF (7, 26). Therefore, it seems that to increase the activity of antioxidant enzymes, the use of antioxidant compounds can be a good treatment option for the treatment of pulmonary fibrosis. Despite advances in understanding the pathogenesis of pulmonary fibrosis, treatment options are currently limited. Pirfenidone is one of the main drugs used in the treatment of IFP which is approved by the FDA. Pirfenidone is an anti-fibrosis drug that has been shown in *in vitro* studies to have antipyretic properties (11). Because, pirfenidone does not completely cure the disease, research continues to find drugs with fewer side effects. One of the possibilities in this regard is antioxidants such as selenium (14). Selenium is a key component of a number of functional selenoproteins and exerts its effects by participating in the construction of selenoproteins (27). The most important known use of this element is its role in the construction of antioxidant enzymes such as glutathione peroxidase (GPx), which is the main enzyme in removing the reactive oxygen species (28). Selenium has antioxidant effects and the use of antioxidant compounds could be a good option for the treatment of pulmonary fibrosis. Therefore, this study was performed to evaluate the effectiveness of combination therapy of pirfenidone and selenium supplementation in patients with idiopathic pulmonary fibrosis.

The present study was performed on 10 patients with IPF who were randomly divided into two equal groups. Both groups of participants received pirfenidone as the main choice of treatment, but one of the groups was given selenium supplement in addition to pirfenidone. There was not significant differences between the groups which received selenium and the control group. According to the conditions of this experiment, selenium did not change the amount of factors examined in this experiment and the level of FEV, FVC, FEV / FVC and FEF25 was the same as control group. Of course, one reason for the insignificant results could be the small number of patients who entered the experiment. Unfortunately, due to the coincidence of the time of this study with the pandemic of covid-19, which affects the respiratory system, it was very difficult to find patients to enter the study, and many patients refused to continue in the middle of the experiment. Nevertheless, the time of the drug administration was regularly reminded to patients and/or family members through phone calls and text messages, some of them regret to use supplementation regularly. And, since the patients were

not hospitalized, it was not possible to fully monitor the use of drugs. These were the limitation of the study. It is possible that if more volunteers participated in the experiment, there would be a significant difference between the

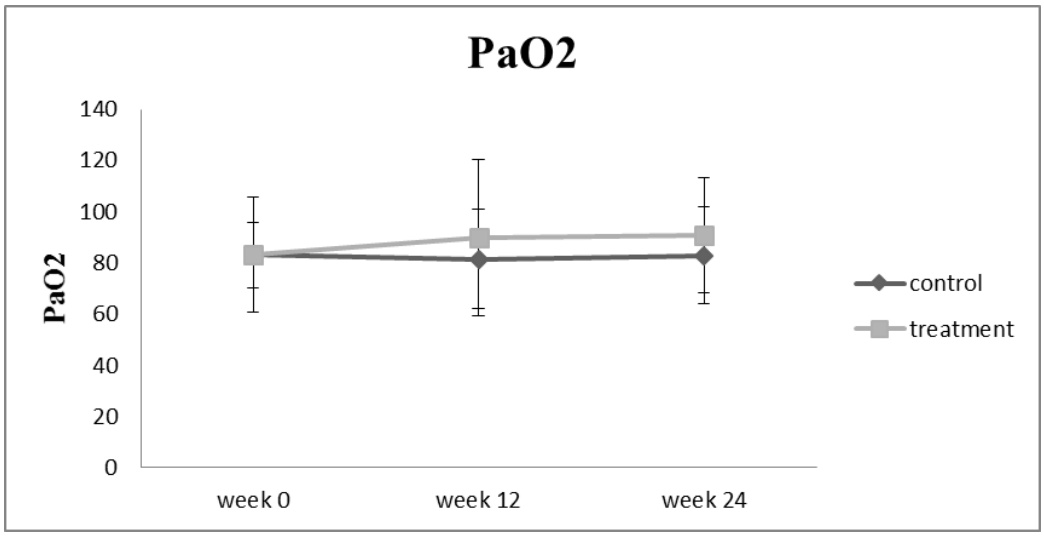

Figure 1: Arterial blood oxygen pressure in the study groups during the experiment. No significant differences were observed between different groups. Results are reported as Mean ± SD

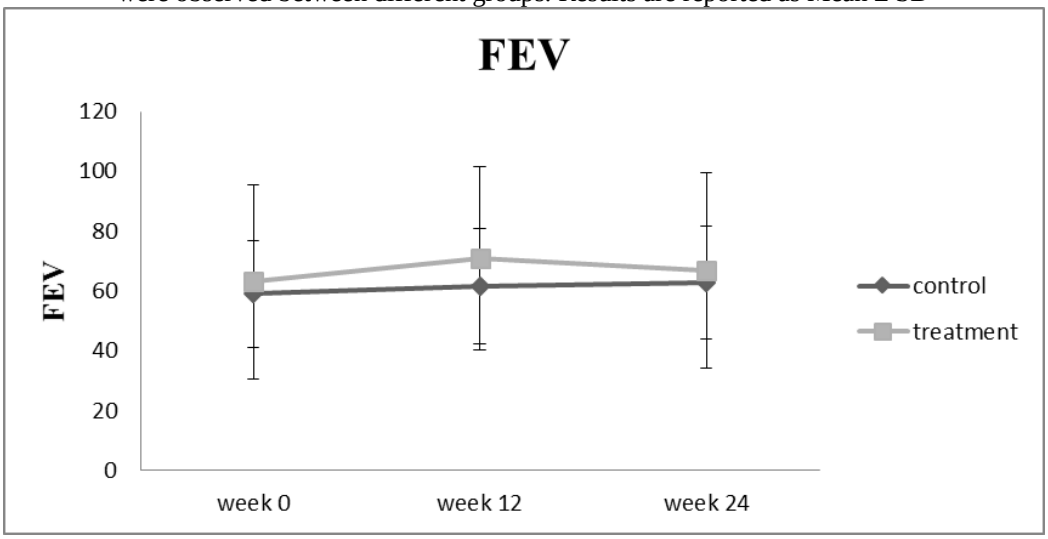

Figure 2: The amount of forced expiratory volume in the study groups during the experiment. No significant differences were observed in different groups. Results are reported as Mean ± SD

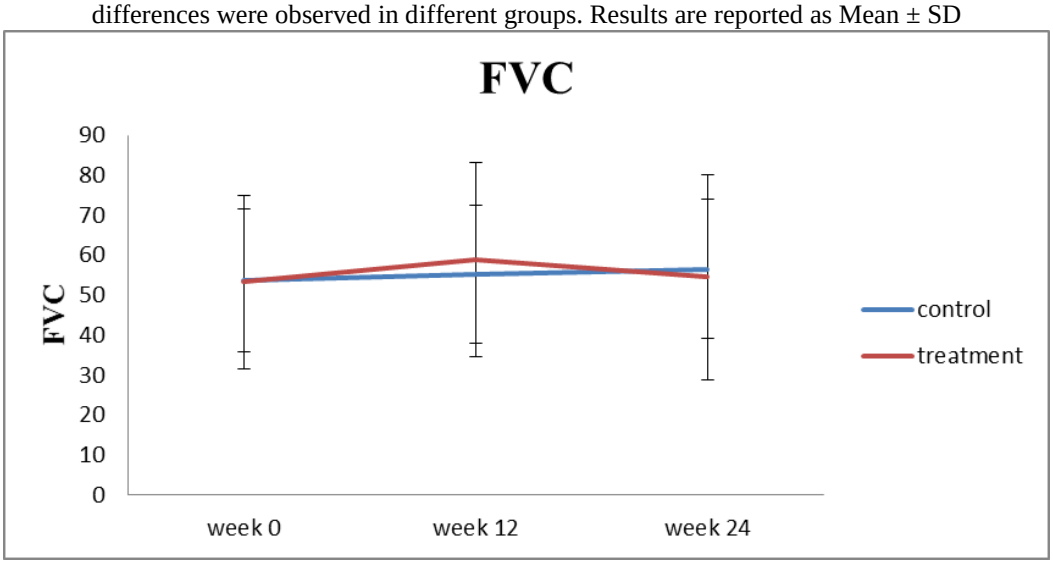

Figure 3: Forced vital capacity in the experimental groups during the 24 weeks of the experiment. No significant differences were observed between different groups. Results are reported as Mean ± SD.

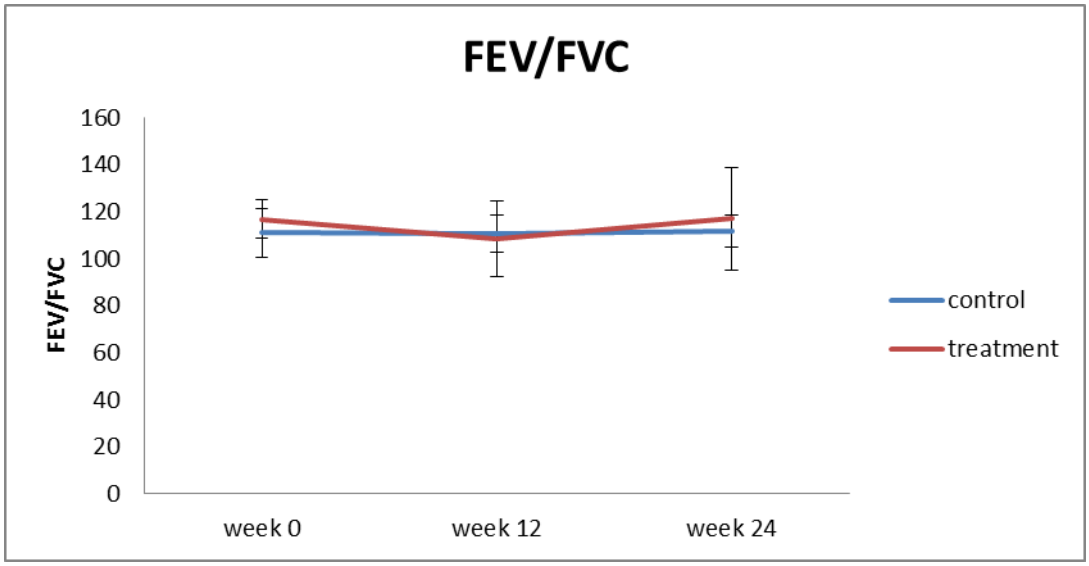

Figure 4: FEV / FVC levels in the experimental groups during the 24 weeks of the experiment. No significant differences were observed between different groups. Results are reported as Mean ± SD.

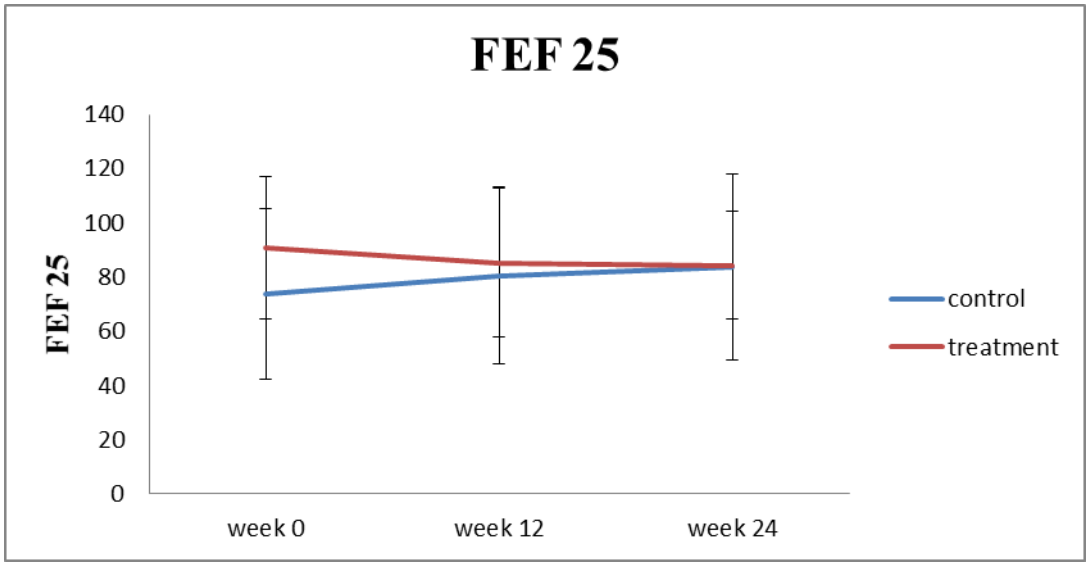

Figure 5: FEF25 levels in the experimental groups during the 24 weeks of the experiment. No significant differences were observed between different groups. Results are reported as Mean ± SD.

experimental groups.

There are a few studies on the effects of selenium on IFP patients. A recent study by Hazem (29) showed that following selenium supplementation in people with IFP, the rate of FVC and FEV was significantly higher than the control group which received N-acetylcysteine. The difference between the results of the present study and Hazems' study can be for several reasons. The most important of which are the number of participants and the duration of the intervention. In addition, the rate of disease progression and the stages of the disease can also be another important factor of difference in the study results. Therefore, if the intervention was performed for a longer period or patients who were in the early stages of the disease or had milder degrees of the disease with a possibility of repair or improvement of lung fibrous tissue included in the experiment, the better results could be obtained. However, further studies are needed to elucidate the various aspects of the role of selenium supplementation in patients with pulmonary fibrosis. Larger sample size as well as serum test and lung imaging are suggested in future study to determine the effect of antioxidant supplementation in IPF patients.

5. Conclusion

The results of the present study showed that selenium supplementation had no statistically significant effect on the measured factors in patients with idiopathic pulmonary

fibrosis. It is possible that with increasing the number of patients or increasing the duration of treatment, significant effects are observed. Thus, it is recommended to evaluate the effect of the supplement in different doses, in more patients, and in the early stages of the disease.

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7. Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

8. Funding source

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9. Author contribution

None.

10. Reference

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