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INTERSTITIAL LUNG DISEASE

Evaluation of Renin and Soluble (Pro)renin Receptor in Patients with IPF. A Comparison with Hypersensitivity Pneumonitis

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Abstract

Introduction Idiopathic pulmonary fibrosis (IPF) is a lethal disease with an unclear pathogenic mechanism. Components of the renin–angiotensin system (RAS) have a role in the pathogenesis of IPF, specifically, the aspartyl protease renin acts as a profibrotic factor in the lung. However, the concentration of the RAS components renin and soluble (pro)renin receptor (sPRR) have not been previously evaluated neither in serum nor in bronchoalveolar lavage fluid (BAL) of patients with IPF or chronic Hypersensitivity pneumonitis (cHP), a disease which may be confused with IPF.

Methods The serum levels of renin [IPF patients ($n=70$), cHP patients ($n=83$), and controls ($n=26$)] and sPRR [IPF ($n=28$), cHP (37), and controls ($n=20$)] were measured by ELISA. Renin was also quantified in BALs of IPF patients and controls by Western blot.

Results We found that the levels of renin were higher in serum samples from IPF patients when compared with cHP patients and controls. Furthermore, BALs from IPF patients had more renin than BALs from controls. Unlike renin, the serum levels of sPRR were lower in IPF and cHP patients than in control individuals.

Conclusions The high levels of renin in sera and BALs of IPF patients suggest that renin might play a major role in the pathogenesis of IPF. Results from BAL confirm that renin is produced locally in the lung. Serum levels of renin could be used to differentiate IPF from cHP.

Keywords Idiopathic pulmonary fibrosis · Hypersensitivity pneumonitis · Serum levels of renin · Bronchoalveolar levels of renin

Abbreviations

IPF	Idiopathic pulmonary fibrosis
cHP	Hypersensitivity pneumonitis
BAL	Bronchoalveolar lavage fluid
EMT	Epithelial to mesenchymal
sPRR	Soluble (pro)renin receptor
PRR	(Pro)renin receptor
RAS	Renin–angiotensin system

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Introduction

Idiopathic pulmonary fibrosis (IPF) has a poor prognosis with an average survival rate of 3–5 years, but there is an appreciable heterogeneity among individual patients in the course of the disease. Currently, there are two FDA approved medications for IPF which only slow down the progression of the disease, leaving limited therapeutic options for patients with this progressive disease [1, 2]. To date, the etiology and pathogenic mechanisms of the disease are unknown. IPF is characterized by an epithelial activation process that leads to an increased migration and proliferation of lung fibroblasts. Fibroblasts further differentiate into myofibroblasts that synthesize excessive extracellular matrix proteins, which results in the destruction of the pulmonary architecture [3]. Though many profibrotic molecules have been described to date, transforming growth factor beta 1 (TGF-β1) is one of the most important in IPF. This protein increases the expression of collagen and promotes fibroblast proliferation and epithelial to mesenchymal transition (EMT) [4–6]. Thus,

stimuli that increase TGF-β1 expression are of special interest to better understand the pathophysiology of IPF. Different components of the renin–angiotensin system (RAS) have been reported to promote the synthesis of TGF-β1 such as angiotensin II (Ang II) and renin [7, 8].

The aspartyl protease renin and its receptor, i.e., (pro) renin receptor (PRR), belong to the RAS and are implicated in fibrotic process in kidney, heart, and lung [8–10]. Binding of renin to PRR enhances the expression of TGF-β1 and collagen I in lung fibroblasts. Renin is overexpressed in IPF lung fibroblasts, but its levels in serum and bronchoalveolar lavage (BAL) fluid have not been measured previously [10]. PRR is a protein of 350 amino acids with a single transmembrane domain. A soluble form of this receptor, i.e., soluble PRR (sPRR), is obtained when PRR is processed by the protease furin [11, 12]. sPRR can be detected in serum, plasma, and urine, and high concentrations of sPRR are associated with gestational diabetes mellitus, however, levels of sPRR are unknown in IPF [13, 14]. Thus, the aim of this study was to measure the serum and BAL levels of renin and the serum levels of sPRR in IPF. Furthermore, we also evaluated its diagnostic value to differentiate IPF from hypersensitivity pneumonitis (cHP).

Methods

Patients Characteristics

All serum samples used in this study were obtained from the Biobank of the Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (Mexico City). Blood samples were obtained from the subjects diagnosed with IPF (according to the 2011 ATS/ERS/JRS/ALAT statements [15]) and cHP. Samples were centrifuged to separate the sera which were stored at − 70 °C until use.

Measurements of Serum Renin and sPRR

Quantifications of serum renin and sPRR were carried out using two ELISA kits (R&D Systems, Minneapolis, MN, Immuno-Biological Laboratories Co., Ltd., Japan respectively) according to the manufacturer's instructions.

Bronchoalveolar Lavage (BAL)

BALs were obtained from the Biobank of Pulmonary Interstitial Disease in the Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas. BALs are obtained as part of the diagnostic procedure, aliquoted, and stored at − 70 °C. BALs were concentrated 10 times using centrifugal filter units (Amicon Millipore, Darmstadt, Germany) according to the manufacturer's instructions. Total protein was then quantified with Bradford dye reagent (Bio-Rad, Hercules, CA).

Western Blot

Renin in BAL was evaluated by Western blot under denaturant/reducer conditions using 10 µg of total protein. All samples were preincubated for 5 min at 100 °C. After that, they were loaded onto a 14% polyacrylamide gel for electrophoresis. Proteins were then transferred to a polyvinylidene difluoride (PVDF) membrane and incubated with anti-renin primary antibody (Cell Signaling, Danvers, MA) followed by incubation with HRP-conjugated secondary antibody. A chemiluminescent substrate (Thermo Scientific, West Femto) was used to detect the protein in ChemiDoc XRS system. Densitometric analysis was performed with the ImageLab software (Bio-Rad, Hercules, CA) [16].

All samples from the Biobank in INER had the approval signature of informed consent from patients, to allow the use of the samples in research.

Statistical Analysis

Data were analyzed with measures of central tendency and dispersion in univariate analysis, study groups were compared using ANOVA followed by Tukey's test, and Fisher exact test was used for population characteristics. $p < 0.05$ was considered statistically significant.

Results

Study Population

We studied renin levels in 70 IPF patients, 83 patients diagnosed with cHP, and 26 control individuals. Characteristics of the cohorts are summarized in Table 1. The average ages in the groups were: IPF 64 years, cHP 52 years, and the control group 68 years. The levels of sPRR were evaluated in 28 IPF patients, 37 cHP patients and 20 controls individuals (Table 2).

Serum Levels of Renin and sPRR

Serum renin levels (Fig. 1) were higher in samples of IPF patients when compared with the control group (1360.1 pg/mL ± 116.59 pg/mL vs. 297.49 pg/mL ± 73.13 pg/mL; $p < 0.0001$). Furthermore, IPF serum samples had more renin than samples from the cHP group (475.86 pg/mL ± 45.60 pg/mL; $p < 0.0001$).

Serum levels of sPRR (Fig. 2a) were lower in cHP patients when compared with the IPF group (4887 pg/

Table 1 Characteristics of the renin study population

	IPF $n=70$	HP $n=83$	Controls $n=26$	p
Age, average (min–max)	64 (36–84)	52 (30–73)	68 (47–78)	<0.0001
Gender (female:male)	19:51	72:11	20:6	<0.0001
Hypertension (%)	23	27	15	0.03
Smoking former (%)	52	22	23	<0.0001
Time of symptoms (months)*	25 ± 32	29 ± 28	NA	0.25
FVC (% predicted)	67 ± 25	59 ± 20	88 ± 21	<0.0001
DLCO (% predicted)	52 ± 20	44 ± 24	106 ± 24	<0.0001
Sp_{O_2} at rest (%)	84 ± 8	84 ± 9	94 ± 2	<0.0001
Sp_{O_2} after exercise (%)	79 ± 8	76 ± 9	91 ± 7	<0.0001
6MWT (m)	249 ± 174	355 ± 144	379 ± 187	0.001

Table 2 Characteristics of the sPRR study population

	IPF $n=28$	HP $n=37$	Controls $n=20$	p
Age, average (min–max)	65 (47–78)	50 (16–73)	69 (60–87)	<0.0001
Gender (female:male)	6:22	36:1	15:5	<0.0001
Hypertension (%)	42	16	20	0.2
Smoking former (%)	56	31	26	0.0039
Time of symptoms (months)*	33 + 27	26 + 21	NA	0.28
FVC (% predicted)	76 + 21	59 + 20	87 + 25	0.051
DLCO (% predicted)	56 + 22	51 + 24	95 + 26	0.0059
Sp_{O_2} at rest (%)	89 + 5	86 + 8	94 + 2	<0.0001
Sp_{O_2} after exercise (%)	80 + 6	76 + 8	92 + 5	<0.0001
6MWT (m)	460 + 95	420 + 142	450 + 161	0.0902

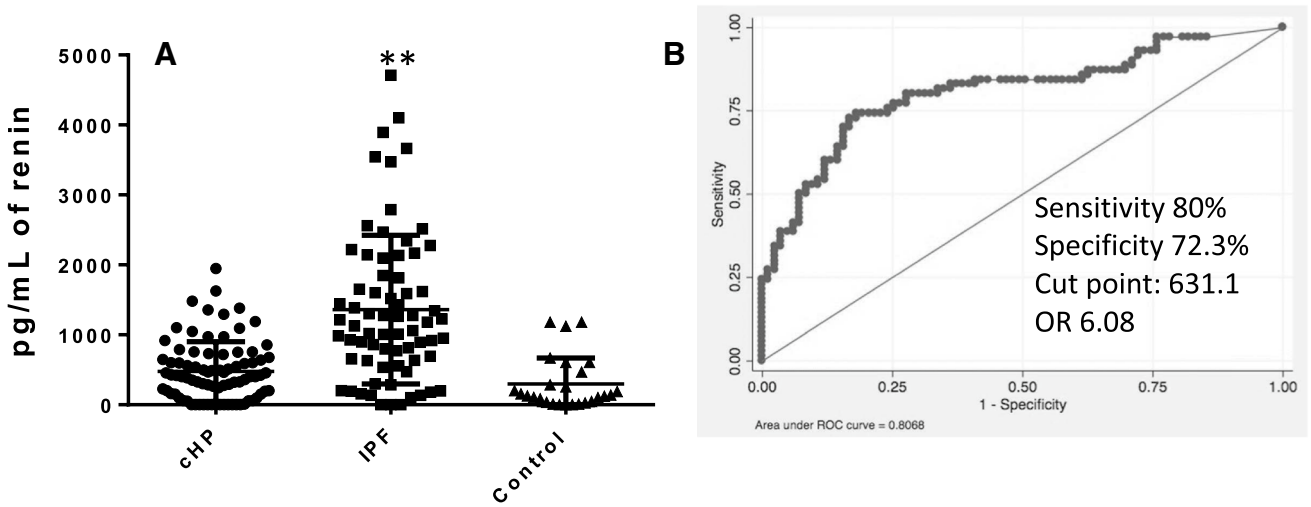

Fig. 1 Panel **a** Serum levels of renin in cHP, IPF, and control. Renin is significantly increased in IPF samples when compared with control (**$p<0.0001$) and cHP samples (**$p<0.0001$). Panel **b** Receiver operating characteristics curve used to evaluate the serum renin as a diagnostic biomarker for IPF

mL ± 533.4 pg/mL vs. 15,105 pg/mL ± 1372, $p<0.0001$) and the control group (4887 pg/mL ± 533.4 pg/mL vs. 20,090 pg/mL ± 1467 pg/mL, $p<0.0001$).

BAL Levels of Renin

Renin levels in BAL were measured by Western blot. We found that renin was more abundant in BALs from IPF

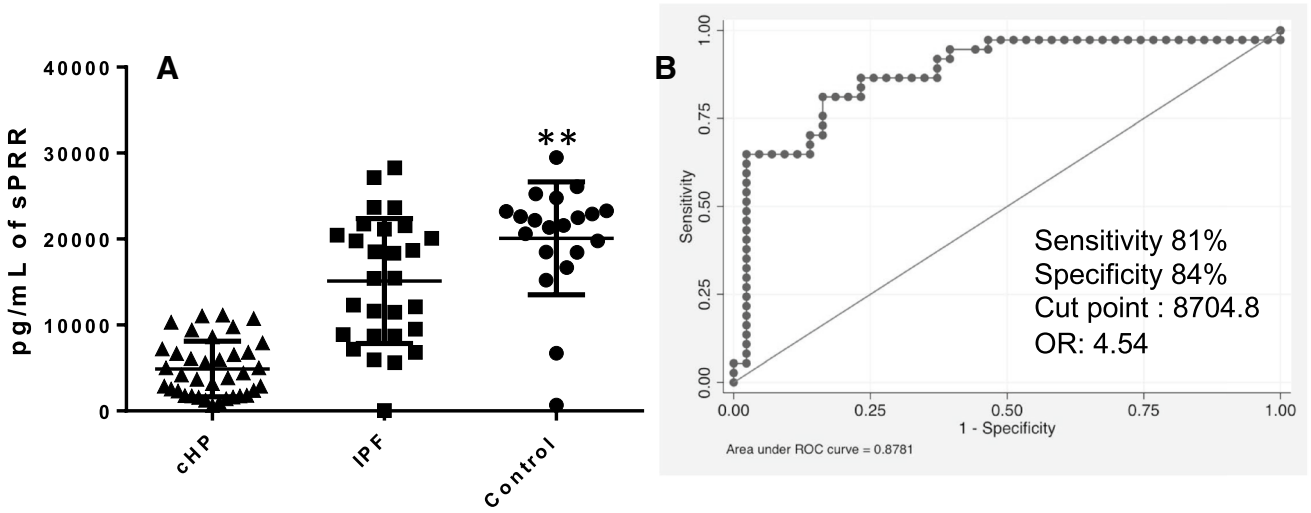

Fig. 2 Panel **a** Serum levels of sPRR in cHP, IPF, and Control. sPRR is significantly decreased in IPF samples when compared with control (**$p=0.0096$). Serum levels were lower in cHP samples than in IPF patients (**$p<0.0001$). Panel **b** Receiver operating characteristics (ROC) curve used to evaluate the serum sPRR as a diagnostic biomarker for IPF

patients than control individuals ($p=0.0015$) (Fig. 3).

Renin and Prorenin Receptor Cutpoint for Differential Diagnosis Between IPF and cHP

We evaluated if the measurements of serum renin and sPRR could be used to distinguish between IPF and cHP by performing a receiver operating characteristic (ROC) analysis. For renin, we found that, with a cut point of 631.1 pg/mL, the sensitivity was 80%, the specificity 72.3% and the OR of 6.08 (95% CI 2.9–12.7) (Fig. 1b). ROC analysis for sPRR resulted in a cut point of 8704.8 pg/mL, with a sensitivity of 81%, a specificity of 84%, and an OR of 4.54 (Fig. 2b).

Discussion

IPF is a devastating disease with neither a cure nor an effective treatment. Many profibrotic molecules have been evaluated over the years (e.g., CCL18, sL1-CAM, SP-D, SP-A, KL-6, CC16, MMP1, and MMP7) with the objective of finding prognostic biomarkers as well as aiding in the differential diagnosis of IPF from other interstitial lung diseases. All studies without definitive results [17–21].

Profibrotic effects of renin, independent of Ang II peptides, have been reported in kidney, heart, and lung. In these tissues, renin and its receptor (PRR) can increase the expression of TGF-β1 and collagen I. Our group reported

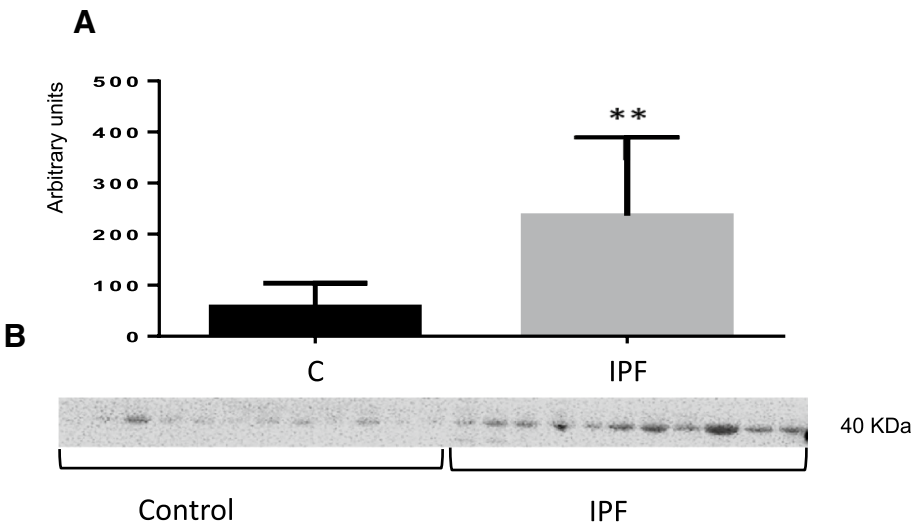

Fig. 3 Renin levels in BAL of IPF and control samples (**$p=0.0015$). Panel **a** densitometric analysis of Western blot (Panel **b**). Levels of renin in BAL are higher in the IPF group when compared with the control group

previously increased levels of renin in the fibroblasts from IPF patients; moreover, stimulation of the control fibroblast with renin resulted in an augmented fibrotic profile. Therefore, to study renin in body fluids such as serum and BAL is of great importance [7, 8, 22].

In this study, we showed for the first time that renin is upregulated in sera from IPF patients relative to the control individuals. Besides, renin levels were significantly increased in IPF when compared with cHP, an inflammatory disease that could also evolve into pulmonary fibrosis in its chronic phase. According to our results, the measurement of serum renin could be used to distinguish between IPF and cHP with a cut point of 631.1 pg/mL and OR 7.79 (Fig. 1b).

Renin is elevated in BALs of patients with IPF when compared with BALs from controls. This finding reinforces the idea of local generation of renin in the lungs of IPF patients, and this idea is supported in a previous report that lung fibroblasts from IPF patients have increased levels of renin [10]. Hence, renin would extend its effects to other cell types (e.g., alveolar epithelial cells, lymphocytes, and macrophages) spreading its profibrotic effects and promoting a pulmonary fibrotic environment. A renin receptor had been reported in T lymphocytes, natural killer cells, and monocytes [23]. Analogous to lung fibroblasts, renin induces ERK1/2 phosphorylation in these cells, suggesting that PRR is functional [10, 23–26].

Moreover, renin has the capacity to process angiotensinogen thus promoting local generation of Ang II peptides in the lung, which has been reported to induce the synthesis of TGF-β1 in lung fibrosis [7]. Excessive deposition of extracellular matrix proteins is the hallmark of fibrotic disorders, hence, renin has a profibrotic role in the lung by upregulating TGF-β1 and collagen I. For the first time, we have demonstrated that renin is overexpressed in BALs and sera of IPF patients, suggesting that renin could be implicated in several profibrotic cellular processes, e.g., proliferation, migration, etc. It would be interesting to study the mechanism that regulates the elevated expression of renin in pulmonary fibrosis and its role as a prognostic biomarker in IPF patients [27].

Unlike renin, sPRR levels were decreased in IPF serum samples when compared with the control group. Furthermore, serum levels of sPRR were even lower in cHP patients than in IPF patients and control individuals. The levels of sPRR in our control group were similar to previously reported values for healthy individuals (19.3- 27.4 ng/mL) [28].

Future Directions

A limitation of our study is that the levels of renin and sPRR were determined for differential diagnosis only (before the treatment), so we were unable to correlate with prognosis or treatment response. It would be interesting to evaluate this in future clinical studies. Likewise, the precise role of the sPRR in IPF is controversial since it has been reported that sPRR is not only able to bind renin and prorenin but also interacts with the receptor frizzled 8. This interaction activates the β-catenin pathway that, like RAS, has a role in IPF development [29, 30]. Both mechanisms need further investigation in patients with IPF and cHP.

Compliance with Ethical Standards

Conflict of interest None declared.

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