Research Article

CLINICAL INVESTIGATION

A clinical study to evaluate the effects of yoga and pharmacotherapy on pulmonary functions, mechanism of inflammation, and quality of life in bronchial asthma patients

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Abstract

Background:

Aim: To evaluate the effects of pharmacotherapy and along with Yoga on airways inflammation and quality of life in bronchial asthma patients.

Methods: Patients with a clinical diagnosis of mild to moderate bronchial asthma were recruited as per the inclusion criteria and randomized into Group I (conventional anti-asthma pharmacotherapy) - and Group II (yogic intervention + pharmacotherapy). This latter group was given yogic intervention daily for 50 min in addition to conventional pharmacotherapy. Pulmonary functions, inflammatory markers, oxidative stress markers, and quality of life were assessed in both groups at baseline and after 1, 2, and 3 months of therapy and compared.

Results: The results showed that Group II patients (pharmacotherapy+yogic intervention) showed a significant and persistent improvement in pulmonary functions and greater reductions in inflammatory markers, viz. IL-6, TNF-α, and FENO, as compared to Group I (pharmacotherapy alone). The oxidative stress marker, MDA was significantly reduced whereas, anti-oxidant markers, SOD and GSH, were elevated in Group II, as compared to Group I. These differences were seen at 1, 2, and 3 months, with maximal effects being at 3 months. There was also a significant improvement in the quality of life in Group II as compared to Group I patients, as assessed by the Asthma Quality of life Questionnaire.

Conclusion: It was inferred that introducing Yoga as an adjunct therapy in patients of bronchial asthma improved asthma symptoms and the quality of life, which may possibly be due to modulations of cellular and molecular markers of inflammation and immunity.

Keywords: bronchial asthma • corticosteroids • yoga • pulmonary functions • inflammatory markers • AQLQ

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Introduction

Bronchial asthma is a chronic inflammatory disease of the airway leading to airway obstruction with considerable impact on the quality of life in the long term. In developing countries like India, urbanization and westernization are responsible for the gradual escalation in the prevalence of the disease. Approximately more than 300 million people worldwide currently suffer from asthma and this is likely to rise to 400 million by the year 2025 [1,2].

The primary goal of asthma management is to achieve and maintain control of the disease in order to prevent

exacerbations and reduce the risk of morbidity and mortality. Medical management generally focuses on treating airway inflammation and control symptoms. Conventional pharmacotherapy suppresses airway inflammation and reduces bronchial hyperreactivity and airway obstruction of asthma. Various types of medication are available for the management of acute and chronic asthma. The current pharmacotherapy depends on anti-inflammatory agents (corticosteroids) and bronchodilators (beta-agonists) by the inhalation route, but systemic use is also sometimes advocated.

Glucocorticoids (corticosteroids) are the cornerstone of conventional pharmacotherapy of asthma because of its genomic and non-genomic mechanisms of action [3] Glucocorticoid drugs are widely used for their anti-inflammatory effect in bronchial asthma. Bronchodilators are not effective in inhibition of the progression of chronic asthma but are effective in acute asthma relief. The glucocorticoid efficacy in asthma patients was first discovered in 1950. Since then, several synthetic more potent glucocorticoids have been formulated without the unwanted mineralocorticoid side effects. In acute emergencies like status asthmaticus, steroids are usually given by the intravenous route e.g. methylprednisolone or hydrocortisone. Irrespective of their mode of administration, steroids are commonly associated with some disturbing adverse effects (more with systemic administration). Inhaled corticosteroids are used in regular maintenance therapy, whereas systemically administered corticosteroids are used in patients not achieving symptomatic control with standard treatments or experiencing an exacerbation [4] Oral corticosteroids, short periods of treatment is effective in managing acute exacerbations [5] Patients who do not get treatment response at high-dose inhaled corticosteroids they are prescribed oral corticosteroids [4].

Such drug treatments are accompanied by many severe side effects typically seen with chronic use of glucocorticoid drugs [6]. Administration of Inhaled corticosteroids at higher doses is associated with the risks of diabetes onset and diabetes progression [7,8]. In long-term use by oral route causes systemic side effects-impaired growth in children, osteoporosis, cataract, hypertension, diabetes mellitus, and immune suppression [9,10]. Others include secondary osteoporosis and the leading cause of nontraumatic osteonecrosis [11,12] HPA axis suppression leading to broad endocrine disturbances, delay puberty, mood disturbances [13]. Several oral conditions such as dental caries, candidiasis, ulceration, gingivitis, periodontitis, and taste changes have been observed with inhalation therapy [14]. Since corticosteroids are the cornerstones in asthma treatment, care must be exercised in rationalizing therapy, reducing the dose and adverse effects, reducing costs, and hence ensuring better compliance.

Increasing evidence has suggested that predominant anti-inflammatory effects of glucocorticoid drugs with undisputable side effects, it is therefore important to open the door for new and better strategies in the management of asthma. Nowadays, Yoga is being practiced as a complementary and alternative medicine which is a form of mind-body medicine and the renaissance of Yoga is being remarked across the world [15]. Complementary and alternative medicine or therapy are defined as a therapy that complements the mainstream conventional treatment by ameliorating the efficacy of standard treatment. According to Herzberg and Tschudin [16] "While scientific medicine works on the disease by keeping in mind symptoms and the specific cause of disease, complementary therapy emphasizes the restoration of health.

The present study was a randomized, open-label, parallel design, controlled clinical study to evaluate the effects of Yoga and conventional treatment (Corticosteroids) on pulmonary functions, clinical symptoms, and cellular/molecular markers of inflammation and immunity and quality of life parameters, in patients of bronchial asthma. Since bronchial asthma is a chronic inflammatory disorder with associated immunological changes; markers of inflammation, immunity was assessed in both controls as well as Yoga practicing Groups. In subjects with asthma, the levels of Fractional Exhaled Nitric Oxide (FENO) have been shown to have excellent correlation with eosinophilic airway inflammation as represented by blood, sputum, Bronchoalveolar Lavage (BAL), and mucosal eosinophilia [17]. Therefore, in the present study, FENO was used as a biomarker to assess the response of asthmatic patients to yogic intervention. Oxidative stress has been implicated in bronchial asthma, and thus the effects of Yoga were evaluated on oxidative stress markers in the study subjects. Accordingly, the lipid peroxidation product, Malondialdehyde (MDA), and endogenous antioxidants (GSH and SOD) were measured. In addition, other markers of inflammation

and immunity viz. cytokines IL-6 and TNF-α were measured. Further, the effects of Yogic intervention on lung function tests and biochemical findings were correlated with quality of life parameters which were assessed by using the Asthma Quality of Life Questionnaire (AQLQ).

Materials and Methods

Study Design

This was a prospective, open-label, randomized, parallel design study, in patients of bronchial asthma, selected from the outpatient department of the Vishwanathan Chest Hospital, Vallabhbhai Patel Chest Institute, Delhi. The study was carried out jointly by the Clinical Pharmacology Unit, Department of Pharmacology and Vishwanathan Chest Hospital, Vallabhbhai Patel Chest Institute. The study was initiated after obtaining the approval of the Institutional Ethical Committee and conducted according to the ICH-GCP guidelines.

Subjects

In this study, total of 100 patients of mild to moderate bronchial asthma from outdoor facilities were enrolled and randomly assigned to two Groups, after taking into consideration the laid down exclusion and inclusion criteria. After the dropout of 15 patients, 5 patients from Group I, and 10 patients from Group II, there were 45 patients in Group I (conventional treatment) and 40 patients in Group II (conventional treatment with Yoga therapy) in the study. Written informed consent as per Performa was obtained prior to the commencement of the study.

Treatment intervention

After enrolment of patients, they were randomly allocated into two groups as per a computer-generated randomization chart and two different treatment regimens were given, Group I was given conventional treatment (inhaled corticosteroids with long-acting β-agonist) for 3 months. Group II was given conventional treatment with yogic intervention for 50 minutes daily for 3 months under the guidance of a trained Yoga instructor as per the list of yogic practices. Both the Groups were given SOS short-acting β-agonist in case of exacerbation of asthma. After recording the baseline parameters, Patients of both the group were followed up for 3 months and a comparison of parameters was done between Group I and Group II at 4 weeks, 8 weeks and 12 weeks of Yoga training and conventional drug treatment. At each follow-up, patients were physically examined and Pulmonary function, inflammatory parameters and other clinical measurements were assessed and quality of life was evaluated using AQLQ. The list of yogic practices of study is given in the supplementary table1.

Outcome measures and techniques of measurement

Pulmonary Functions

Pulmonary Function Tests (PFT) were performed with a spirometer which is the most reliable method to assess the lung functions, specifically the amount (volume) and/or speed (flow) of air that can be inhaled and exhaled. PFT is an important set of investigations used for monitoring lung functions of patients with respiratory pathology [18]. A computerized portable spirometer with ultrasonic flow head (Model: NDD Easy one PC Ultrasonic spirometer, USA) was used for the assessment. The components of the respiratory cycle are labeled as lung volumes and lung capacities (a capacity is the sum of one or more volumes) are the components of the respiratory cycle. Spirometry is the most effective way of determining the severity, allowing calculation of percentage (%) predicted FEV_1 and FVC from the resulting volume-time curve produced. The predicted values were calculated according to the age, height, and weight of the patient and compared to the corresponding measured data, customize according to the north Indian population with ethnic group correction to measure FEV_1, FVC, FEV%.

Fractional Exhaled Nitric Oxide (FENO)

The level of Fractional Exhaled Nitric Oxide (FENO) is far the most well-established biomarker for bronchial asthma and is correlated with eosinophilic airway inflammation. The FENO analyzer (Model: 09-1000, Aerocine AB, Sweden) was used for measuring NO levels in expired air according to guidelines established by the American Thoracic Society. It is a validated and reproducible non-invasive marker with a high discriminatory capacity and could be used with more than 90% specificity for the assessment of asthma in both adults and children [17]. Exhaled NO is reported directly from NO analyzers in parts per billion in exhaled breath [19]. Measures refer to the study participant exhaling directly into the instrument.

Medical and scientific value NO is generally accepted as a marker of airway inflammation. FENO values

are generally higher in individuals with asthma than in healthy controls which reflect lower airway inflammation. The populations with asthma have shown higher levels of FENO than that in a population without asthma, which decreased in response to corticosteroids [20]. In general, the upper value of the normal range for the measure of FENO is 25 ppb in adults. FENO greater than 45 ppb suggests eosinophilic inflammation and corticosteroid-responsive asthma [19]. The FENO analyzer was used for measuring NO levels in expired air in both Groups I and II.

Estimation of lipid peroxidation

The assay for lipid peroxidation was done by following the method of Okhawa et al, [21]. Malondialdehyde (MDA) is a marker of lipid peroxidation following damage by reactive oxygen species. MDA is a major reactive aldehyde resulting from the peroxidation of biological membranes. MDA was assessed in blood samples of both Groups: I and II at 0, 4, 8, and 12 weeks and compared.

Malondialdehyde assay (MDA) -Malondialdehyde was determined as described by Okhawa et al [21]. Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium lauryl sulfate, 1.5ml of 20% acetic acid (pH 3.5), and 1.5ml of 0.8% aqueous solution of thiobarbituric acid, and 0.1 ml of serum sample. The mixture was made up to 4ml with distilled water and heated at 95 ◦C for 60 min. After cooling with tap water, 5ml of n-butanol and pyridine (15:1, v/v) and 1ml of distilled water was added and centrifuged. The organic layer was separated and its absorbance was measured at 532 nm using a UV-Vis spectrophotometer (UV 5740 SS, ECIL), and MDA content was expressed as nmol/ml.

Estimation of superoxide dismutase

Superoxide Dismutase (SOD), which catalyzes the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. The assay of superoxide dismutase was performed by a method devised by Nandi and Chatterjee [22]. SOD was assessed in blood samples of both Groups: I and II at 0, 4, 8, and 12 weeks and compared.

Superoxide Dismutase (SOD) assay-serum sample was used for estimation of the SOD by using the method of Nandi and Chatterjee [22]. Assay mixture containing 2.86 ml of tris HCl buffer (50 mM, pH 8.5), 0.1 ml of Na-EDTA (30 mM), and 20 µl serum sample, after that 20 µl of pyrogallol was added. The increase in the absorbance at 420 nm was recorded on a spectrophotometer (UV 5740 SS, ECIL) from 30 s to 2 min, the lag period of 30 s was allowed for steady-state or auto-oxidation of pyrogallol to be attained. The concentration of pyrogallol was so adjusted that the rate of change of absorbance was approximately 0.020 to 0.023 per minute. The increase in the absorbance at 420 nm after the addition of pyrogallol was inhibited by the presence of SOD. One unit of SOD was defined as the amount of the enzyme required to cause 50% inhibition of pyrogallol auto-oxidation per 3 ml of the assay mixture, and the results were expressed in U/mg protein.

Estimation of glutathione (GSH)

GSH is a substrate in reactions catalyzed by GST, an endogenous antioxidant which is present in cytosol, protects from cell-damaging by binding with NAPQI and it also plays a key role in the neutralization of free radicals. The assay of GSH was performed by a method devised by the Ellman method [23].

The Ellman or thiol reagent, 5-5'-dithiobis (2-nitrobenzoic acid) (DTNB) reacts with the thiol group of Glutathione (GSH) to yield 5-thionitrobenzoic acid (TNB) which was measured by spectrophotometrically at 412 nm wavelength. The serum sample 0.1 ml was treated with 2 ml phosphate buffer (pH 8.4) and 0.5 ml of DTNB. Further 0.4 ml double distilled water was added at last and the reaction mixture was vortexed. Absorbance was measured by spectrophotometrically at 412 nm within 15 min and GSH was expressed as µ moles/ml.

Assay for IL-6 levels

IL-6 mediates the changes occurring during inflammation changing from acute to chronic situation [24]. The levels of serum cytokines IL-6 level was assessed by ELISA by commercially available kits in blood samples of both the Groups: I and II at 0, 4, 8, and 12 weeks and compared.

Blood (serum) samples were assayed for IL-6 levels using commercially available ELISA kits (Diaclone, France). Briefly, a microtiter plate pre-coated with an antibody highly specific for IL-6 was used. Blood samples were then added to the appropriate wells of the microtitre plate with biotinylated anti-IL-6 and incubated at room temperature for 1 hour. After incubation, the microtiter plate was washed with a washing solution. Then, streptavidin horse-radish peroxidase was added into all wells and again

incubated for 30 minutes at room temperature. After washing, TMB substrate was added and incubates for 12-15 minutes in dark to produce a colored reaction product. The enzyme-substrate reaction was quickly stopped by adding H_2SO_4. The absorbance was read at a wavelength of 450 nm using ELISA microplate reader and the results were expressed in pg/ml.

Estimation of TNF- α

TNF-α is an amplifying inflammatory mediator. The levels of serum cytokines TNF-α level, was assessed by ELISA by commercially available kits in blood samples of both the Groups: I and II at 0, 4, 8, and 12 weeks and compared.

Blood (serum) samples were assayed for TNF-α levels using commercially available ELISA kits (Diaclone, France). Briefly, a microtiter plate pre-coated with an antibody highly specific for TNF-α was used. Serum samples were added to the appropriate wells of the microtitreplate with biotinylated anti-TNF-α and incubated at room temperature for 3 hours. After incubation, microtiter plate was washed with washing solution. Then, streptavidin horse-radish peroxidase was added into all wells and again incubated for 30 minutes at room temperature. After washing, TMB substrate was added and incubates for 12-15 minutes in dark to produce a coloured reaction product. The enzyme-substrate reaction was quickly stopped by adding H_2SO_4. The absorbance was read at a wavelength of 450 nm using ELISA microplate reader and the results were expressed in pg/ml.

Asthma Quality of Life Questionnaire (AQLQ)

The quality of life was assessed by the Asthma Quality of Life Questionnaire (AQLQ) developed by Professor Elizabeth Juniper, McMaster University, Canada. It is an evaluative and discriminative test in bronchial asthma. It is available in bilingual forms for study, English, and Hindi. The Asthma Quality of Life Questionnaire (AQLQ) is an evaluative questionnaire to measure Quality of Life (QOL). It has also been validated by Chhabra and Kaushik [25] this version of AQLQ is suitable and acceptable in Indian asthma subjects and it can be used in clinical and research studies in asthma patients in India. The Asthma Quality of Life Questionnaire (AQLQ) is an evaluative questionnaire that is sensitive to small changes over time and was therefore appropriate for capturing the effects of an intervention in a clinical trial [26]. The AQLQ measure Quality Of Life (QOL) under sub-domains; QOL symptoms, QOL activity limitation, QOL emotional function, and QOL environmental stimuli. The AQLQ is a 32-item disease-specific questionnaire that has been validated to measure the problems that adult patients with asthma experience in their daily lives. The total 32 questions or items of AQLQ, are categorized under sub-domains; 12 questions in symptoms, 11questions in activity limitation, 5 questions in emotional function, and 4 questions in response to environmental stimuli. It gives an accurate estimate of the amount of impairment experienced by individual patients. Patients responded to each item on a 7-point scale. The overall score was the mean of all the items, the maximum score of 7 means no impairment and 1 score indicates maximum impairment of quality of life. It can measure the small improvement in patients after treatment. These diseases specific questionnaire of AQLQ, can assess control and uncontrolled asthma and measure the severity of the disease. The list of Asthma Quality of Life Questionnaire (AQLQ) of the study is given in supplementary annexure-1.

Statistical Analysis

Statistical analysis was carried out using the SPSS and graph pad prism (version 5.01) for Windows. The data are expressed as Mean ± SEM and analyzed by one-way ANOVA for repeated measures followed by post hoc Tukey's multiple comparison test and paired t-test variable appropriate. A minimum p-value <0.05 was used as a level of significance in all statistical tests.

Results

Socio-demographic data

Analysis of the data showed that there was no significant difference found statistically in both age and sex distribution, both groups were also comparable at baseline with respect to the severity of asthma. The male: female sex ratio for Group I was 21:24 and Group II was 23:17 mean age for Group I was 36 ± 11.99 and Group II was 32 ± 10.33. The severity of asthma results is summarized in Table 1.

Table 1. Distribution of patients as per asthma severity in Group I and Group II

Bronchial Asthma	Group I (45)		Group II (40)	
	No.	%	No.	%
Mild	34	75.6	28	70
Moderate	11	24.4	12	30
Total	45	100	40	100

Pulmonary functions test

Analysis of data showed that FEV_1 and FEV_1/FVC (%) values were improved in both the Groups after treatments, but statistically significant results were observed in Group II as compared to Group I at 1, 2, 3 months of treatment. Group, I patients showed significant improvement in FEV_1 and FEV_1/FVC (%) after 1 month of pharmacotherapy, but surprisingly the percentage improvement in FEV_1 and FEV_1/FVC (%) decreased, after 2nd and 3rd months. Interestingly, the results showed that Group II patients, who received the adjunct yogic intervention, showed significant improvement in PFT throughout the period of follow up i.e. for three months, thus suggesting a greater reduction in airway obstruction. The results are summarized in Table 2.

Inflammatory marker Fractional exhaled nitric oxide (FENO)

The analysis of data showed that significant and magnitudinal reduction in exhaled nitric oxide was observed in Group II patients than in Group I from 1 month. These results are summarized in Table 3.

Oxidative markers

The overall analysis of data showed that reduction of MDA and increase in GSH and SOD was found in both groups but significant changes were found in Group II after 3 months of treatment. These results are summarized in Table 4.

Inflammatory markers Interleukin 6 (IL-6) and Tumor Necrosis Factor Alpha (TNF-α)

In analysis of data showed that a significant and remarkable decrease of serum TNF- α and IL-6 was found in Group II patients than in Group I after 3 months of treatment in bronchial asthmatic patients. These results are summarized in Table 5.

Asthma Quality of Life (AQLQ)

The data of all the aspects of the Asthma Quality of Life Questionnaire, i.e. symptoms, activity limitation, emotional function, and response to environmental

Table 2. Comparison of pulmonary functions test of the patients of bronchial asthma of Group I (conventional treatment) and Group II (conventional treatment with Yoga therapy)

Parameters	Groups	Baseline	1 month	2 month	3 month
FEV_1 (L)	Group I	2.247 ± 0.12	2.539± 0.30 *	2.253 ± 0.16	2.416 ± 0.11
	Group II	2.196 ± 0.09	2.506± 0.13 *	2.658 ± 0.15 *	2.701 ± 0.12 **
FVC(L)	Group I	3.096 ± 0.12	3.333± 0.48	3.032 ± 0.13	3.034 ± 0.12
	Group II	3.208 ± 0.15	3.582 ± 0.17	3.304 ± 0.19	3.299 ± 0.16
FEV_1/FVC (%)	Group I	73.53 ± 1.94	78.37± 2.65 *	76.87 ± 3.30	77.23 ± 1.48
	Group II	71.42 ± 1.81	74.88 ± 2.07 *	76.18 ± 2.04 *	80.00 ± 1.31**

All data are expressed as mean ± SEM. *p <0.05, **p<0.01 based on paired t test comparisons with baseline values at 0 month

Table 3. Comparison of FeNO of the patients of bronchial asthma of Group I (conventional treatment) and Group II (conventional treatment with Yoga therapy)

Parameters	Groups	Baseline	1 month	2 month	3 month
FeNO (ppb)	Group I	38.97 ± 3.47	33.95 ± 3.12	29.04 ± 2.40	25.09 ± 2.28 *
	Group II	43.54 ± 4.31	31.86 ± 4.37*	22.59 ± 2.48 *	18.40 ± 1.49 **

All data are expressed as mean ± SEM. *p < 0.05, **p < 0.01 vs 0 month value, based on one way ANOVA followed by Tukey's multiple comparison test.

Table 4. Comparison of oxidative markers in the patients of bronchial asthma of Group I (conventional treatment) and Group II (conventional treatment with Yoga therapy)

Parameters	Groups	Baseline	1 Month	2 Months	3 Months
MDA (nmoles/ml)	Group I	6.250 ± 0.72	4.368 ± 0.86	4.168 ± 0.86	3.983 ± 0.63
	Group II	5.808 ± 0.79	4.529 ± 0.84	3.123 ± 0.93	2.791 ± 0.48 *
GSH (µmoles/ml)	Group I	0.8742 ± 0.06	1.022 ± 0.08	1.044 ± 0.11	1.076 ± 0.06
	Group II	0.9555 ± 0.06	1.137 ± 0.09	1.302 ± 0.10	1.507 ± 0.09 *
SOD (U/mg protein)	Group I	237.6 ± 27.88	500.2 ± 93.89	352.3 ± 97.41	253.1 ± 35.59
	Group II	230.3 ± 33.84	519.9 ± 72.45	416.0 ± 100.9	268.9 ± 45.92 *

All data are expressed as mean ± SEM. *p < 0.05 vs Baseline value, based on one way ANOVA followed by Tukey's multiple comparison test.

Table 5. Comparison of Interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNF-α) levels of the patients of bronchial asthma of Group I (conventional treatment) and Group II (conventional treatment with Yoga therapy)

Parameters	Groups	Baseline	1 month	2 months	3 months
IL-6 (pg/ml)	Group I	128.40 ± 22.54	106.00 ± 20.22	86.28 ± 17.21	70.11 ± 11.67
	Group II	150.90 ± 15.82	97.77 ± 17.52	82.29 ± 18.29	60.64 ± 9.401*
TNF-α (pg/ml)	Group I	54.24 ± 11.59	35.66 ± 8.577	29.50 ± 6.932	24.22 ± 3.665
	Group II	70.62 ± 11.97	49.50 ± 12.03	29.62 ± 13.25	13.49 ± 1.539 *

All data are expressed as mean ± SEM. *p <0.05 vs 0 month value, based on one way ANOVA followed by Tukey's multiple comparison test.

Table 6: Comparison of Quality of life parameters of the patients of bronchial asthma of Group I (conventional treatment) and Group II (conventional treatment with Yoga therapy)

Parameters	Groups	Baseline	1 month	2 month	3 months
SD	Group I	5.008 ± 0.16	5.620 ± 0.16	6.085 ± 0.13 *	6.059 ± 0.10 **
	Group II	4.492 ± 0.24	5.492 ±0.21*	6.187 ± 0.19 **	6.398 ± 0.11 ***
AL	Group I	4.491 ± 0.21	5.093 ± 0.22	5.393 ± 0.34	5.304 ± 0.19 *
	Group II	4.162 ± 0.19	5.365 ± 0.22 *	5.567 ± 0.16 **	5.753 ± 0.13 ***
EF	Group I	3.924 ± 0.21	4.773 ± 0.35	4.920 ± 0.35	5.276 ± 0.25 *
	Group II	3.779 ± 0.23	4.750 ± 0.42	5.705 ± 0.27 *	5.897 ± 0.22 **
ES	Group I	3.019 ± 0.28	3.942 ± 0.32	3.864 ± 0.45	4.073 ± 0.30
	Group II	2.988 ± 0.27	3.703 ± 0.45	4.182 ± 0.36 *	4.758 ± 0.24 **

SD: symptom domain; AL: Activity Limitation; EF: Emotional function; ES: Environmental stimuli. All data are expressed as mean ± SEM. *p < 0.05, **p < 0.01 vs 0 month value, based on one way ANOVA followed by Tukey's multiple comparison test.

stimuli were improved but a greater magnitude of improvement was observed in Group II patients after 1, 2, and 3 months of adjunct Yoga treatment than Group I. These results are summarized in Table 6.

Discussion

The results of this study showed that the improvement in pulmonary function was found after conventional treatment but the improvement was not persistent after 4 weeks of treatment. The conventional treatment consisted of Inhaled Corticosteroid (ICS) with Long-Acting β-Agonist (LABA), it has been reported that glucocorticoids (corticosteroids) are the most effective anti-inflammatory treatments for many inflammatory and immune diseases, including asthma. However, a few patients with these diseases show a poor or absent response even to high doses of Corticosteroids. Corticosteroid resistance or insensitivity is a major obstacle to the treatment of inflammatory diseases including asthma. This may occur due to chronic glucocorticoid drug use at high doses for prolonged periods and even small doses for only a few days may lead to suppression of the hypothalamic-pituitary-adrenal axis through negative feedback, the recovery of the HPA axis is much delayed. This may lead to secondary adrenal insufficiency [27-29]. However, the patients who received the adjunct yogic intervention, showed significant improvement in PFT throughout the period of follow up i.e. for three months, thus suggesting a greater reduction in airway obstruction and persistent control of the disease.

The larger magnitude of (%) reduction in IL-6 and TNF-α levels was measured in patients who were on Yogic intervention as an adjunct as compared to patients who were on conventional treatment only. In asthma, IL-6 is inversely correlated with lung function, progressive loss of lung function in untreated asthma. In allergic asthmatic patients elevation of IL-6 is associated with loss of central airway function. IL-6 is associated with airway inflammation and during the progression of obstructive airway disease, and a key player in the transition between acute to chronic stage inflammations. TNF-α may have an important amplifying effect on asthmatic inflammation. TNF-α is a powerful pro-inflammatory factor that regulates many aspects of macrophage function and is considered as a main mediator of inflammation by activating the secretion of cytokines from a variety of cells in the airways [30,31]. Both pro-inflammatory cytokines, TNF-α and IL-6, act as master regulators of inflammation, in which NF-KB (transcription factor) leads to increased expression of multiple inflammatory genes, including those of cytokines, chemokines, and adhesion molecules [32,33]. All these indicate that these cytokines are cytotoxic, pro-inflammatory, and acts as an immunomodulatory agent. In closing, both these cytokines show synergistic effects with each other and amplify inflammation [24,34]. Hence,

these cytokines are valid and potential biomarkers in airway inflammation. The effects of conventional treatment (ICS+LABA) and conventional treatment plus Yogic intervention were assessed on pro-inflammatory cytokines in patients of bronchial asthma. This present finding strongly suggests the anti-inflammatory effects of Yoga when used as an adjunct to conventional therapy in patients of bronchial asthma.

Proinflammatory mediators lead to increased production of ROS and the gaseous molecule Nitric Oxide (NO) [35]. High ROS production is correlated with a decrease in Forced Expiratory Volume in 1 second (FEV_1) [36], which indicates an increase in asthma symptoms with enhanced oxidative stress. The human lung has an antioxidant system to combat ROS and RNS injury. GSH is an endogenous antioxidant and SOD is endogenous antioxidant enzyme, important for maintaining a redox balance of cells by reacting with reactive oxygen molecules and other free radicals. The major enzymatic antioxidant in the airways, Superoxide Dismutase (SOD), plays an important role in offering protection to the airways against oxidative stress. It converts superoxide radicals to molecular oxygen and hydrogen peroxide which is eliminated by glutathione peroxidase. SODs get inactivated by reactive oxygen and nitrogen species. Thus, the antioxidant defense is impaired in hyperactive airways. The situation is further aggravated during acute exacerbations of asthma. In asthma, a greater amount of oxidative stress significantly decreases SOD activity as compared to normal humans- thus serving as a sensitive marker of asthma severity. The other antioxidant enzymes were not found to be affected in this asthma study. Corticosteroid treatment may stimulate SOD production and restoration of intracellular SOD activity, and such anti-inflammatory and anti-oxidative stress property of corticosteroids have been suggested [37]. Another major endogenous antioxidant produced by cells is reduced Glutathione (GSH). A Thiol group of cysteine is able to donate a reducing equivalent ($H^+ + e^-$) to other unstable molecules such as ROS, xenobiotics to maintain a reducing environment of the cell. The capacity to recycle GSH makes it the main antioxidant in cellular defense, which gets depleted during oxidative burden [38]. In view of this, the effects of conventional treatment and conventional treatment plus adjunct Yoga therapy were evaluated on oxidative stress markers in patients of bronchial asthma. Inadequate GSH and SOD activity potentiates cellular damage and tissue injury through reduced scavenging of free radicals. The results of this study showed that the levels of antioxidant GSH were increased and MDA levels (a marker of lipid peroxidation) were decreased in both groups, but more marked results were found in patients of adjunct Yoga therapy after 3 months. There were increases in SOD after 1 month of treatment in both groups, and this may have been due to corticosteroid-induced stimulation of SOD production and restoration of intracellular SOD activity. In Group II patients, significant and remarkable increases in SOD were found, and this finding was consistent with the above results thus reaffirming and emphasizing the protective effects of yogic intervention against oxidative damage seen in asthma patients.

In asthma, the level of FENO is an excellent marker of eosinophilic airway inflammation, which proves a decrease in eosinophilic airway inflammation. After corticosteroids treatment the levels of FENO were reduced in both the groups after 3 months of treatment. Similar to earlier observations, the magnitude of response was much more and consistent in patients of the adjunct Yoga therapy group as compared to the conventional treatment group. The quality of life was assessed by the Asthma Quality of Life Questionnaire (AQLQ). All the aspects of the AQLQ i.e. symptoms, activity limitation, emotional function, and response to environmental stimuli, were established in this study to evaluate disease severity and efficacy of treatments. Although, all the factors of the AQLQ viz. symptoms, activity limitation, emotional function, and response to environmental stimuli were found to be improved in both groups, but more remarkable results were seen in patients of conventional treatment along with the Yogic intervention group. The results suggest that yogic intervention as an adjunct therapy improves the quality of life by achieving resilience against environmental stimuli, emotional stability which provides psychophysiological benefits and may work to decrease symptoms and lead to efficient pulmonary functions.

Conclusion

In conclusion, the long-term pharmacotherapy with corticosteroids (and beta-adrenergic agonists) in bronchial asthma has its limitations and can influence treatment compliance resulting in poor disease control. In general, the risk of secondary adrenal insufficiency increases with the dose and duration

of corticosteroid therapy. Hypothalamic-pituitary-adrenal axis suppression is frequently unrecognized; some patients come to light when an adrenal crisis is precipitated by physical stress. Alternative/complementary modes of therapy are required to address this problem. Our findings revealed that introducing Yoga as an adjunct therapy in patients of bronchial asthma improved pulmonary functions and the quality of life, which could be attributed to modulations of disease-specific inflammatory and immune markers as well as by restoration of endogenous adrenal (steroid hormone) axis. Such complementary therapy can make significant contributions to improve the quality of life and disease control in patients of bronchial asthma.

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Executive summary

Background: Co-amoxiclav is associated with Drug-Induced Liver Injury (DILI). HLA genotype is an important predictor of DILI susceptibility but it is likely that non-HLA risk factors also contribute. This study aimed to characterize non-HLA risk factors in larger cohorts than previously. Although KCNJ1 is known for its regulation of kidney activities through modulation of potassium homeostasis, the association seen between this gene and co-amoxiclav DILI, detected in a previous Genome-Wide Association Study (GWAS), which was close to p-value threshold significance, was stimulating to investigate its role in DILI susceptibility.

Methods: A variant (rs2855790) in KCNJ1 was genotyped to extend the previous findings. 73 co-amoxiclav adjudicated DILI cases and 75 community controls were genotyped using RFLP-PCR assay. Drug causality of the cases was assessed using the RUCAM method. The data obtained were analyzed for its odds ratio (OR) value and Fisher's exact test was used to generate a p-value.

Results: Although, variant allele frequency in cases were much lower (15.8%) than in controls (24.7%); however, the difference was not statistically significant (OR=0.58, 95% CI=0.29-1.1; P=0.13). Hence, the previously reported association with KCNJ1 (rs2855790) could not be confirmed in this new cohort of co-amoxiclav DILI.

Conclusion: Given the biological role of KCNJ1 in the kidney, not the liver, this gene was not a very biologically plausible candidate as a DILI gene so the final result obtained for this is not too surprising.

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