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Role of transforming growth factor beta in assessment of severity of hypersensitivity pneumonitis: a single center study

Yasmine H. EL Hinnawy[1], Aymen S. Salem[1], Hebatallah H. Ahmed[1], Radwa M. Abdel Halim[2] and Naglaa B. Ahmed[1*]

Abstract

Background Hypersensitivity pneumonitis (HP) is a more frequently diagnosed picture of diffuse parenchymal lung disease. It is an inflammation of the lung tissue, provoked by immune mechanisms, which happens to prone individuals as a reaction to a wide range of antigens. There are different degrees of fibrosis and inflammation. A group of extracellular mediators both proinflammatory and profibrotic claimed to be involved in the pathogenesis of HP. Among these mediators, a significant role is played by transforming growth factor-beta (TGF-β).

Aim Correlation between the severity of hypersensitivity pneumonitis and the serum level of TGF beta.

Patients and methods Sixty subjects were included in the study who were classified into 30 patients newly diagnosed with hypersensitivity pneumonitis and 30 healthy subjects served as controls. All the participants were subjected to complete history taking, physical examination, spirometry, 6-min walk distance test, HRCT, and serum levels of TGF-β.

Results The serum level of TGF beta is elevated in newly diagnosed HP cases (fibrotic and non-fibrotic) in relation to control participants showing statistical significance p value < 0.001, and the serum level of TGF beta in the fibrotic group of HP patients is more than that in non-fibrotic group with statistical significance p value 0.012.

Conclusion The serum level of transforming growth factor can be used in the assessment of the severity of hypersensitivity pneumonitis as regards the intensity of lung parenchymal changes.

Keywords Hypersensitivity pneumonitis, TGF beta serum level, HRCT

Introduction

Hypersensitivity pneumonitis (HP) is one of the diffuse parenchymal lung diseases (DPLD) [1]. The etiological agents are numerous being either organic [2, 3] or inorganic [3]. The most common special habit associated with the disease is exposure to birds [4] due to the exposure to different antigenic products of various birds, especially pigeons [5]. However, identification of the offending agent is often difficult [6–8]. Thus, the term cryptogenic HP is described [9]. HP is increasing in incidence and is becoming one of the commonest DPLDs [10]. It has different phenotypes, making the differentiation from other interstitial lung diseases challenging [9]. Epidemiologic data for the disease seem to be lacking in Egypt.

The diagnosis of HP is usually not easy [11], as there is usually a wide range of vague clinical presentations in the form of varying degrees of dyspnea and dry cough [12]. HRCT provides an important method in the diagnosis, especially if a history of exposure has been elucidated

*Correspondence:
Naglaa B. Ahmed
naglaa.bakry@kasralainy.edu.eg
[1] Chest Department, Faculty of Medicine, Cairo University, Cairo, Egypt
[2] Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt

[13]. Diagnosis can always be confirmed by a bronchoscopic lavage rich in lymphocytes [14].

However, in some cases of uncertainty, lung biopsies should be obtained [15].

Numerous proinflammatory and profibrotic extracellular agents have been identified as contributing factors in the development of HP. Among these agents, transforming growth factor beta (TGF-β) is a significant mediator.

As an important profibrotic growth factor, transforming growth factor (TGF)-β aggravates pulmonary fibrosis and increases the expression of α-smooth muscle actin (α-SMA) which stimulates myofibroblast differentiation, a contractile stress fiber. It also provokes fibronectin excretion, leading to the gathering of lung fibroblasts at the location of fibrosis [16].

The aim of our study is to assess the role of TGF-β as a marker of disease severity and correlation with fibrosis.

Patients and methods

This is a prospective study performed in the Department of Chest Diseases in cooperation with the Chemical Pathology Department Hospitals of Kasr Alainy, Cairo University, during the period between August 2019 and February 2020.

The study is conducted after obtaining the ethical committee approval letter with IRB number: Ms-203–219.

It included 60 participants put in 2 groups. Group 1 included 30 patients who presented to our department fulfilling the inclusion criteria the newly diagnosed definite HP according to the guidelines, and all patients have typical HRCT findings (not started corticosteroids) [9]. The history of exposure to bird raising was elicited in all patients to fit the criteria of definite HP, a variable duration of illness. There are 2 age of 18 years old and older, and both sexes are included (there was no special selection). After the inclusion of these 30 patients, they were divided into 2 subgroups (fibrotic 12 patients and non-fibrotic 18) according to HRCT findings. Group 2 had 30 healthy control subjects age- and sex-matched, and they were selected from totally healthy (by history and clinical examination) relatives of the patients. Exclusion criteria are as follows: patients with DPLD cases other than HP or patients known as HP and on regular steroids or other treatments. Any patients with a disease known to cause elevated TGFB (e.g., malignancies) were excluded.

The sample size was calculated according to the prevalence of HP among cases presented to our department and considering a dropout rate of 10%.

Patients with other forms of DPLD, any other chest disease, or any other medical conditions associated with elevated levels of TGF beta were excluded.

Proper history taking and physical examination with concern to dyspnea grading according to New York Heart Association (NYHA) functional classification system 1994 [17].

> Class I, no limitation of physical activity: ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
> Class II (mild), slight limitation of physical activity: comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
> Class III (moderate), marked limitation of physical activity: comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
> Class IV (severe), unable to carry out any physical activity without discomfort: symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

Spirometry was done using Master screen PFT 2012, CareFusion 234 GmbH, Germany (V-781267–057 version 03.00). Interpretation of spirometry (*ATS-1994*) Restriction if FEV1/FVC > 805, FVC less than 80%, and then classified into mild from 80 to 60%, moderate from 60 to 40%, and severe if less than 40% [18].

HRCT chest (Siemens 16-channel MDCT) was done on all patients. HRCT findings suggestive of HP: centrilobular ground-glass, centrilobular nodular opacities, evidence of air-trapping in the mid to upper portion of the lung lobes. Head-cheese sign: a combination of GGO, mosaic parenchyma, and normal lung tissue. HRCT findings in chronic HP are the combination of reticular, ground-glass, and centrilobular nodular opacities associated with signs of "fibrosis" (i.e., interlobular septal thickening, lobar volume loss, traction bronchiectasis, and honeycombing) [19, 20].

Arterial blood gases (GEM premier 3000): according to the National Institute of Health, typical normal values are:

- pH: 7.35–7.45
- Partial pressure of oxygen (PaO2): 75 to 100 mmHg
- Partial pressure of carbon dioxide (PaCO2): 35–45 mmHg
- Bicarbonate (HCO3): 22–26 mEq/L
- Oxygen saturation (O2 Sat): 94–100% [21]

Six-min walk test (6MWT): The 6MWT was performed using the methodology specified by the American Thoracic Society (ATS-2002). The patients were instructed that the objective was to walk as far as possible for 6 min. The 6MWT was performed in a flat, long, corridor which was 30-m long, meter-by-meter marked. Heart rate and oxygen saturation were collected at the beginning and at the end of the 6MWT.

When the test was finished, the distance covered was calculated [22].

A collection of 3 mL of a sample of whole blood was done in plain Vacutainer tubes (BD, Franklin Lakes, NJ, USA). Then they were left for clotting for 20 min at the usual room temperature and then centrifugation at 3000 rpm for 20 min was done to it.

Kits of enzyme-linked immunosorbent assay that are commercially available were used to measure the serum levels of human TGFB-1 (IBL INTERNATIONAL GMBH (Hamburg Germany), and the assay was done according to the manufacturer's protocol.

Coding and entering data were done using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Summarization using mean, standard deviation, minimum, maximum, and median, in analysis of quantitative data, and we used, for categorical data, frequency (count) and relative frequency (percentage).

We compared quantitative variables by using the non-parametric Kruskal–Wallis and Mann–Whitney tests [23]. Chi-square (χ^2) test was used to compare categorical data. An exact test was used instead when the expected frequency was less than 5 [24].

We did quantitative variables correlations using the Spearman correlation coefficient [25].

Statistical significance is considered when *P*-value is less than 0.05.

Results

The sex was almost near in the two groups, where the females were 83.3% and 90% in groups 1 and 2, respectively.

In group 1, the mean age was 43.63 with SD 15.68, and in group 2, mean was 38.63 with SD 10.14.

As regards exposure to HP risk factors, most of the patients (90%) gave a history of raising birds, and the other 10% of patients without a known history of exposure to offending agents.

The descriptive clinical presentations of the patients regarding the degree of dyspnea assessed by NYHA, oxygenation on room air, and the clubbing with its degree are shown in Table 1.

HRCT picture of the patients showed that 18 patients (60%) had non-fibrotic HP while 12 (40%) had fibrotic HP.

According to spirometry, almost all the patients [28] were presented with restrictive patterns. Mild and moderate cases were 11 each while 6 patients had severe restrictive patterns.

The 6MWT showed a mean distance of 266.6 ± 117 m. The mean oxygen saturation pretest was 91.47 while the post-test was 75.8 shown in Table 2.

Table 1 Dyspnea assessment, oxygenation, and clubbing among the studied HP patients

		Count	%
NYHA	**2**	19	63.3%
	3	11	36.7%
SO_2 on RA	**Hypoxic**	8	26.7%
	Not hypoxic	22	73.3%
Clubbing	**Yes**	22	73.3%
	No	8	26.7%
Degree of clubbing	**1st**	8	36.4%
	2nd	13	59.1%
	3rd	1	4.5%

SO_2 oxygen saturation, *RA* room air. Not hypoxic means oxygen saturation ≥ 90%. Hypoxic means SO_2 on RA > 90%

The serum level of TGF beta (pg/ml) is elevated in newly diagnosed HP cases in relation to the control group with statistical significance *p* value of < 0.001 (Table 3).

There was statistical significance regarding the fibrotic group and hypoxemia, clubbing, and degree of dyspnea with *p* values 0.034, 0.010, and 0.001, respectively (Tables 4 and 5).

Table 6 showed that patients in the fibrotic group had more restrictive patterns than those in the non-fibrotic group with a statistical significance *p* value of 0.015.

The patients in the fibrotic group had a more restrictive pattern than those in the non-fibrotic group with a mean of FVC% of 62.49 in the non-fibrotic group and 45.53 in the fibrotic group,and the distance is less in the fibrotic group (mean 195.67 m) than the non-fibrotic group (mean 313.89) (Table7).

The serum value of TGF beta in the fibrotic group of HP patients is more than that in the non-fibrotic group with a statistical significance *p* value of 0.012 (Table 8).

The serum value of TGF beta is higher in hypoxic patients than non-hypoxic, those with clubbing and with severe degree of restriction as well as dyspnea score without any statistical significance (Table 9).

In our study, we found no significant correlation between the serum level of TGF beta and the distance covered by the patients in 6 MWT (*p* value 0.128) or desaturation occurred at the end of the test (*p* value 0.556) (Table 10).

Discussion

Hypersensitivity pneumonitis (HP) is a more frequently diagnosed picture of diffuse parenchymal lung disease. Numerous proinflammatory and profibrotic extracellular agents have been identified as contributing factors in the development of HP. Transforming growth factor

Table 2 6MWD test of studied HP patients

	Mean	SD	Median	Minimum	Maximum
Pre-test oxygen saturation %	91.47	4.96	92.00	80.00	98.00
Post-test oxygen saturation %	75.80	8.01	75.00	61.00	89.00
Distance, meters	266.60	117.00	250.00	30.00	600.00

6MWD 6-min walk distance

Table 3 Comparison between serum level of TGF beta (pg/ml) in newly diagnosed HP patients and normal healthy control subjects

	Mean	SD	Median	Minimum	Maximum	*P* value
HP cases	1991.83	1300.52	1380.00	730.00	5250.00	< 0.001
Control	556.40	183.93	555.00	240.00	1035.00	

Table 4 Comparison between fibrotic and non-fibrotic HP patients regarding oxygenation and clubbing

		HRCT				
		Non-fibrotic		Fibrotic		*P* value
		Count	%	Count	%	
SO_2 on RA	**Hypoxic**	2	11.1%	6	50.0%	0.034
	Not hypoxic	16	88.9%	6	50.0%	
Clubbing	**Yes**	10	55.6%	12	100.0%	0.010
	No	8	44.4%	0	0.0%	
Degree of clubbing	**1st**	6	60.0%	2	16.7%	0.074
	2nd	4	40.0%	9	75.0%	
	3rd	0	0.0%	1	8.3%	

ISO_2 oxygen saturation

Table 5 Comparison between dyspnea score of fibrotic and non-fibrotic HP patients

		HRCT				
		Non-fibrotic		Fibrotic		*P* value
		Count	%	Count	%	
NYHA	2	16	88.9%	3	25.0%	0.001
	3	2	11.1%	9	75.0%	

NYHA New York Heart Association

Table 6 Comparison between the pulmonary function of fibrotic and non-fibrotic HP patients

		HRCT				
		Non-fibrotic		Fibrotic		*P* value
		Count	%	Count	%	
Spirometry	**Normal**	2	11.1%	0	0.0%	0.015
	Mild restriction	10	55.6%	1	8.3%	
	Moderate restriction	4	22.2%	7	58.3%	
	Severe restriction	2	11.1%	4	33.3%	

Table 7 Comparison between pulmonary function and 6MWT of fibrotic and non-fibrotic HP patients

		FVC% (spirometry)	Pre-test saturation (6MWT)	Post-test saturation (6MWT)	Distance (6MWT)
Non-fibrotic	**Mean**	62.49	93.89	78.00	313.89
	SD	16.50	3.69	7.88	109.35
	Median	65.15	95.00	77.50	310.00
	Minimum	22.90	85.00	65.00	120.00
	Maximum	91.10	98.00	89.00	600.00
Fibrotic	**Mean**	45.53	87.83	72.50	195.67
	SD	12.59	4.45	7.32	91.97
	Median	45.90	88.50	73.00	205.00
	Minimum	24.90	80.00	61.00	30.00
	Maximum	72.00	95.00	87.00	358.00
***P* value**		0.004	< 0.001	0.095	0.005

FVC forced vital capacity, *6MWT* 6-min walk test

Table 8 Comparison between serum level of TGF beta in fibrotic and non-fibrotic HP patients

	TGF beta					
	Mean	**SD**	**Median**	**Minimum**	**Maximum**	***P* value**
Non-fibrotic	1497.22	905.80	1325.00	730.00	4825.00	0.012
Fibrotic	2733.75	1479.94	2562.50	1265.00	5250.00	

Table 9 Correlation between serum level of TGF beta to hypoxia, clubbing, degree of restriction, and dyspnea score in newly diagnosed HP cases

		TGF beta					
		Mean	**Standard deviation**	**Median**	**Min**	**Max**	***P* value**
SO_2 on RA	**Hypoxic**	2294.38	1551.71	1340.00	1135	5250	0.909
	Not hypoxic	1881.82	1218.82	1382.50	730	4960	
Clubbing	**Yes**	2091.14	1321.33	1380.00	730	5250	0.730
	No	1718.75	1285.72	1392.50	745	4825	
Degree of clubbing	**1st**	1797.50	1334.36	1320.00	730	4960	0.475
	2nd	2201.15	1369.58	1380.00	1000	5250	
	3rd	3010.00		3010.00	3010	3010	
Spirometry	**Normal**	1005.00	388.91	1005.0	730	1280	0.052
	Mild restriction	1334.09	403.02	1370.0	745	2380	
	Moderate restriction	2402.27	1424.09	1660.0	1215	4960	
	Severe restriction	2774.17	1718.22	2610.0	1025	5250	
NYHA	**2**	1783.42	1262.04	1370	730	5250	0.145
	3	2351.82	1346.44	1495	1135	4960	

NYHA New York Heart Association, SO_2 oxygen saturation, *RA* room air

beta (TGF-β) is among those mediators that can induce fibrosis [16].

The study at hand included 30 patients newly diagnosed with HP. It was noticed that 25 patients were females (83.3%) and only 5 patients were males (16.7%). The female predominance noticed in our study could be related to the initiating agent or to the pathogenesis of the disease itself. The mean age of the included patients was 43.6 years (± 15.6 SD). This was also found by Akl et al. (2017) as they studied demographics of hypersensitivity pneumonitis in Egypt and reported that out of 118 HP patients, females were ten times more affected

Table 10 Correlation between serum level of TGF beta, distance in 6MWT, and desaturation at the end of the test

	TGF beta		
	Correlation coefficient	***P* value**	***N***
Pre-test saturation (6MWT)	−0.068	0.723	30
Post-test saturation (6MWT)	0.056	0.770	30
Distance (6MWT)	−0.284	0.128	30
Desaturation	−0.112	0.556	30

6MWT 6-min walk test

than males with a ratio of 108:10 and the mean age was 42.7 ± 12.5 years [26–28]. Thus, an explanation is that females have a lengthy stay at home (where they breed birds) than men and, therefore, are more prone to more prolonged exposure to pet birds, and females are usually the ones to take care of them [29].

Dyspnea was the most common complaint among all the patients with different grades (mild and moderate grades at almost 90% of them) matching as the main symptom of DPLD as well as dry cough [27–30]. A restrictive pattern in spirometry was prominent with a decrease in oxygen saturation [30].

Although there is no reference for risk stratification of HP patients according to the 6MWDT, the mean value was 266.6 ± 117, and the mean SO_2 by pulse oximetry was 91.4% ± 4.9 before the test and desaturated after the test to a mean SO_2 of 75.8% ± 8 [29].

The fibrotic group showed more clinical worsening regarding dyspnea score, hypoxia, and clubbing and showed greater restrictive lung physiology than the non-fibrotic group, and this could be explained by more scarring of the lung.

The serum level of TGF beta was elevated in newly diagnosed HP cases in relation to the control group showing a statistical significance p value of < 0.001 [31].

With the same concept, a study showed TGF-β_1 with higher immunoexpression levels in sarcoidosis patients [32]. Also, in IPF patients' mean (SD), TGF-β1 values were higher than in the control subjects significantly [33].

As regards serum level of TGF beta, it was higher in the fibrotic group of HP patients than the non-fibrotic group which can be explained by TGF-beta 1 expressed in epithelial cells of patient's lungs with fibrosis where the presence of TGF-beta 1 was not disease-specific but evidence of the chronicity of the lung injury [34, 35].

In this study, we found no significant correlation between the serum level of TGF beta and the distance covered by the patient in 6 MWT (p value 0.128) or desaturation occurred at the end of the test (p value 0.556), and these results were matching with the study conducted by Azmy et al. (2017) [31].

This study also showed that the level of TGF beta in the serum was elevated in hypoxic patients with clubbing and more worsened dyspnea but with no statistical significance and showed elevated serum level of TGF beta in patients with severe degree of restriction but also with no statistical significance. These findings were also retrieved from another study with sarcoidosis patients [30].

TGF-β could participate in the pathogenesis of fibrotic HP and can be a biomarker of value with a negative prognosis.

Conclusion

The serum level of transforming growth factor beta can be used in the assessment of the severity of hypersensitivity pneumonitis as regards the intensity of lung parenchymal changes and a potential target for treatment as it is elevated in newly diagnosed HP cases in relation to the control group, and its level in tyeh fibrotic group of HP patients is more than that in the non-fibrotic group.

Abbreviations

HP	Hypersensitivity pneumonitis
DPLD	Diffuse parenchymal lung diseases
TGF	Transforming growth factor
6MWT	6-Min walk test

Authors' contributions

Main idea: Prof Ayman Salem. Data collection: Heba Hisham, Yasmine Hamdy, Naglaa Bakry, Radwa Marawan. Data analysis: Heba Hisham, Yasmine Hamdy, Naglaa Bakry, Radwa Marawan. Paper review: Ayman Salem, Heba Hisham, Yasmine Hamdy, Naglaa Bakry. All authors shared in the final revision and accepted it.

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Declarations

Competing interests

The authors declare that they have no competing interests.

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