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Original Research Article

To Know the Association Between IRISIN and Spirometry, 6 Minute Walk Test, Clinical Features, Dyspnea Score and Quality of Life

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Conflict of interest: Nil

Abstract:

Introduction: Chronic obstructive pulmonary disease (COPD) is one of the most common causes of disease burden both globally and in India it needs more emphasis than ever, the third leading cause of death in India and globally. There is a need for more diagnostic and therapeutic options. We studied Serum IRISIN in hope of finding a new biomarker for COPD. Serum IRISIN is a skeletal muscle protein associated with physical activity, the levels of which are increased in COPD patients after 8 weeks of pulmonary rehabilitation. There is no Indian data available on IRISIN in COPD patients.

Objective:

Primary objective of our study is to assess the level of serum IRISIN level in COPD before starting Pulmonary Rehabilitation and after 8 weeks of Pulmonary Rehabilitation.

Secondary objective is to study correlation between IRISIN and spirometry, 6 minute walk test, Clinical features, Dyspnea score and Quality of life.

Methodology: Cases were selected from OPD department of Pulmonary Medicine JSS Hospital Mysore. 80 subjects satisfying inclusion and exclusion criteria underwent PFT, 6MWT, SGRQ-C, CAT score, BODE index and ELISA for serum IRISIN levels. Statistical analysis included, Chi2 test/Fisher extract test, One, Independent t Test, Mann Whitney test, Pearson correlation test and spearman correlation test.

Results: The mean age of the study group was 62.4 ± 9.8. Male subjects in the study were 50 and female subjects were 30. There is a significant increase in IRISIN levels between COPD (control group, no pulmonary rehabilitation) and COPD (intervention group, with pulmonary rehabilitation) with significant p value <0.001.

Conclusion: IRISIN levels are significantly high in COPD patients with pulmonary rehabilitation compared to COPD patients without pulmonary rehabilitation as hypothesized. (p value <0.001). Serum IRISIN levels correlate well with quality of life suggesting IRISIN may also reflect changes in domains other than airflow limitation in COPD patients. There is no significant difference in serum IRISIN levels among male and female COPD patients, suggesting that levels of IRISIN are independent of age.

Keywords: IRISIN, COPD, pulmonary.

Introduction

1- Exposure to particles

- Tobacco smoke – 10 packs/years, 50% of smokers develop COPD.
- Indoor air pollution – from heating and cooking with biomass fuel in poorly ventilated homes (at least 25 years of exposure).

2- **Occupational dust** - organic and inorganic (attributable Risk 15%in American population) [1].

a) Automobile – drivers, vehicular mechanics, fertilizer manufacturing, chlorinated oraginic compunds dyes, explosives, rubber products, metal itching, plastics, ammonia exposure in refrigeration and petroleum refining, grain dust and funguses in farmers, textile mill manufacturing. Leather manufacturing, food products manufacturing and sales. Beauty care workers and welders in automotive industries.

b) Exposure to cystalline silica; cement industry, Brick manufacturing, Pottery and ceramic work. Silica sand, granite and diatomaceous earth industries, gold mining and iron and steel founding.

3- Outdoor air pollution Reduced lung volume

- Previous tuberculosis (28-68% cases of post treated tb <2.9-6.6 folds increase risk.
- Early childhood recurrent lower respiratory tract infections.

c) Lung growth and development.

d) Poor nutrition.

- Female gender.
- Old age.
- Low socioeconomic status.

Genes [2] severe hereditary deficiency of alpha-1 antitrypsin.

- SERPINA1 encoding AAT1.
- MMP12, Matrix metalloproteinase 12.
- HHIP, hedgehog interacting genes.
- FAM13 A.
- HMOX1, Heme Oxygenase 1, ADRB2, b2 adrenergic receptor.
- TGFB3, transforming growth factor.

Pathogenesis of COPD

COPD development is implicated by protease and anti protease imbalance, oxidant and antioxidant imbalance, excessive reduction of cytokines and chemokine's and recurrent viral and bacterial infection. [3,4] COPD involve destruction of alveoli and remodeling of airways. COPD patients are associated with frequent and recurrent exacerbation which is associated with poor pulmonary innate immunity causing respiratory symptoms like cough with sputum production and dyspnea. Lung function decline with every episode of exacerbation as a major cause of mortality and morbidity. [5]

Inflammation of the small airways is usually seen in people who smoke, the normal protected response is amplified in COPD leading to tissue destruction and disruption in repair mechanism and impairment of defense mechanism that limits such destruction. The inflammatory and structural changes in the airway persists even after smoking cessation there is an imbalance between protease and anti protease and imbalance between oxidant and antioxidant in the lung.

Inflammatory cells – COPD is characterized by increased number of neutrophils macrophages and lymphocytes, these inflammatory cells release cytokines and chemokine's that causes the structural and inflammatory damage.

Inflammatory mediators – many inflammatory mediators are increased in COPD including LTB4, which is a neutrophil and T cell attractant produce by neutrophils, macrophages and epithelial cells, these attract cell from the systemic

circulation and amplified the inflammatory response , the other chemotactic factors are interleukin 8, growth related oncogene, TGF alpha are some of the known inflammatory mediators.

Molecular Mechanism in Pathogenesis of COPD

The cells primarily involved in COPD inflammation are neutrophils, macrophages and lymphocytes. These inflammatory cells further release a battery of inflammatory mediators like cytokines, chemokine and chemo attractants which perpetuate the inflammation leading to an uncontrolled cascade.

Neutrophils by releasing chemo attractants like interleukin-8 (IL-8) and leukotriene B4 (LTB4) further attract neutrophils to the site.6 Proteolytic enzymes such as elastases, proteinase-3, cathepsin b, cathepsin G and matrix metalloproteinase (MMP) released by neutrophils cause damage to elastic lung tissue. [7]

Macrophages release cytokines and chemokine such as IL-6, IL-8, IL10, TNFα, LTB4, etc and reactive oxygen species which attract and activate various inflammatory cells, and series of proteinases, particularly MMPs such as MMP-2, MMP-9, MMP-12, MMP-14, with tremendous elasteolytic potential and elastin lytic cysteine proteinases such as cathepsin K, L and S. [8]

CD8+ lymphocytes release destructive enzymes such as perforins and granzyme B which have the ability to induce apoptosis of the alveolar epithelial cells and CD4 Lymphocytes induce autoimmune response to the lung tissue. [9]

A series of COPD related pathological changes have also been attributed to oxidative stress such as oxidative inactivation of anti proteases and surfactants, mucus hyper secretion, membrane lipid peroxidation, alveolar epithelial injury, remodeling of extracellular matrix, and apoptosis, reduction in elastin collagen synthesis and fragmentation of these skeletal proteins and steroid unresponsiveness. [10]

Importance of scientific knowledge in COPD

Rate of decline in FEV1 is considered the gold standard for measuring COPD disease progression hence clinical trials that relay on decline in FEV1 to assess the effectiveness of therapy requires thousands of patients and years of follow up making the test of new treatment inefficient and expensive , numerous COPD clinical trials used markers of stress and inflammation for measuring the disease progression but these markers are not specific and can be confounded by other coexisting disease condition.[11]

- There is an urgent need for a new blood biomarker for
- Detecting disease activity and progression.
- Therapeutic response.
- Exacerbation.

- Fast and slow decliners.
- Identification of such non invasive biomarkers may help In better management of disease and development of newer therapies and help in understanding COPD. [12]

Evidence supporting Serum IRISIN a New biomarkers associated with Physical Activity in COPD patients.

IRISIN is a recently identified hormone secreted by skeletal myocytes which has been proposed to mediate the beneficiary effects of exercise. Physical activity has been one of the principal targets of treatment for COPD. Circulating IRISIN could be used to evaluate physical activity in COPD patients and increased after an 8 week of exercise training, Serum IRISIN level may prove to be a valuable biomarker in clinical follow up COPD.

In a study by IJIRI et al, [13] 2015 reported that Serum IRISIN level did not significantly correlate with any pulmonary parameter and 6MWD, however serum IRISIN level was associated with the physical activity levels with all subjects. In COPD patients acute exercise did not affect serum IRISIN levels but an 8 weeks exercise training was linked to significant increased in levels.

Hypothesis: we hypothesize that serum IRISIN levels directly correlate with physical activity as well as quality of life in COPD patients. [13]

Materials and Methods

1. Source of Data: Patients with COPD attending the Department of Pulmonary Medicine, JSS HOSPITAL MYSURU meeting inclusion and exclusion criteria will be taken into study.
2. Duration of Study: From October 2016 to September 2018
3. Type of Study: Cohort study
    - Study site: JSS Medical College & Hospital, Mysore.
    - Study design: Cohort study.
    - Study period: This study has been carried out for a period of 2 years.

Method of collection of data:

Sample size: 80 COPD PATIENTS (40 in intervention group and 40 in control group)

The study will be conducted over a period of 2 years at pulmonary medicine Department, JSS Hospital Mysuru, it includes COPD patients coming to JSS Hospital will be screened for inclusion and exclusion criteria after explaining them about the study, patients satisfying inclusion criteria will be included in the study after obtaining informed consent, patient serum will be collected and analyzed for serum IRISIN level in patients with COPD at baseline and after 8 weeks of pulmonary rehabilitation.

Inclusion criteria:

1. COPD Patients who are not on pulmonary rehabilitation program
2. Age >40 years of either gender
3. Able to undergo informed consent process
4. Able to perform the study related process

Exclusion criteria:

1. Subjects in acute exacerbation /too sick/require hospitalization for any illness.
2. Subjects with contraindication for any of the study related procedure like recent MI. Recent stroke, Eye surgery Recent pneumothorax, Stress incontinence, Hemoptysis, Deep vein thrombosis,
3. Patient not giving consent
4. Patient less than 18 years

Observation and Results

During the study period (OCTOBER 2016 - SEPTEMBER 2018)

A total of 80 patients with COPD who visited Pulmonology Department; of which all patients could perform Spirometry. 24 patients were excluded from the analysis for one of the following reasons: 8 patients did not give consent and 16 patients were not willing for pulmonary rehabilitation and regular follow up. Finally,68 patients (136 blood samples) were included for further analysis.

In this study following groups were included-

A total of 80 subjects were studied, whom include, 50 patients with COPD (Smoker COPD) and 30 subjects with COPD (biomass exposure/non smoker COPD).

Table 1: Serum IRISIN levels between control and intervention group (baseline and after 8 weeks of pulmonary rehabilitation)

	Group						P
	Control			Intervention			
	Median	Q1	Q3	Median	Q1	Q3	
SERUM IRISIN ng/ml	1.34	0.94	1.61	1.80	1.27	2.59	0.01
SERUM IRISIN ng/ml AFTER 8 WEEKS	1.86	1.15	2.57	5.09	4.40	5.54	<0.001
Increase in SERUM IRISIN ng/ml	0.49	-0.08	1.07	2.88	1.55	4.02	<0.001

Mann Whitney test

There is a statistical significance increase in serum IRISIN levels after 8 weeks of pulmonary rehabilitation in intervention group.

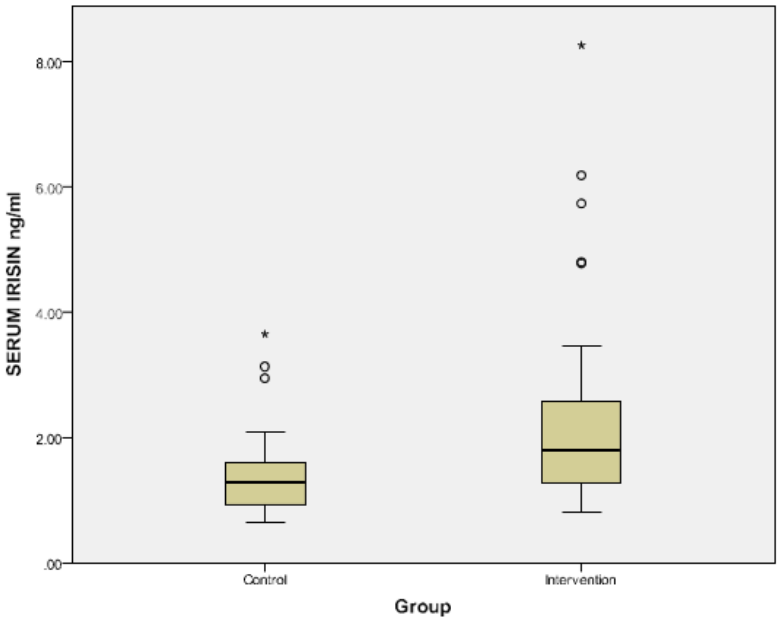

Figure 1: Comparison of serum IRISIN in control and intervention group at base line

There is increase in serum IRISIN in intervention group compare to control group before pulmonary rehabilitation.

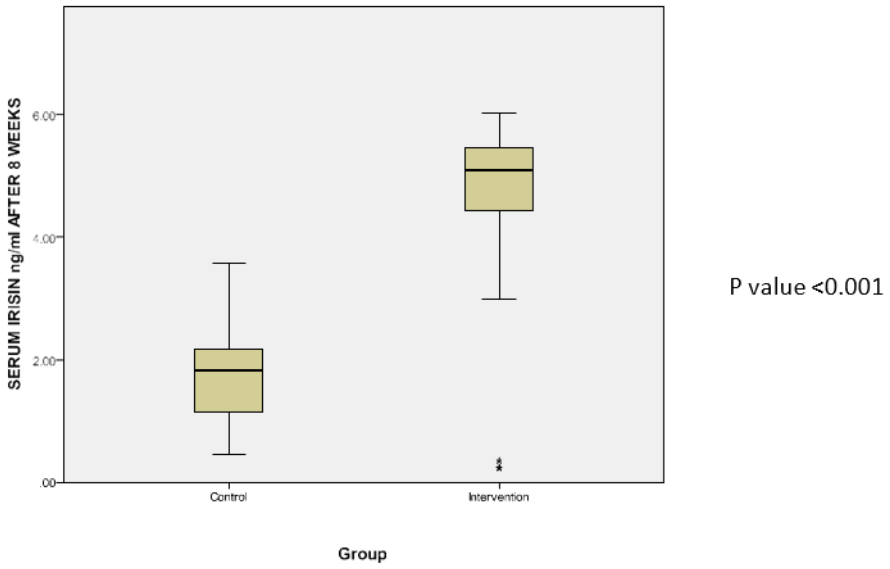

Figure 2: There is a significant increase in serum IRISIN level after 8 weeks in intervention group compare to control group

Two patients had significant decrease in serum IRISIN level after 8 weeks of pulmonary rehabilitation.

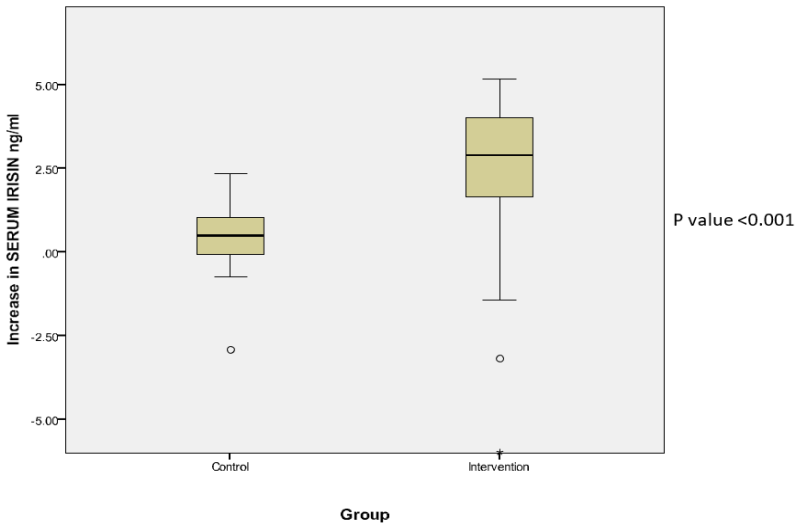

Figure 3: Comparison of increase in serum IRISIN level between control and intervention group after pulmonary rehabilitation

Table 2: Comparison of serum IRISIN with PFT parameters

		Pre FVC test	Pre FVC percent	Post FVC test	Post FVC percent	FVC change percent	FEV1 Pred	Pre FEV1 test	Pre FEV1 percent	Post FEV1 test
SERUM IRISIN ng/ml	r	0.329	0.172	0.311	0.137	-0.028	0.159	0.332	0.175	0.307
	p	0.003	0.127	0.005	0.227	0.808	0.160	0.003	0.121	0.006
	N	80	80	80	80	80	80	80	80	80
		Post FEV1 percent	FEV1 change percent	FEVF VC ratio pred	pre-FEVFV C ratio-test	pre FEVFV C ratio percent	post FEVF VC-ratio test	post FEVFV C ratio percent	Percentag e Change	
SERUM IRISIN ng/ml	r	0.142	-0.028	-0.121	0.029	0.051	0.049	0.078	0.023	
	p	0.210	0.807	0.285	0.796	0.651	0.663	0.490	0.840	
	N	80	80	80	80	80	80	80	80	

Pearson correlation
There is a statistical significance is between serum IRISIN and pre FVC test and Post FVC test.
There is statistical significance is between serum IRISIN and pre FEV1 test and post FEVI test.
There is no significant difference between serum IRISIN and other parameters of PFT.

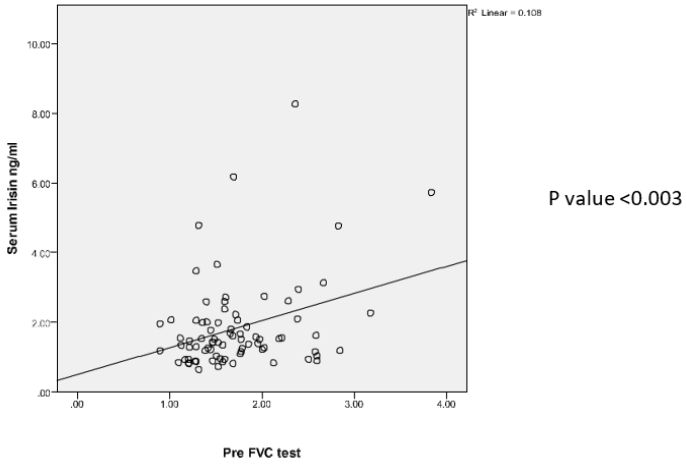

Figure 4: Comparison of serum IRISIN with pre FVC test

There is statistical significance with p value 0.003, as pre FVC increases serum IRISIN also increases linearly.

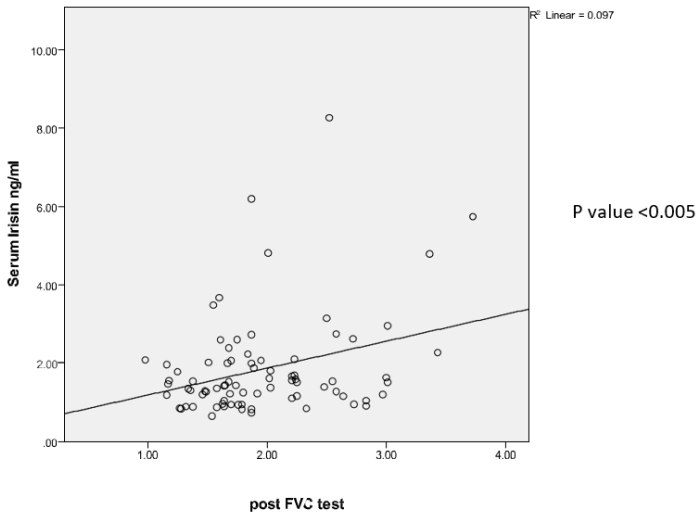

Figure 5: Comparison of serum IRISIN and post FVC Test

Statistically significance with p value 0.05, as post FVC Increases serum IRISIN also increases linearly.

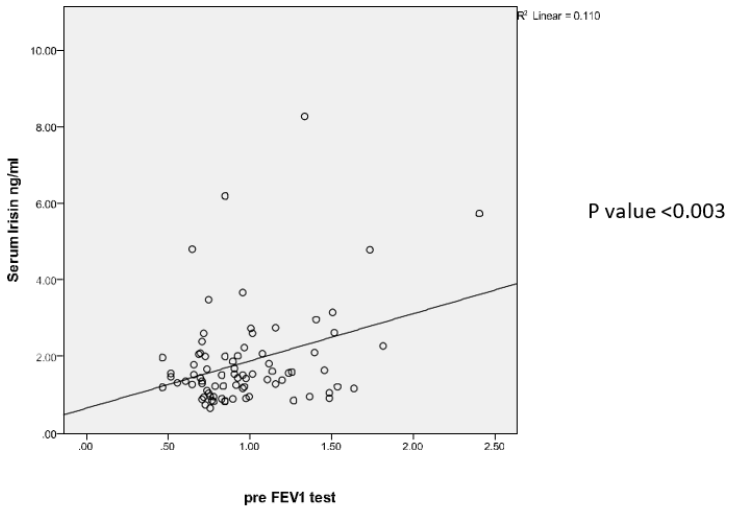

Figure 6: Comparison between serum IRISIN level and pre FEV1 test

Statistical significance with p value 0.003, as pre FEV1 Increases serum IRISIN also increases linearly.

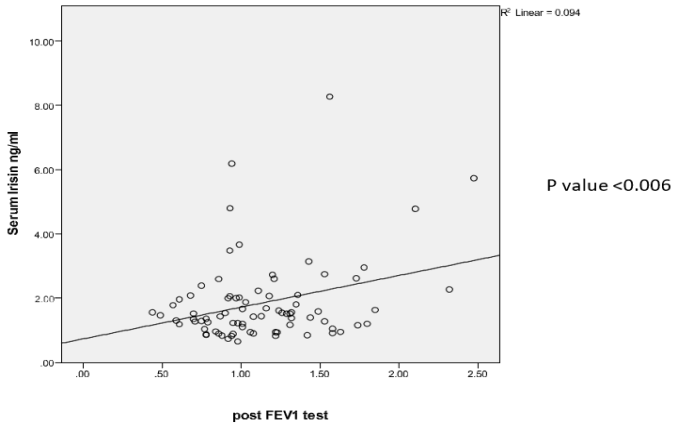

Figure 7: Comparison between serum IRISIN and post FEV1 test

No statistical significance with p value 0.06.

Table 3: Comparison between serum IRISIN and pack years, biomass index, CAT score, BODE index

		PACK YEAR	BIOMASS_IN DEX	CAT SCORE	BODE INDEX
SERUM IRISIN ng/ml	R	0.189	-0.169	-0.078	-0.079
	p	0.093	0.133	0.492	0.488
	N	80	80	80	80

No statistical significance between serum IRISIN and smoking pack year, biomass index, CAT score and BODE index.

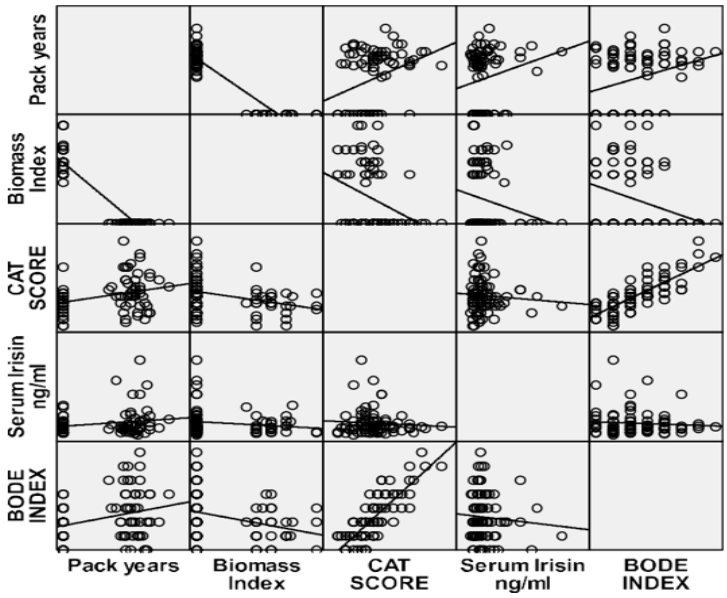

Figure 8:

Table 4: Comparison of serum IRISIN and 6MWD, SGRQC

		Pred 6MWT Distance	6MWT Distance Walked	6MWD Percent	Symptoms	Activity	Impact	TOTAL SGRQ-C score	SGRQ-C percent
SERUM IRISIN ng/ml	Pearson Correlation	-0.044	0.055	0.115	-0.029	0.033	-0.012	0.022	0.029
	Sig. (2-tailed)	0.697	0.626	0.310	0.800	0.769	0.919	0.848	0.799
	N	80	80	80	80	80	80	80	80

No statistical significance between serum IRISIN and 6MWD, SGRQC

Table 5: Comparison between MMRC, GOLD grade, ABCD assessment tool and serum IRISIN

		SERUM IRISIN ng/ml			P
		Median	Percentile 25	Percentile 75	
MMRC	0.00	1.58	1.20	2.26	0.9
	1.00	1.51	1.04	2.06	
	2.00	1.39	1.21	1.99	
	3.00	1.51	1.20	1.66	
GOLD GRADE	1.00	2.26	.90	2.94	0.8
	2.00	1.52	1.15	2.06	
	3.00	1.35	1.10	1.99	
	4.00	1.49	1.17	1.53	
ABCD ASSESSEMENT TOOL	A	1.43	1.15	2.07	0.9
	B	1.30	.94	2.05	
	C	1.53	1.10	1.66	
	D	1.52	1.26	1.99	

Kruskall Wallis test
No statistical significance between MMRC, GOLD grade, ABCD assessment tool and serum IRISIN.

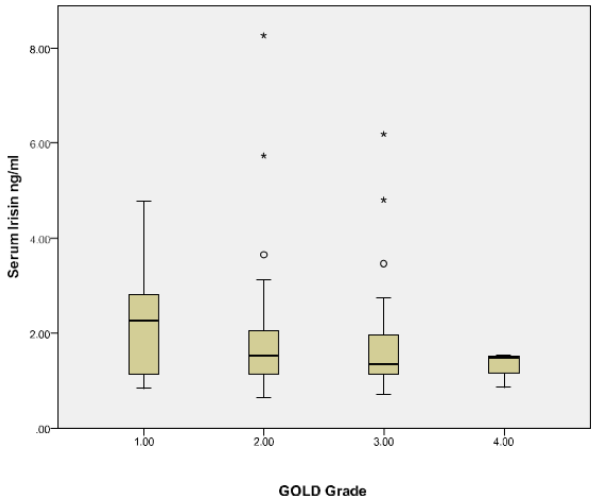

Figure 9: Comparison between serum IRISIN and gold grade

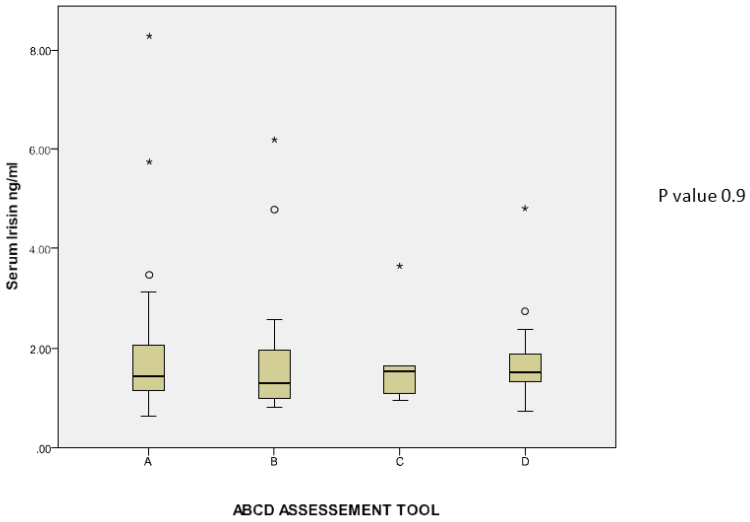

Figure 10: Comparison between serum IRISIN and ABCD assessment tool

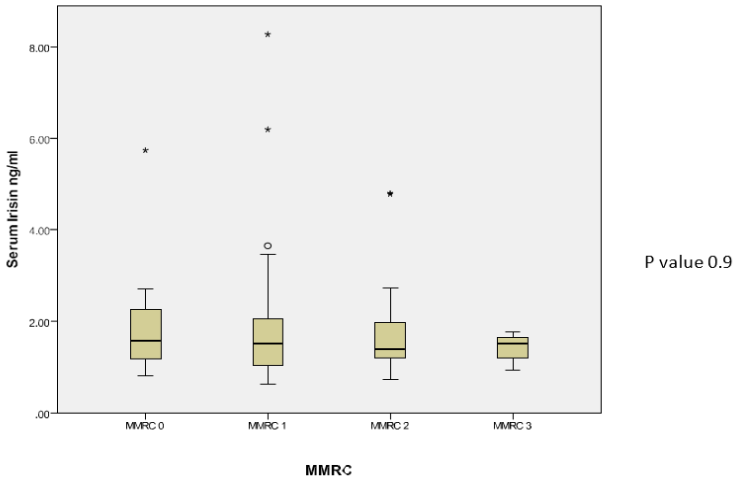

Figure 11: Comparison between serum IRISIN and MMRC

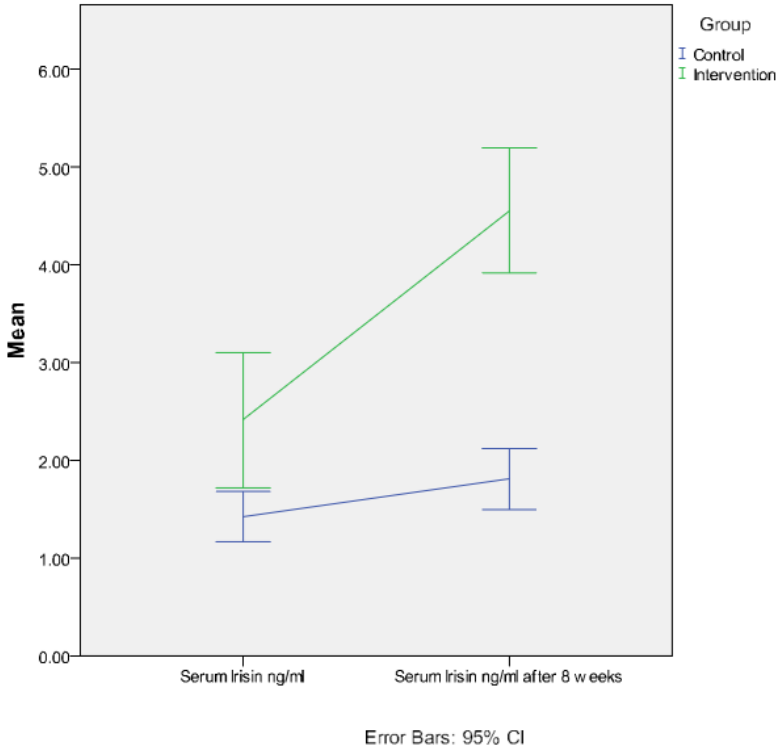

Figure 12: Mean serum IRISIN levels in control and intervention group (after 8 weeks)

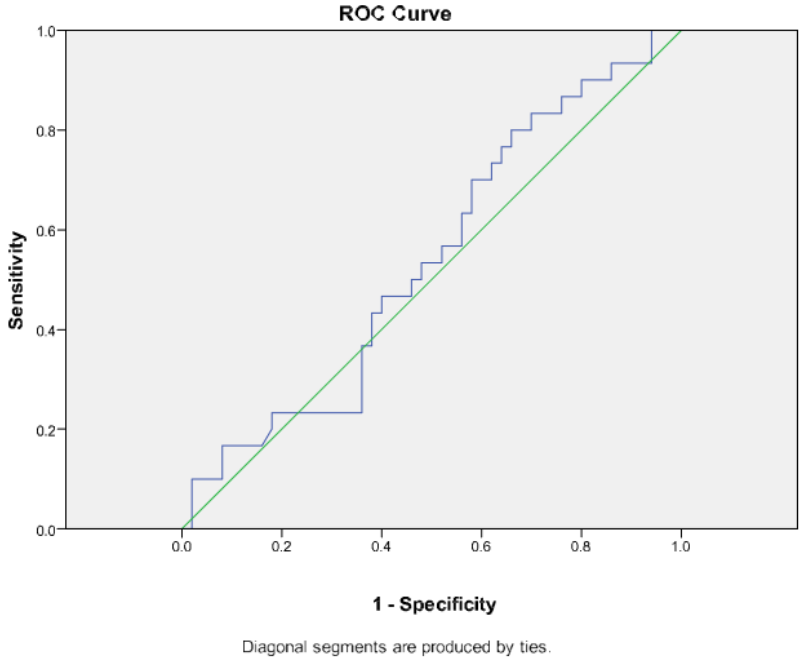

Figure 13: ROC curve

Area Under the Curve Test Result Variable(s): Serum Irisin ng/ml

Table 6:

Area	Std. Error[a]	p	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.541	0.065	0.528	0.414	0.668

The test result variable(s): Serum Irisin ng/ml has at least one tie between the positive actual state group

and the negative actual state group. Statistics may be biased.

1. Under the nonparametric assumption
2. Null hypothesis: true area = 0.5

Discussion

As per recent global burden of disease 2017 published in Lancet, COPD is second leading cause of death morbidity in India. Due to lack of circulating predictive biomarkers of severity of disease, it has been challenge to manage the pharmacotherapy in patients with COPD. A growing body of evidence from Western world suggest that circulating serum IRISIN levels is reliable marker of skeletal muscle in patients of COPD whom undergoing pulmonary rehabilitation. Whether this association between circulating serum IRISIN levels and lung function decline in exist in Indian COPD population has not been investigated. To best of our knowledge based on the literature survey, this is a first study in Indian population to show that impact of pulmonary rehabilitation on serum IRISIN levels in COPD patients.

Secondly, we also investigated whether this association holds true among variables i.e. 6MWT, PFT, BOLD SCORE, GOLD, MMRC, SGRQC., Did not found any significant association with all other variables. Together, these results support existing hypothesis that circulating increased serum IRISIN levels is a novel biomarker in patients with COPD on pulmonary rehabilitation. Six-minute walk test (6MWT) was performed for all the control and intervention group and we correlated the distance walked with serum IRISIN levels, we found statistically insignificant differences between Control and intervention group.

There are limited studies correlating serum IRISIN levels with exacerbation frequency and quality of life among COPD patients. In our study, as per from exclusion criteria we did not take patients with acute exacerbation as studies showed that during exacerbation serum IRISIN was increased.

We also attempted to assess association between serum IRISIN levels and quality of life of patients with COPD using CAT score, BODE index and Dyspnoea (MMRC score). We noted no statistically significance between control and intervention group.

We did not find any significant difference in the mean levels of serum IRISIN between males and females. The difference could possibly be due to discrepancy in sample size or this could be the case in Indian population or it could be because of confounding factors. Larger study group is required to further study this difference in Indian population.

It is important that any putative biomarker is stable in the biological fluid when assessed over a period of time after storage. From the literature, it is suggested that serum IRISIN is relatively stable and can be preserved for month and years. [37] This was evaluated in a subset of 80 individuals who were COPD patients either male and female.In our study the levels of serum IRISIN varied between Control group (COPD with no pulmonary rehabilitation) and intervention group (COPD with pulmonary rehabilitation), with intervention group showing significantly higher IRISIN levels. Similar result was seen in a study conducted by Watz et al [14] and our study result is also consistent with those from previous studies. [15,16,17]

In our study, we found consistent associations between increased serum IRISIN levels and pulmonary rehabilitation. These observations are consistent with those from previous studies on serum IRISIN in COPD patients.

Table 7: Various Studies Comparing serum IRISIN Levels With pulmonary rehabilitation

Study	Serum IRISIN	Serum IRISIN after pulmonary rehabilitation
Watz *et al* [14]	Low	Increased
Greulich *et al* [15]	Low	Increased
Papp C *et al* [18]	Low	Increased
Frode Northem *et al*	Low	Increased
Our study	Low	Increased

Whether the relationship between serum IRISIN levels, and pulmonary rehabilitation and development of COPD are causal or are confounded by other unmeasured factors cannot be conclusively determined from our data.

The protective molecular mechanisms of IRISIN that could protect against development of COPD are unknown. There are possibly anti-inflammatory and anti- oxidative activities of this molecule in the lung. [19]

Serum IRISIN levels are lower in COPD patients without pulmonary rehabilitation compared to COPD patient undergone pulmonary rehabilitation suggesting that pulmonary rehabilitation may have a strong role in increased serum IRISIN levels in COPD patients. Similar findings were seen in few studies on serum IRISIN and COPD. [14,15,16]

Conclusion

IRISIN levels are significantly high in COPD patients with pulmonary rehabilitation compared to COPD

patients without pulmonary rehabilitation as hypothesized. (p value <0.001). Serum IRISIN levels correlate well with quality of life suggesting IRISIN may also reflect changes in domains other than airflow limitation in COPD patients. There is no significant difference in serum IRISIN levels among male and female COPD patients, suggesting that levels of IRISIN are independent of age. There is no significant difference in serum IRISIN levels among smokers and nonsmoker patients. FEV1 is significantly lower in patient with decrease serum IRISIN level before pulmonary rehabilitation (p value 0.003). FVC is significantly lower in patient with decrease serum IRISIN level before pulmonary rehabilitation (p value 0.005).

References

1. Mannino DM, Buist AS. Global burden of COPD: risk factor prevalence, and future trends. Lancet 2007; 370:765-73.
2. Wan ES, Silverman EK. Genetics of COPD and Emphysema. Chest 2009; 136:859-866.
3. Nakamura H. Genetics of COPD. Allergol Int 2011. Jul 25 (Epub ahead of print http://chestjournal.chestpubs.org/externalref?access_num=10.3109/00365516309051324andlink_type=DOI
4. MacNee W. Pathology, pathogenesis, and pathophysiology: BMJ. 2006 May 20; 332(7551):1202-4.
5. Soler-Cataluna JJ, Martinez-Garcia MA, Roman Sanchez P, Salcedo E, Navarro M, Ochando R. Severe acute exacerbations and mortality in patients with chronic obstructive pulmonary disease. Thorax. 2005; 60(11):925-31.
6. Stockley RA; Neutrophils and the Pathogenesis of COPD. Chest 2002; 121:151S-155S.
7. Tetley TD. Macrophages and the Pathogenesis of COPD. Chest 2002121:156S- 159S.
8. Barnes PJ, Cosio MG. Characterization of T Lymphocytes in Chronic Obstructive Pulmonary Disease PLOS Medicine 2004; 1:e20
9. William Mac Nee, MD. Oxidants/Antioxidants and COPD. Chest 2000;117:303S–317S
10. Sapey E, Bayley D, Ahmad A, New bold P, Snell N, et.al . Inter-relationships between inflammatory markers in patients with stable COPD with bronchitis: intra-patient and inter-patient variability. Thorax. 2008; 63(6):493-9.
11. Soriano JB, Abajobir AA, Abate KH, Abera SF, Agrawal A, Ahmed MB, et al. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet Respiratory Medicine.5(9):691- 706
12. Naoki I jiri , Hiroshi Kanazawa, Kazuhisa Asai, Tetsuya Watanabe, Kazuto Hirata et al. Respirology: Official Journal of the Asian Pacific Society of Respirology 2015, 20 (4): 612-7
13. Chatila WM, Wynkoop WA, Vance G, Criner GJ. Smoking patterns in African Americans and whites with advanced COPD. Chest. 2004; 125:15
14. Castillo-Quan JI. From white to brown fat through the PGC-1α-dependent myokine IRISIN: implications for diabetes and obesity. Dis. Model. Mec
15. Greulich T, Nell C, Koepke J, Fechtel J, Franke M, Schmeck B, Haid D, Apelt S, Filipovic S, Kenn K et al. Benefits of whole body vibration training in patients hospitalised for COPD exacerbations—a randomized clinical trial. BMC Pulmonary Medicine. 2014; 14: 60–68
16. Lim JH, Kim EN, Kim MY et al (2012) Age-associated molecular changes in the kidney in aged mice. Oxidative Medicine and Cell Longevity 2012:171383
17. Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. Environment Health Perspective. 1985;64:111–126
18. Catalfo G, Crea L, Lo Castro T, et al. Depression, body mass index, and chronic obstructive pulmonary disease – a holistic approach International Journal of Chronic Obstructive Pulmonary Disease. 2016 ; 11:239–249
19. Pedersen BK, Akerstrom TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. Journal of Applied Physiology 2007; 103:1093-8