Comparison of Prednisolone Monotherapy *vs*. Combined Prednisolone and N-Acetylcystiene Dual Therapy to Treat Idiopathic Pulmonary Fibrosis

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Abstract

Background: Idiopathic pulmonary fibrosis is a chronic progressive disease with poor prognosis. Some recent studies revealed that N-acetylcysteine slowed the deterioration in IPF. This trial tried to find out whether addition of N-acetylcysteine to prednisolone is superior to prednisolone monotherapy in Idiopathic Pulmonary Fibrosis (IPF).

Materials and Methods: We conducted this single blind, randomized, placebo controlled trial in the National Institute of Diseases of the Chest and Hospital (NIDCH), Mohakhali, Dhaka, Bangladesh over a period of one year from January 2018 to December 2018 to assess effectiveness of high dose oral N-acetylcysteine (600 mg three times daily) added to standard therapy with prednisolone (start with 40 mg and tapered 2.5 mg after each 10 days) alone. Drug trial was conducted for a period of 12 weeks. Total 65 patients had been clinically diagnosed as Idiopathic pulmonary fibrosis confirmed by high resolution computed tomography of the chest and excluding other known causes of interstitial lung disease. Group-I patients (N_n=35) got N-acetylcysteine (600 mg three times daily for 12 weeks) with prednisolone (start with 40 mg and tapered 2.5 mg after each 10 days). Group-II patients (N_p=30) got placebo (three times daily for 12 weeks) with prednisolone (start with 40 mg and tapered 2.5 mg after each 10 days). Within this period, total 12 patients (18%); 5 patients (14%) from group-I and 7 patients (23%) from group-II were dropped out, so ultimately 30 patients in group-I and 23 patients in group-II came for final follow-up. Pulmonary function tests with spirometry and carbon monoxide diffusing capacity (DL_{CO}) was performed in each case at the beginning and at the end of study, after 12 weeks. The primary end points were changes between baseline and week 12 in vital capacity (in liter and % of predicted value) and single breath carbon monoxide diffusing capacity (DL_{CO}) (in ml/min/kPa and % of predicted value) between two groups to assess the N-acetylcysteine efficacy.

Results: A total number of 65 patients with IPF were randomly enrolled and assigned to treatment. Among them 35 patients were treated with N-acetylcysteine and prednisolone (Group-I) and the remaining 30 patients were treated with placebo and prednisolone (Group-II). Ultimately 30 patients (85.71%) in group-I and 23 patients (76.67%) in group-II came for final follow-up. The mean (± SD) difference of MRC dyspnea index at base line and follow-up during 12th week was found 0.067 ± 0.365 and -0.652 ± 0.647 in group-I & group-II respectively, which was statistically significant (p<0.05). Thus current study revealed a significant deterioration of dyspnoea in placebo group in relation to N-acetylcysteine group. Absolute mean difference at 12th week at 95% CI between two group was -0.571 (-0.989 to -0.153). The mean (± SD) difference of forced vital capacity in liter and in percentage at base line and follow-up during 12th week was found -0.05 ± 0.14, 0.22 ± 0.34 and -0.78 ± 5.59, 8.36 ± 11.19 in group-I and group-II respectively, which was statistically significant in both cases (p<0.05). The absolute mean difference at 12th week with 95% CI was -0.27 (-0.40 to -0.13) and -9.14 (-13.85 to -4.43) respectively which signified less deterioration of forced vital capacity (L and %) with N-acetylcysteine in the current study. The mean (± SD) difference of carbon monoxide diffusing capacity in ml/min/kPa and in percentage at base line and follow-up during 12th week was found -1.41 ± 2.11, 2.22 ± 2.24 and 6.14 ± 11.51, 7.55 ± 10.18 respectively in group-I and group-II, which was statistically significant (p<0.05) in both cases. Thus the absolute mean difference at 12th week with 95% CI was -3.62 (-4.83 to -2.42) and -13.69 (-19.79 to -7.59) respectively which signified less deterioration of carbon monoxide diffusing capacity in ml/min/kPa and in percentage with N-acetylcysteine in our current study. Mortality during the study was 3% among patients taking

acetylcysteine and 10% among those taking placebo (P=). There were no significant differences in the type or severity of adverse events between patients taking N-acetylcysteine and those taking placebo, except for a significantly lower rate of fever (P=0.014), respiratory tract infection (P=0.001) in the group taking N-acetylcysteine (P=0.03).

Conclusion: Therapy with N-acetylcysteine at a dose of 600 mg three times daily, added to prednisolone in patients with Idiopathic pulmonary fibrosis preserves vital capacity and DL_{CO} and patients experienced better symptomatic improvement than prednisolone monotherapy.

Introduction

Idiopathic Pulmonary Fibrosis (IPF) is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause with poor prognosis. It occurs primarily in older adults. It is limited to the lungs, and associated with the histopathologic and/or radiologic pattern of Usual Interstitial Pneumonia (UIP) [1]. It has been proposed that a pathogenic mechanism of IPF is repeated lung injury, with progressive aberrant progressive fibrotic reaction. If this is the case, mot may explain why treatment with corticosteroid results in only slight therapeutic benefit.

An oxidant-antioxidant imbalance may contribute to the disease process in IPF. Acetylcysteine, a precursor of major antioxidant glutathione, given at a daily dose of 1800 mg, has been showed to restore depleted pulmonary glutathione level and to result in a statistically significant improvement in patient with IPF after 12 weeks of treatment.

The joint official statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS), the Japanese Respiratory Society (JRS), and the Latin American Thoracic Association (ALAT), 2011 for IPF jointly approved the current guide line where IPF can be diagnosed when fulfilling the following criteria: a) Exclusion of other known causes of Interstitial Lung Disease (ILD) (e.g., domestic and occupational environmental exposures, connective tissue disease, and drug toxicity). b) The presence of a UIP pattern on High-Resolution Computed Tomography (HRCT) in patients not subjected to surgical lung biopsy. c) Specific combinations of HRCT and surgical lung biopsy pattern in patients subjected to surgical lung biopsy.

A study from the United States estimated the incidence of IPF to be between 6.8 and 16.3 per 100,000 persons using a large database of healthcare claims in a health plan [2]. Prevalence estimates for IPF have varied from 2 to 29 cases per 100,000 in the general population [3,4] lungs of patients with IPF show varying degrees of fibrosis and inflammation. As it has been long thought that inflammation precedes fibrosis, therapeutic regimens for IPF have included corticosteroids and immunosuppressive agents (e.g. azathioprine) [5]. In patients with severe fibrosis, treatments targeting inflammation, however, have not been shown to improve survival or quality of life. Some studies did find that patients with IPF treated with high-dose N-acetyl cysteine (NAC), an antioxidant, in addition to a standard regimen (e.g. corticosteroids and azathioprine) had better lung function compared to those on the standard regimen alone [5]. An oxidant-antioxidant imbalance may contribute to the disease process in IPF [6-15].

In a study with 18 patients with established diagnosis of IPF, treatment with 600 mg N-acetylcysteine three times a day for 12 weeks in addition to their latest immunosuppressive therapy resulted in significant improvement of pulmonary function tests [6]. A recent double-blind, randomized, placebo-controlled multicenter study, known as IFIGENIA (Idiopathic Pulmonary Fibrosis International

Group Exploring NM-acetylcysteine I Annual) trial, assessed the effectiveness over one year of a high oral dose of N-acetylcysteine (600 mg three times daily) added to standard therapy with prednisolone plus azathioprine [5]. This landmark trial showed that N-acetylcysteine slowed the deterioration of Vital Capacity (VC) and carbon monoxide diffusing capacity (DL_{CO}) at 12 months without significant differences in the type or severity of adverse events.

This study tried to evaluate the effect of NAC in IPF by matching forced vital capacity and DL_{CO} in treatment group and control group at the beginning and after 12 weeks of study.
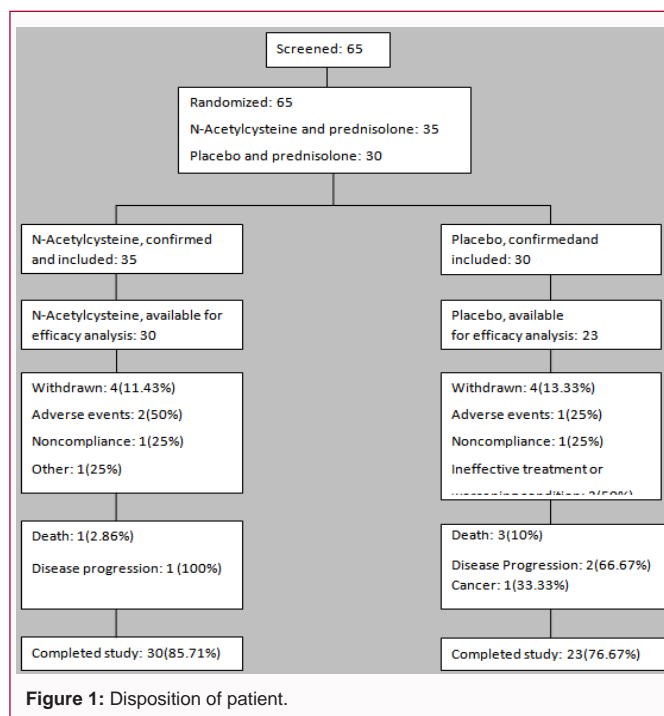
Materials and Methods

Study design

This single blind, randomized, placebo controlled, parallel group clinical trial was carried out from January 2018 to December 2018. Drug trial in each case was conducted for a period of 12 weeks. Patient's enrollment was going on up to 24 weeks from the beginning of study. Patients were considered to have withdrawn from the study if they discontinued the follow-up visits for any reason. This study was carried out in the National Institute of Diseases of the Chest and Hospital (NIDCH), Mohakhali, Dhaka, Bangladesh. Hospitalized patients with IPF were enrolled after fulfilling the selection criteria. Initially 65 patients were enrolled according to inclusion and exclusion criteria, after taking written informed consent.

Each case was included in this study by purposive sampling method and each case was put in a group by simple random sampling method. Patients were randomized in two groups; one group was given effervescent NAC tablet (600 mg, three times daily for 12 weeks) and prednisolone (start with 40 mg and tapered 2.5 mg after each 10 days) (Group -I) and other group was given placebo and prednisolone (start with 40 mg and tapered 2.5 mg after each 10 days) (Group -II). The study protocol was approved by the institutional ethical committee of NIDCH. All patients gave written informed consent and were free to withdraw at anytime. All information gathered was kept secret and was only used for medical research and analysis.

The diagnosis of IPF based on "an official ATS/ERS/JRS/ALAT Statement: Idiopathic Pulmonary Fibrosis: Evidence-based Guidelines for Diagnosis and Management", 2011.

Inclusion and exclusion criteria

Patients of both sexes aging 30 years and above, who were able to return for reassessment after 12 weeks from the time of enrollment had been clinically diagnosed as IPF (including insidious onset of otherwise unexplained dyspnoea on exertion, dry cough, bibasilar end inspiratory fine crackles) confirmed by HRCT scan of the chest (According to ATS IPF 2011 statement) were included. Bronchoalveolar Lavage (BAL) had been taken if patient aged less than 50 years and or if there were features supporting alternative diagnoses. Patients were included with vital capacity of no more than 80% of the predicted value or single breath carbon monoxide

Figure 1: Disposition of patient.

diffusing capacity (DL_{CO}) less than 80% of the predicted value.

Patients were excluded if prednisolone was contraindicated or not justified for them or if they presented with a known intolerance to N-acetylcysteine, presence of other known causes of ILD e.g. drug toxicities, environmental and occupational exposures and connective tissue diseases, presence of significant co morbidities interfering with respiratory status e.g. congestive cardiac failure, COPD, HRCT chest demonstrating a pulmonary disease other than IPF as the predominant pulmonary abnormality. Other reasons for exclusion were patients with advanced kidney disease or with severe hepatic impairment, pregnancy and breast feeding, concurrent use of nitroglycerin, patient with risk of upper gastrointestinal tract hemorrhage e.g. esophageal varices, peptic ulcer disease and those who refused to give consent to be included in the study.

Group I patients were treated with N-acetylcysteine, 600 mg, three times daily for 12 weeks in association with Prednisolone (start with 40 mg and tapered 2.5 mg after each 10 days) and group II were treated with placebo, three times daily for 12 weeks in association with prednisolone (start with 40 mg and tapered 2.5 mg after each 10 days). At the end of 12th week, patient was advised to come with some report of routine investigations including Full Blood Count (FBC), Alanine Aminotransferase (ALT), serum creatinine and Random Blood Sugar (RBS). The patients underwent clinical examination, assessment of adverse events and drug compliance to evaluate patient's condition. At 12th week, repeat spirometry and DL_{CO} were performed. Finally, 5 patients from group I and 7 patients from group II dropped out for different reasons. So, 30 patients in group I and 23 patients in group II completed the study.

At the end of the study, all documented information were gathered and analyzed by SPSS (Figure 1).

Results

Enrollment, patient characteristics and follow-up

Between January, 2012 to December, 2012, 65 patients were

included in analysis and randomly assigned to treatment. Drug trial in each case was conducted for a period of 12 weeks. Patient's enrollment was going on up to 24 weeks from the beginning of study. Hospitalized patients with IPF were enrolled after fulfilling the selection criteria. Initially 65 patients were enrolled according to inclusion and exclusion criteria, after taking written informed consent. Finally, as 5 patients from group I and 7 patients from group II dropped out for different reasons, Ultimately 30 patients in group-I and 23 patients in group-II came for final follow up at week 12. No significant differences in the baseline characteristics were found between the 30 patients assigned to N-acetylcysteine and 23 assigned to placebo (Table 1).

The mean (± SD) age of patients in group-I was 52.7 ± 9.07 years and in group-II, it was 57.1 ± 12.24 years. Male was 46.7% and 65.2% in group-I and group-II respectively. Female was 53.3% and 34.8%. In group-I, most of the patients were nonsmoker (60.0%) followed by former smoker (26.7%) but in group-II, most of them were former smoker (47.8%) followed by nonsmoker (39.2%). In both group, least of the patients were current smoker (13.3% & 13.0% in group-I and group-II respectively).

In both groups, HTN was predominant co-morbidities (23.3% & 21.7% respectively) followed by DM (3.3% & 8.7% respectively) and both DM & HTN was present in 10.0% & 17.4% patients respectively.

HRCT scan of chest was performed in 100% of cases in this study. Typical UIP pattern of IPF was detected in 73.4% and 69.6% cases in group-I and group-II respectively followed by possible UIP (23.3% & 30.4%) and only a single case, belonged to group-I 3.3% cases had inconsistent UIP pattern.

Bronchoalveolar Lavage (BAL), taken by Fibre Optic Bronchoscopy (FOB) for cytological study was performed in selected cases to rule out some differential diagnoses of IPF. Total 21 patients (39.62%) underwent FOB which revealed total count of cell in BAL (/ml) was 381 ± 133 and 417 ± 184 in group-I and group-II respectively with neutrophil (%) was 35.08 ± 19.65 and 30.22 ± 20.75, lymphocyte (%) was 20.17 ± 15.02 and 20.67 ± 13.57 and histiocyte (%) was 43.67 ± 22.95 and 47.44 ± 26.35 in group-I and group-II respectively.

At the start of this study, all (100%) of the patients were presented with shortness of breath followed by cough (100% & 95.7% in group-I & group-II respectively), chest pain (16.7% and 21.7%) and sputum production (10.0% and 4.3%). Only a single patient (3.3%) from group-I presented with hemoptysis. Baseline clinical examination revealed bibasilar end inspiratory crackles in 100% cases in both groups followed by clubbing (36.7% & 60.9% in group-I & group-II respectively) and cyanosis (26.7% & 21.7%) and edema (13.3% and 17.4%). Near half (47.2%) of the total cases had clubbing.

At the end of the study, 100% patients of both groups had shortness of breath and cough. Clinical examination at the end of the study explored that bibasilar crackles were detected in all cases (100.0%) in both groups followed by cyanosis and edema 6.7% , 10.0% in group-I and 30.4%, 13% of patients in group-II respectively. The mean (± SD) MRC dyspnoea index in group-I at baseline was 2.80 ± 0.664, range was 2 to 4 and follow-up during 12th week was 2.73 ± 0.740, range was 2 to 4. The mean (± SD) difference of MRC dyspnoea index at base line and follow up during 12th week was 0.067 ± 0.365, which was statistically not significant (p>0.05) whether in other group, the mean (± SD) MRC dyspnoea index at baseline was 2.65 ± 0.885, range was 1 to 4 and follow-up during 12th week was

Table 1: Baseline characteristics of the patients (N=53).

Characteristics	Group-I (N$_n$=30)	Group-II (N$_p$=23)
Age (year)	52.7 ± 9.07	57.1 ± 12.24
Sex (%)		
Male	46.7	65.2
Female	53.3	34.8
Smoking status (%)^ξ		
Never smoked	60.0	39.2
Former smoker	26.7	47.8
Current smoker	13.3	13.0
Co-morbidities (%)		
Diabetes mellitus (DM)	3.3	8.7
Hypertension (HTN)	23.3	21.7
DM & HTN	10.0	17.4
HRCT scan of chest (%)		
UIP Pattern$^·$	73.4	69.6
Possible UIP Pattern	23.3	30.4
Inconsistent UIP Pattern	3.3	0.0
Bronchoalveolar lavage (BAL)	(N$_n$=12)	(N$_p$=9)
Total count of cell (/ml)	381 ± 133	417±184
Neutrophil (%)	35.08 ± 19.65	30.22 ± 20.75
Lymphocyte (%)	20.17 ± 15.02	20.67 ± 13.57
Histiocyte (%)	43.67 ± 22.95	47.44 ± 26.35

Group-I =NAC and prednisolone =N$_n$, Group-II = Placebo and prednisolone =N$_p$
ξ Current smokers were defined as those actively smoking, irrespective of the number of cigarettes smoked per day
ξ Former smokers were defined as those who had stopped smoking at least three months previously
·UIP: Usual interstitial pneumonia (defined as ATS-IPF, 2011)

3.30 ± 0.765, range was 2 to 4. The mean (± SD) difference of MRC dyspnoea index at base line and follow up during 12th week was -0.652 ± 0.647, which was statistically significant (p<0.05). The mean (± SD) difference of MRC dyspnoea index at base line and follow-up during 12th week was found 0.067 ± 0.365 and -0.652 ± 0.647 in group-I and group-II respectively, which was statistically significant (p<0.05). So the absolute mean (± SD) difference of MRC dyspnoea index at 12th week with 95% CI was -0.571 (-0.989 to -0.153).

The mean (± SD) respiratory rate at baseline in was 23.37 ± 2.606 and follow-up during 12th week was 16.40 ± 2.061. The mean (± SD) difference of respiratory rate at base line and follow up during 12th week was 6.967 ± 2.810, which was statistically significant (p<0.05), whether in other group, the mean (± SD) respiratory rate at base line was 23.48 ± 3.203 and follow-up during 12th week was 21.57 ± 4.132. The mean (± SD) difference of respiratory rate at base line and follow up during 12th week was 1.913 ± 4.842, which was statistically not significant (p>0.05). The mean (± SD) difference of respiratory rate at base line and follow-up during 12th week was found 6.967 ± 2.810 and 1.913 ± 4.842 in group-I and group-II respectively, which was statistically significant (p<0.05).

Effects on primary end points

The analysis included 53 patients (30 receiving acetylcysteine and prednisolone- group-I and 23 receiving placebo and prednisolone- group-II) for vital capacity and DL$_{CO}$ analysis. There was a slower rate of loss of vital capacity in the group receiving acetylcysteine and prednisolone. The analysis of DL$_{CO}$ yielded similar results. In group-I, the mean (± SD) FVC in liter at baseline was 1.699 ± 0.54 and follow-up during 12th week was 1.75 ± 0.53. The mean (± SD) difference of FVC in liter at base line and follow up during 12th week was -0.05 ± 0.14, which was statistically not significant (p>0.05). In group-II, the mean (± SD) FVC in liter at baseline was 1.65 ± 0.62 and follow-up during 12th week was 1.43 ± 0.48. The mean (± SD) difference of FVC in liter at base line and follow up during 12th week was 0.22 ± 0.34, which was statistically significant (p<0.05). The mean (± SD) difference of FVC in liter at base line and follow-up during 12th week was found -0.05 ± 0.14 and 0.22 ± 0.34 in group-I and group-II respectively, which was statistically significant (p<0.05).

The mean (± SD) FVC in percentage in group-I at baseline was 55.76 ± 14.56 and follow-up during 12th week was 56.54 ± 13.12. The mean (± SD) difference of FVC in percentage at base line and follow up during 12th week was -0.78 ± 5.59, which was statistically not significant (p>0.05) whether the mean (± SD) FVC in percentage In group-II at baseline was 54.60 ± 20.298 and follow-up during 12th week was 46.24 ± 17.28. The mean (± SD) difference of FVC in percentage at base line and follow up during 12th week was 8.36 ± 11.19, which was statistically significant (p<0.05). The mean (± SD) difference of FVC in percentage at base line and follow-up during 12th week was found -0.78 ± 5.59 and 8.36 ± 11.19 in group-I and group-II respectively, which was statistically significant (p<0.05).

The mean (± SD) FEV1/FVC (in %) in group-I at baseline was 87.69 ± 9.77 and follow-up during 12th week was 91.18 ± 12.06. The mean (± SD) difference of FEV1/FVC (in %) at base line and follow up during 12th week was -3.49 ± 10.91, which was statistically not significant (p>0.05). In group-II, the mean (± SD) FEV1/FVC (in %) at baseline was 90.67 ± 13.57 and follow-up during 12th week was 95.21 ± 12.298. The mean (± SD) difference of FEV1/FVC (in %) at base line and follow up during 12th week was -4.54 ± 13.62, which was statistically not significant (p>0.05). The mean (± SD) difference of FEV1/FVC (in %) at base line & follow-up during 12th week was found -3.49 ± 10.91 and -4.54 ± 13.62 in group-I & group-II respectively, which was statistically not significant (p>0.05) (Table 2).

The mean (± SD) DL$_{CO}$ in ml/min/kPa in group-I at baseline was 8.23 ± 4.46 and follow-up during 12th week was 9.64 ± 3.83. The mean (± SD) difference of DL$_{CO}$ in ml/min/kPa at base line and follow up during 12th week was -1.41 ± 2.11, which was statistically significant (p<0.05). In other group, the mean (± SD) DL$_{CO}$ in ml/min/kPa at baseline was 7.12 ± 2.71 and follow-up during 12th week was 4.90 ± 2.36. The mean (± SD) difference of DL$_{CO}$ in ml/min/kPa at base line and follow-up during 12th week was 2.22 ± 2.24, which was statistically significant (p<0.05). The mean (± SD) difference of DL$_{CO}$ in ml/min/kPa at base line and follow-up during 12th week was found -1.41 ± 2.11 and 2.22 ± 2.24 in group-I and group-II respectively, which was statistically significant (p<0.05).

The mean (± SD) DL$_{CO}$ in percentage in group-I at baseline was 37.45 ± 19.42 and follow-up during 12th week was 43.59 ± 16.78. The mean (± SD) difference of DL$_{CO}$ in percentage at base line and follow-up during 12th week was -6.14 ± 11.51, which was statistically significant (p<0.05). On the other hand, the mean (± SD) DL$_{CO}$ in percentage in group-II at baseline was 31.23 ± 10.36 and follow-up during 12th week was 23.68 ± 12.11. The mean (± SD) difference of DL$_{CO}$ in percentage at base line and follow up during 12th week was 7.55 ± 10.18, which was statistically significant (p<0.05). The mean (± SD) difference of DL$_{CO}$ in percentage at base line and follow-up during 12th week was found -6.14 ± 11.51 and 7.55 ± 10.18 in group-I

Table 2: Effects of NAC on both forced vital capacity (FVC) (in liter and in %) and single breath carbon monoxide diffusing capacity (DL$_{CO}$) (in ml/min/mm of Hg and in %) at the end of study (at 12th week).

Measurement	No. of patients		Value for patients		Mean difference	P value
	Group-I (N$_n$)	Group-II (N$_p$)	Group-I (N$_n$)	Group-II (N$_p$)		
FVC (in liter)						
Baseline	35	30	1.699 ± 0.54	1.65 ± 0.62	0.05	0.751^{ns}
Follow up at 12th week	30	23	1.75 ± 0.53	1.43 ± 0.48	0.32	0.028^s
Difference P value			-0.05 ± 0.14 0.06^{ns}	0.22 ± 0.34 0.005^s	-0.27	<0.001^s
Absolute mean difference:-0.27 at 12th week with 95% CI(-0.40 to 0.13)						
FVC (in %)						
Baseline	35	30	55.76 ± 14.56	54.60 ± 20.298	1.15	0.811^{ns}
Follow up at 12th week	30	23	56.54 ± 13.12	46.24 ± 17.28	10.29	0.017^s
Difference P value			-0.78 ± 5.59 0.451^{ns}	8.36 ± 11.19 0.002^s	-9.14	<0.001^s
Absolute mean difference:-9.14 at 12th week with 95% CI (-13.85 to -4.43)						
DL$_{CO}$ (ml/min/kPa)						
Baseline	35	30	8.23 ± 4.46	7.12 ± 2.71	1.11	0.297^{ns}
Follow up at 12th week	30	23	9.64 ± 3.83	4.90 ± 2.36	4.74	<0.001^s
Difference P value			-1.41 ± 2.11 0.001^s	2.22 ± 2.24 <0.001^s	-3.62	<0.001^s
Absolute mean difference:-3.62 at 12th week with 95% CI(-4.83 to -2.42)						
DL$_{CO}$ (in %)						
Baseline	35	30	37.45 ± 19.42	31.23 ± 10.36	6.22	0.171^{ns}
Follow up at 12th week	30	23	43.59 ± 16.78	23.68 ± 12.11	19.91	<0.001^s
Difference P value			-6.14 ± 11.51	7.55 ± 10.18	-13.69	<0.001^s
Absolute mean difference:-13.69 at 12th week with 95% CI (-19.79 to -7.59)						

Group-I =NAC and prednisolone =N$_n$, Group-II = Placebo and prednisolone =N$_p$
s: Significant; ns: Not significant
P value reached from unpaired t-test, P value reached from paired t-test
CI denotes for confidence interval, Plus- minus values are means ± SD

Table 3: Adverse events occurring in at least 5 percent of patients§.

Adverse Events	Acetylcholine Group (N$_n$=30)		Placebo Group (N$_p$=23)		Total		P value
	No	(%)	No	(%)	No	(%)	
Fever	2	6.7	8	34.8	10	18.9	0.014^s
RTI Ω	4	13.3	13	56.5	17	32.1	0.001^s
Abdominal pain∑	2	6.7	2	8.7	4	7.5	1.000^{ns}
Abnormal LFT's £	6	20	2	8.7	8	15.0	
Increased Blood Glucose €	3	10	4	17.4	7	13.2	
Bone marrow toxicity ¥	2	6.7	3	13	5	9.4	

§ All diagnoses were based on the clinical judgement of the investigators and were blindly coded by qualified persons
Ω Lower respiratory tract infections, pneumonia, bronchopneumonia, acutebronchitis, rhinitis, sinusitis and nasopharyngitis are included
∑ Gastritis is included
£ Increased or abnormal aspartate aminotransferase, alanine amino transferase, Alkaline Phosphatase and Y-glutamyl transferase
€Hyperglycemia is included
¥ Anemia, Leucopenia, Lymphopenia, Neutropenia, Thrombocytopenia and decreased Red cell count Area included
s: Significant; ns: Not Significant; RTI: Respiratory Tract Infection; LFT: Liver Function Test

and group-II respectively, which was statistically significant (p<0.05).

Discussion

Our study shows that the addition of N-acetylcysteine to prednisone in patients with Idiopathic Pulmonary Fibrosis (IPF) significantly slows the rate of deterioration of the primary pulmonary surrogate end points vital capacity and DLco. Although we could not establish that the N-acetylcysteine-related reduction in the decline of vital capacity and DLco translates into a survival benefit, our data suggest that the effects of N-acetylcysteine on the primary end points may slow disease progression.

As per our opinion, the effects of N-acetylcysteine on the primary end points are of clinical relevance. Table 3 show that, in our study,

N-acetylcysteine reduces the declines in vital capacity and DLco after 12 weeks of treatment. However, the present study did not document the finding of other studies that changes in vital capacity and DLco are associated with survival. Other recent studies indicated that the six minute walk test may be a predictor of survival as well. Such tests were not performed in this trial.

The main rationale for the present study was based on previous findings that an oxidant-antioxidant imbalance existed in idiopathic pulmonary fibrosis that depleted glutathione levels were restored by high doses of N-acetylcysteine and (in a pilot study) that N-acetylcysteine treatment had concomitant favorable effects on lung function. The favorable effects of N-acetylcysteine on lung function have, indeed, been confirmed by the present trial.

We enrolled total 65 patients with IPF were from the National Institute of Diseases of the Chest and Hospital (NIDCH), Mohakhali, Dhaka, Bangladesh during January, 2018 to December, 2018. Among them 35 patients were treated with N-acetylcysteine (600 mg three times daily for 12 weeks) and prednisolone(started with 40 mg and tapered 2.5 mg every 10 days) (Group-I) and the remaining 30 patients were treated with placebo and prednisolone (started with 40 mg and tapered 2.5 mg every 10 days) (Group-II). Within this period, total 12 patients (18%); 5 patients (14%) from group-I and 7 patients (23%) from group-II were dropped out, so ultimately 30 patients in group-I and 23 patients in group-II came for final follow-up. The main reason for withdrawal was death, disease progression despite taking drugs, non compliance and development of cancer. In Demedts et al. [5] study, fifty-seven of the 80 patients taking N-acetylcysteine (71%) and 51 of the 75 patients taking placebo (68%) completed the study; total dropped out rate was 30% which was well above than this study. Dropped out rate was 10% and 27% in the study conducted by Behr et al. [6] and Tomioka et al. [7] respectively [5-7].

At the beginning of this study, all of the patients (100.0%) were presented with shortness of breath followed by cough (100% & 95.7% in group-I & group-II respectively) and crackles were detected in 100% cases followed by clubbing (36.7% & 60.9% in group-I & group-II respectively) and cyanosis (26.7% & 21.7% in group-I & group-II respectively). Near half (47.2%) of the total cases had clubbing. Similar to the current study in Demedts's study [5], all the patients had shortness of breath and bibasilar inspiratory crackles at the beginning of study [5]. Johnston's study revealed that at least 49% patients (49% to 66% of patients) had clubbing in IPF which was similar to the current study [8]. But in Hunninghake's study [9], the scenario was quite different where only 16% patients had clubbing in IPF. In Hunninghake's study, 100% patients had cough and 84% had dyspnoea in IPF which were dissimilar to the current study [9].

At the end of this study, 100% patients had shortness of breath and cough. Fever, abdominal pain & RTI presented in 6.7%, 6.7% & 13.3% patients in group-I and 34.8%, 8.7% & 56.5% patients in group-II. Crackles were detected in all cases (100.0%) in both groups. At the end of study, significant statistical difference (p<0.05) was found in between two groups regarding fever, RTI & cyanosis and percentage of affected patients were always more in group-II. Demedts's [5] revealed that at the end of study, excess deterioration of cough, RTI, fever & abdominal pain were detected in 16%, 25%, 19% & 15% in NAC group and 21%, 32%, 13% & 9% in placebo group. Abdominal pain was evaluated as an adverse effect of NAC which was relatively more in NAC group in Demedts's study [5] but in the current study it differed. Occurrence of RTI was comparably more in placebo

group in both current & Demedts's study [5]. In Behr's study [6], 17% experienced a single episode of diarrhea having NAC during the treatment period, which ceased in all cases after a treatment pause of 2 to 3 days. Upon continuation of high-dose NAC treatment, there was no recurrence of diarrhea [6].

Regarding the mean (± SD) MRC dyspnoea index at baseline, in group-I, it was 2.80 ± 0.664 and during 12th week was 2.73 ± 0.740. The mean (± SD) difference of MRC dyspnoea index at base line and follow up was 0.067 ± 0.365, which was statistically not significant (p>0.05). In group-II, the mean (± SD) MRC dyspnoea index at baseline was 2.65 ± 0.885 and follow-up during 12th week was 3.30 ± 0.765. The mean (± SD) difference of MRC dyspnoea index at base line and follow up was -0.652 ± 0.647, which was statistically significant (p<0.05). The mean (± SD) difference of MRC dyspnoea index at base line & follow-up was found 0.067 ± 0.365 and -0.652 ± 0.647 in group-I & group-II respectively, which was statistically significant (p<0.05). Thus current study revealed a significant deterioration of dyspnoea in placebo group in relation to NAC group. Absolute mean difference at 12th week at 95% CI between two group was -0.571 (-0.989 to -0.153). By using a different dyspnoea index, Demedts's study [5] revealed that absolute mean difference at 12th month at 95% CI between two group was -0.32 (-1.72 to 1.09) without any significance (P=0.65) where mean dyspnoea index was worse in favor of placebo group at the end. Probably the significance of Demedt's study regarding dyspnoea had been lost due to the four times extended study period in a disease with a median survival time 2 to 3 years [5]. Behr's study revealed that 50% patients having NAC had the impression that their dyspnoea improved during the study period [6].

The mean (± SD) respiratory rate in group-I at baseline was 23.37 ± 2.606 and at follow-up was 16.40 ± 2.061. The mean (± SD) difference of respiratory rate at base line and follow up was 6.967 ± 2.810, which was statistically significant (p<0.05) while in the other group the mean (± SD) respiratory rate at baseline was 23.48 ± 3.203 and at follow-up was 21.57 ± 4.132. The mean (± SD) difference of respiratory rate at base line and follow up was 1.913 ± 4.842, which was statistically not significant (p>0.05). The mean (± SD) difference of respiratory rate at base line & follow-up was 6.967 ± 2.810 and 1.913 ± 4.842 in group-I & group-II respectively, which was statistically significant (p<0.05). Thus NAC had definite and significant impact on normalizing respiratory rate according to the current study.

HRCT scan of chest was taken as a diagnostic tool for IPF in IFIGENIA study [5]. Recent ATS IPF 2011 statement made newer diagnostic criteria for IPF based on HRCT scan of chest where IPF can be declared as 'UIP pattern', 'possible UIP pattern' and 'inconsistent UIP pattern' and this current study followed these UIP patterns [1]. Several studies have documented that the positive predictive value of a HRCT diagnosis of IPF (UIP) is 90% to 100% [16]. HRCT scan of chest was performed in 100% cases in present study. Typical UIP pattern of IPF was detected in 73.3% and 69.6% cases in group-I and group-II respectively and in case of possible UIP, percentage were 23.3% & 30.4% respectively. In this study, only 1 case (1.9% in total) had inconsistent UIP pattern for IPF diagnosis which belonged to group-I. All these cases included in the current study as surgical lung biopsy was not performed routinely in IPF in our hospital setting, none of the suspected cases failed to evaluate by lung biopsy.

In our study, the mean (± SD) FVC in liter in group-I at baseline was 1.699 ± 0.54 and follow-up during 12th week was 1.75 ± 0.53. The

mean (± SD) difference was -0.05 ± 0.14, which was statistically not significant (p>0.05). Data showed that mean difference was actually improved with NAC. In group-II, the mean (± SD) FVC in liter at baseline was 1.65 ± 0.62 and at 12th week was 1.43 ± 0.48. The mean (± SD) difference of FVC was 0.22 ± 0.34, which was statistically significant (p<0.05). Data showed that mean difference was actually significantly reduced with placebo. The mean (± SD) difference of FVC in liter at base line & follow-up during 12th week was found -0.05 ± 0.14 and 0.22 ± 0.34 in group-I & group-II respectively, which was statistically significant (p<0.05). Thus the absolute mean difference at 12th week with 95% CI was -0.27 (-0.40 to -0.13) which signified less deterioration of FVC (L) with NAC in the current study. In IFIGENIA trial, mean FVC in liter at the beginning of study was 2.29 ± 0.68 & 2.36 ± 0.74 in NAC & placebo group respectively and mean FVC in liter at the end of study was 2.27 ± 0.05 & 2.10 ± 0.05 in NAC & placebo group respectively. Thus the absolute mean difference at the end of study with 95% CI was 0.18 (0.03 to 0.32) which signified less deterioration of FVC (L) with NAC (P=0.02) in IFIGENIA trial similar to current study [5].

In the current study in group-I, the mean (± SD) FVC in percentage at baseline was 55.76 ± 14.56 and follow-up during 12th week was 56.54 ± 13.12. The mean (± SD) difference of FVC in percentage at base line and follow up was -0.78 ± 5.59, which was statistically not significant (p>0.05) but improved functionally. In group-II, the mean (± SD) FVC in percentage at baseline was 54.60 ± 20.298 and at follow-up was 46.24 ± 17.28. The mean (± SD) difference of FVC in percentage at base line and follow up was 8.36 ± 11.19, which was statistically significant (p<0.05) and obviously deteriorated. The mean (± SD) difference of FVC in percentage at base line & follow-up was found -0.78 ± 5.59 and 8.36 ± 11.19 in group-I & group-II respectively, which was statistically significant (p<0.05). Thus the absolute mean difference at 12th week with 95% CI was -9.14 (-13.85 to -4.43) which signified less deterioration of FVC (%) with NAC in the current study. In IFIGENIA trial, mean FVC in percentage at the beginning of study was 64.76 ± 15.41 & 66.57 ± 14.42 in NAC & placebo group respectively and mean FVC in percentage at the end of study was 65.13 ± 1.85 & 60.34 ± 1.85 in NAC & placebo group respectively. Thus the absolute mean difference at the end of study with 95% CI was 4.79 (0.80 to 8.77) which signified less deterioration of FVC (%) with NAC (P=0.02) in IFIGENIA trial similar to current study [5]. In Behr's study which was conducted in Germany, the mean percentage of FVC before NAC trial was 80.5 ± 4.4 which was actually improved after 12 weeks of NAC treatment and became 82.6 ± 4.3 [6]. As the median survival time of IPF is only 2 to 3 years, possibly immediate effect of NAC (after 12 weeks) is better than prolonged effect (after 12 months).

The mean (± SD) DL_{CO} in ml/min/kPa in group-I at baseline was 8.23 ± 4.46 and follow-up during 12th week was 9.64 ± 3.83. The mean (± SD) difference of DL_{CO} in ml/min/kPa at base line and follow up was -1.41 ± 2.11, which was statistically significant (p<0.05) & improved. On the other hand in group-II, the mean (± SD) DL_{CO} in ml/min/kPa at baseline was 7.12 ± 2.71 and at follow-up was 4.90 ± 2.36. The mean (± SD) difference of DL_{CO} in ml/min/kPa at base line and follow up was 2.22 ± 2.24, which was statistically significant (p<0.05) & deteriorated. The mean (± SD) difference of DL_{CO} in ml/min/kPa at base line & follow-up was found -1.41 ± 2.11 and 2.22 ± 2.24 in group-I & group-II respectively, which was statistically significant (p<0.05). Thus the absolute mean difference at 12th week with 95% CI was -3.62 (-4.83 to -2.42) which signified less deterioration of DL_{CO} in

ml/min/kPa with N-acetylcysteine in the current study. In IFIGENIA trial, mean DL_{CO} in ml/min/mmHg at the beginning of study was 11.496 ± 4.21 & 11.645 ± 4.15 in NAC & placebo group respectively and mean DL_{CO} in ml/min/mmHg at the end of study was 11.496 ± 0.51 & 9.256 ± 0.54 in NAC & placebo group respectively. Thus the absolute mean difference at the end of study with 95% CI was 2.24 (0.81 to 3.67) which signified less deterioration of DL_{CO} in ml/min/mmHg with NAC (P=0.003) in IFIGENIA trial similar to current study [5].

In the current study, in group-I, the mean (± SD) DL_{CO} in percentage at baseline was 37.45 ± 19.42 and follow-up during 12th week was 43.59 ± 16.78. The mean (± SD) difference of DL_{CO} in percentage at base line and at follow up was -6.14 ± 11.51, which was statistically significant (p<0.05) & actually improved. In group-II, the mean (± SD) DL_{CO} in percentage at baseline was 31.23 ± 10.36 and at follow-up was 23.68 ± 12.11. The mean (± SD) difference of DL_{CO} in percentage at base line and at follow up was 7.55 ± 10.18, which was statistically significant (p<0.05) & actually deteriorated. The mean (± SD) difference of DL_{CO} in percentage at base line & at follow-up was found -6.14 ± 11.51 and 7.55 ± 10.18 in group-I & group-II respectively, which was statistically significant (p<0.05). Thus the absolute mean difference at 12th week with 95% CI was -13.69 (-19.79 to -7.59) which signified less deterioration of DL_{CO} in percentage with NAC in the current study. In IFIGENIA trial, mean DL_{CO} (%) at the beginning of study was 43.04 ± 13.10 & 44.79 ± 15.15 in NAC & placebo group respectively and mean DL_{CO} (%) at the end of study was 41.60 ± 1.35 & 36.52 ± 1.45 in NAC & placebo group respectively. Thus the absolute mean difference at the end of study with 95% CI was 5.08 (1.17 to 8.99) which signified less deterioration of DL_{CO} (%) with NAC (P=0.01) in IFIGENIA trial similar to current study [5]. In Behr's study which was conducted in Germany, the mean percentage of DL_{CO} before NAC trial was 56.5 ± 4.0 which was actually improved after 12 weeks of NAC treatment and became 61.4 ± 4.6 [6]. As the median survival time of IPF is only 2 to 3 years, possibly immediate effect of NAC (after 12 weeks) is better than prolonged effect (after 12 months).

Currently going on well known PANTHER-IPF trial was designed in part to answer some of the questions that arose from IFIGENIA [17]. This phase III multicentre, randomized double blind placebo controlled trial aims to evaluate the effectiveness of NAC, an anti oxidant, alone (at doses comparable with the IFIGENIA study) and in combination with other established IPF medication in the prevention of lung function decline over 60 weeks. The primary outcome measure is the change in serial forced vital capacity between the study arms. They found that participants treated with triple therapy (NAC, Azathioprine, Prednisolone) had increased mortality, serious adverse events and drug discontinuation without any evidence of therapeutic benefit. Eight (11%) and one (1%) patient died in the triple therapy and placebo arms respectively. Twenty-nine per cent of the triple therapy arm required hospitalization compared with 8% of the placebo arm and 31% of the triple therapy arm experienced a serious adverse event compared with 9% in the placebo arm.

Limitations

Some potential limitations of the study also need to be addressed. First, the evidence supporting the better preservation of vital capacity and DLco in the N-acetylcysteine group should be interpreted with caution, since about 15% of the patients were lost to follow-up at 12 weeks owing to withdrawal. Second, it is unknown whether

N-acetylcysteine would have the same effects when given without prednisolone. Third, as DL_{CO} is a relatively newer technique in this country, it was much difficult to orient people regarding performing the test. Fourth, due to constraints of time, facilities and financial supports, only a short period of follow up was performed and lastly, the study was not powered or designed to detect an effect on survival.

Conclusion

The results of our trial explored that N-Acetylcysteine (NAC) at a dose of 600 mg three times daily, added to prednisolone preserves Forced Vital Capacity (FVC) and carbon monoxide diffusing capacity (DL_{CO}), thus slowing the deterioration of pulmonary function in patients with Idiopathic Pulmonary Fibrosis (IPF) better than prednisolone monotherapy. High dose N-acetylcysteine is, therefore, a rational treatment option for patients with Idiopathic Pulmonary Fibrosis (IPF).

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