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Research Article

**ANALYSIS OF GENE EXPRESSION PROFILING AND
OVERLAPPING TRANSCRIPTIONAL PROFILES IN NON-
SPECIFIC INTERSTITIAL PNEUMONIA**¹Dr. Mahreen Siddique, ²Dr. Muhammad Khalid Abbas, ³Dr. Sadia Rehman¹Women Medical Officer at Children Hospital & Institute of Child Health, Multan²King Edward Medical University, Lahore³ Women Medical Officer at BHU 112/7R, Chichawatni**Abstract:**

Introduction: Idiopathic pulmonary fibrosis, identified histologically as usual interstitial pneumonia, accounts for most cases of idiopathic interstitial pneumonia. IPF affects men and women > 50 in a ratio of 2:1, with a markedly increased incidence with each decade of age. **Aims and objectives:** The basic aim of the study is to analyze gene expression profiling and overlapping transcriptional profiles in non-specific interstitial pneumonia. **Material and methods:** This study was conducted at Children Hospital & Institute of Child Health, Multan with the collaboration of King Edward medical college, Lahore in June 2018 to August 2018. RNA was extracted and hybridized to the Human Gene 1.0 set array from explanted lungs (2001–2008) in 22 patients with clinical diagnosis of sporadic IPF. 10 subjects with clinical diagnosis of idiopathic NSIP and definite histologic pattern of fibrotic NSIP; and 11 normal lung samples (age 52 ± 18 years, 4 females) obtained from the region of normal tissue flanking lung cancer resections in ILD-free patients. **Results:** There were no significant differences in pulmonary function tests, exercise capacity or pulmonary artery pressures. NSIP patients were significantly younger than IPF patients. Genes with significantly increased expression in NSIP, compared to both IPF and normal controls, were involved in regulatory mechanisms of immune reaction, including the alloreactive T cell response. **Conclusion:** It is concluded that gene expression profiling from whole lung explants aligns separate, well-recognizable histopathologic patterns of IIP with distinct transcriptional profiles and differentially expressed genes, and does not support the notion of NSIP as an early manifestation of IPF.

Corresponding author:**Dr. Mahreen Siddique,**Women Medical Officer at Children Hospital & Institute of Child Health,
Multan

QR code



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INTRODUCTION:

Idiopathic pulmonary fibrosis, identified histologically as usual interstitial pneumonia, accounts for most cases of idiopathic interstitial pneumonia. IPF affects men and women > 50 in a ratio of 2:1, with a markedly increased incidence with each decade of age. Current or former cigarette smoking is most strongly associated with the disorder. There is some genetic predisposition; familial clustering occurs in up to 20% of cases [1]. A combination of environmental, genetic, and other unknown factors probably contribute to alveolar epithelial cell dysfunction or reprogramming, which leads to abnormal fibro proliferation in the lung. There is ongoing research into the contributions of genetics, environmental stimuli, inflammatory cells, the alveolar epithelium, mesenchyme, and matrix [2].

Symptoms and signs of idiopathic pulmonary fibrosis typically develop over 6 mo to several years and include dyspnea on exertion and nonproductive cough. Constitutional symptoms, such as low-grade fever and myalgias, are uncommon. The classic sign of IPF is fine, dry, inspiratory crackles (Velcro crackles) at both bases. Clubbing is present in about 50% of cases. The remainder of the examination is normal until disease is advanced, at which time signs of pulmonary hypertension and right ventricular systolic dysfunction may develop [3].

Idiopathic pulmonary fibrosis (IPF) and non-specific interstitial pneumonia (NSIP) are the most common forms of idiopathic interstitial pneumonia (IIP). Better prognosis and response to therapy are reported for NSIP compared to IPF, which is defined by a histologic pattern of usual interstitial pneumonia (UIP) [4]. However, both conditions represent a common indication for lung transplantation (LTx). While the fibrotic process in IPF is believed to be driven by alveolar injury leading to unresolving wound healing and pro-fibrotic signals, the pathogenesis of NSIP is not clear. The clinical-radiographic distinction is challenging, but is particularly important given the differences in prognosis and treatment algorithms. Patients with NSIP will often have a good response to corticosteroids, while IPF can worsen on prednisone, and is currently treated with anti-fibrotic agents [5]. This differential response to treatment further highlights the dissimilarities in the molecular basis that defines IPF and NSIP. Despite these differences, the frequent finding of mixed UIP-NSIP patterns on

lung biopsies supported the hypothesis that NSIP may represent an early form of IPF [6].

Aims and objectives

The basic aim of the study is to analyze gene expression profiling and overlapping transcriptional profiles in non-specific interstitial pneumonia.

MATERIAL AND METHODS:

This study was conducted at Children Hospital & Institute of Child Health, Multan with the collaboration of King Edward medical college, Lahore in June 2018 to August 2018. RNA was extracted and hybridized to the Human Gene 1.0 set array from explanted lungs (2001–2008) in 22 patients with clinical diagnosis of sporadic IPF. 10 subjects with clinical diagnosis of idiopathic NSIP and definite histologic pattern of fibrotic NSIP; and 11 normal lung samples (age 52 ± 18 years, 4 females) obtained from the region of normal tissue flanking lung cancer resections in ILD-free patients. Histopathologic diagnoses were based on whole explanted lungs. IPF cases with atypical radiographic features for UIP, and patients with other types of ILD, connective tissue disease or concomitant emphysema were excluded.

Microarray analysis

RNA was isolated, labeled, and hybridized to the human gene 1.0 set array according to the manufacturer's protocols. Data sets for microarray experiments are available at the Gene Expression Omnibus repository, accession n.GSE110147.

Ethical consideration

This research project was approved by "Departmental Ethics and Research committee" of the hospital. The purpose of the study was explained to the study participants accordingly. Permission was obtained from hospitals research center and nephrology clinic.

Statistical analysis

The data of respiratory function were compared between the smoker and non-smoker groups using the independent t-test for normally distributed data or the Mann-Whitney U test for other distributions. Differences were considered statistically significant at $p < 0.05$.

RESULTS:

There were no significant differences in pulmonary function tests, exercise capacity or pulmonary artery pressures. NSIP patients were significantly younger than IPF patients (Table 1).

Table 01: Demographic, clinical and functional characteristics of the patients

Variable	IPF (n = 22)	NSIP (n = 10)	p value
Male/Female (% males)	17/5 (77%)	2/8 (20%)	0.0051
Age (years)	62 ± 6	45 ± 11	< 0.0001
BMI (m/Kg ²)	26 ± 5	24 ± 3	n.s.
mPAP (mmHg) ⁺	29 ± 12	35 ± 21	n.s.
6MWD (m)	293 ± 103	292 ± 195	n.s.
FVC (% pred)	57 ± 19	49 ± 18	n.s.
TLC (% pred)	65 ± 14	65 ± 18	n.s.
DLCO (% pred)	37 ± 10	51 ± 18	0.0242
Treatment (% of total):			
Prednisone alone	7 (31.8%)	5 (50%)	n.s.
Prednisone + Azathioprine	8 (36.4%)	2 (20%)	n.s.
NAC alone	1 (4.5%)	0 (0%)	n.s.
No specific treatment	6 (27.3%)	3 (30%)	n.s.

Genes with significantly increased expression in NSIP, compared to both IPF and normal controls, were involved in regulatory mechanisms of immune reaction, including the alloreactive T cell response (indoleamine 2–3-dioxygenase-1 [IDO-1]), the humoral arm of innate immunity (long pentraxin-3 [PTX-3], IFN-induced protein 44-like [IFI-44]) and the recruitment of leukocytes into the lung compartment (Endocan, LDL receptor-related protein-2 [LRP-2]). The top 25 genes upregulated in NSIP vs. controls are shown in Additional file 3: Table S3, which notably includes POSTN.

DISCUSSION:

The gene expression profile of NSIP received little attention. Yang *et al.* previously examined IPF and NSIP cases, but only half of cases were from large explant samples, both sporadic and familial cases were considered, and microarray analysis was limited to SAM⁷. Kim *et al.* analyzed a large amount of samples, but most consisted of surgical lung biopsies, rather than lung explants, and no normal controls were considered. In our study, integrated SAM, IPA and GSEA analyses in NSIP pointed to alloreactive T cell response, the humoral arm of innate immunity,

IL-12 production regulation, and recruitment of leukocytes into the lung compartment and granulocyte adhesion as main processes involved in NSIP⁸. These, importantly, are all part of anti-microbial response via the IFN-gamma signaling pathway. IFN-gamma is distinguished from other interferons by its ability to coordinate the transition from innate immunity to adaptive immunity, but its substantial contribution to T cell differentiation and immunoglobulin class switching in B cells underlines also a decisive role in adaptive immune responses in autoimmunity⁹.

The gene signature of IPF has been reassuringly reproducible across several microarray studies. Our findings confirm epithelial-to-mesenchymal transition, myofibroblasts proliferation, collagen deposition and PBMCs recruitment and infiltration as leading mechanisms of IPF at the transcriptional level, with the important addition of senescence [10]. However, another novel finding of this study was the complete lack of specificity in the upregulation of POSTN, OPN, MMP-1, MMP-7 and PROM-1, previously and extensively studied in IPF, but not

considered in NSIP. Not surprisingly, we found that IHC for POSTN, OPN and, as shown previously by Huh *et al.*, MMP-7 does not distinguish IPF from NSIP. However, by cross-checking upregulated genes against both NSIP and normal controls, we were still able to identify a gene signature truly specific for IPF, which includes MUC5B, ACTA-2 and IGFBP-5 [11]. The latter is a critical trigger of senescence, which showed a nearly complete separation between IPF and NSIP groups, with differential expression confirmed by RT-PCR [12].

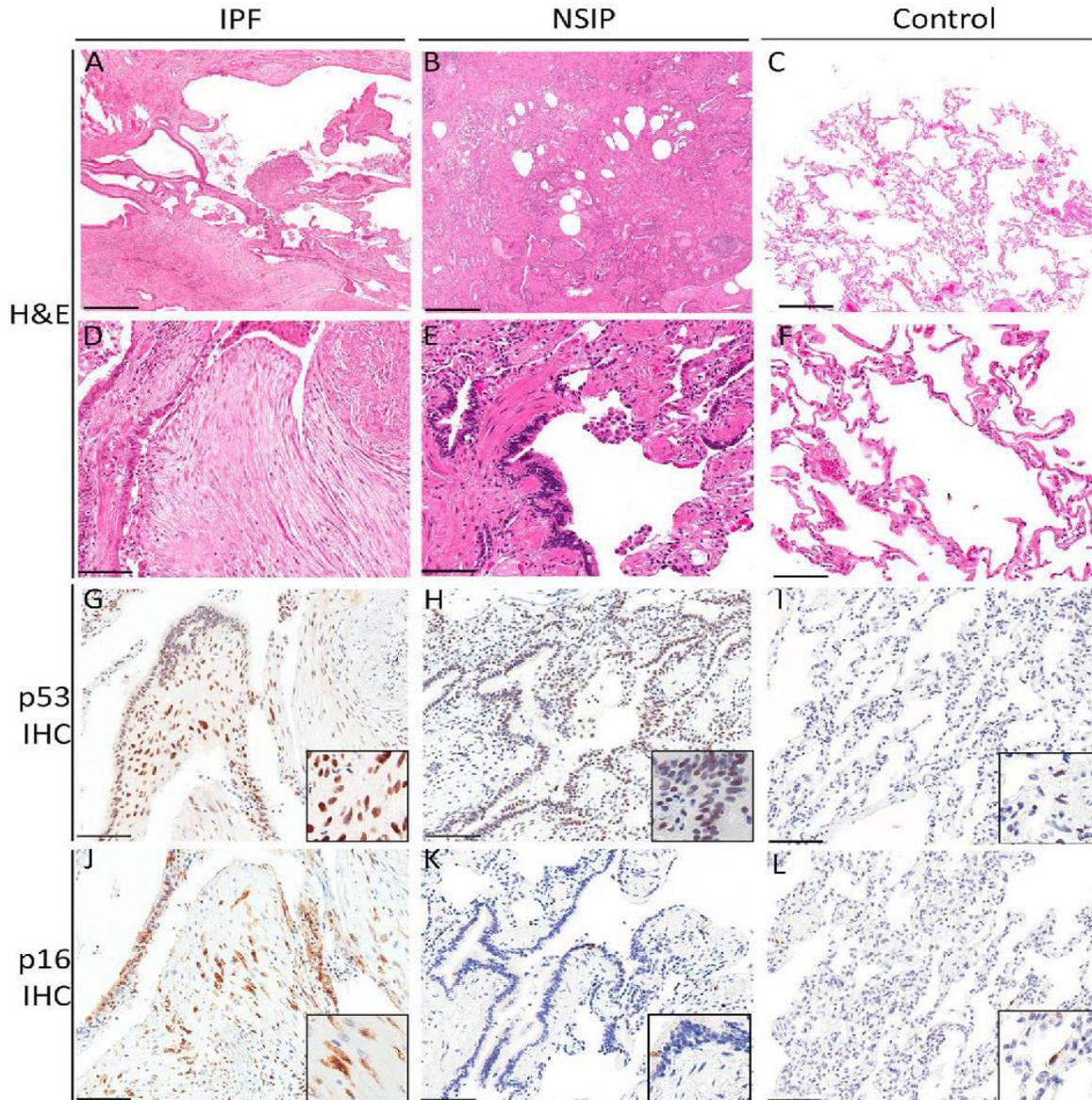


Figure 01: 4 Immunohistochemistry studies. a-f Lower power (4X) and high power (20X) photomicrographs of hematoxylin and eosin stained sections of representative IPF (A,D), NSIP (B,E) cases and normal control lung tissue (C,F). g-l high power photomicrographs (20X) of immunohistochemistry for p53 and p16 of representative IPF (G,J), NSIP (H,K) cases and normal control lung tissue (I,L). Inserts show high power magnification. (Ref: Matthew *et al.*, 2018)

CONCLUSION:

It is concluded that gene expression profiling from whole lung explants aligns separate, well-recognizable histopathologic patterns of IIP with distinct transcriptional profiles and differentially expressed genes, and does not support the notion of NSIP as an early manifestation of IPF, but rather point to a standalone, transcriptionally homogenous condition, even in advanced fibrotic cases.

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