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Anticitrullinated HSP90a In Rheumatoid Arthritis With Interstitial Lung Diseases

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that mostly affects the synovial joints. The arthritis is usually symmetrical, and it causes joint deterioration by eroding cartilage and bone. RA can also cause a variety of extra-articular symptoms **(Esposito A, et al., 2019)**. While cardiac complications account for the majority of RA-related deaths, pulmonary involvement also plays a significant role in morbidity and mortality, accounting for about 10%–20% of all deaths **(Esposito A, et al., 2019)**.

The parenchyma, pleura, airways, or vasculature can all be involved in pulmonary presentation, but parenchymal involvement from ILD is the most prevalent. Pulmonary involvement may occur before the development of articular symptoms in up to 20% of individuals **(Esposito A, et al., 2019).**

Autoantibodies in the blood have been linked to the development of RA-ILD. Smoking raises the risk through causing the development of antibodies as well as raising the citrullination of lung proteins, which increases their affinity for antibodies. **(Shaw M, et al., 2015).**

Anticyclic citrullinated protein antibodies (90 IU/ml) and high titers of rheumatoid factor (100 IU/ml) are indicators linked to an increased risk of developing RA-ILD **(Zhu J, et al., 2014).** Hsp90, a kind of antiicitrullinated protein antibody, shows a specificity of more than 95% for RA-ILD **(Harlow L, et al., 2013).**

The aim of the current study was to assess the association of anti citrullinated Hsp90a to ILD in rheumatoid arthritis patients, and the relationship of Hsp90a to disease parameters.

Methods: The present study including 60 RA patients and 10 IPF patients as control group. Patients with RA who were diagnosed using the American College of Rheumatology/European League Against Rheumatism classification criteria from 2010; they all had definite RA with a score of 6/10. IPF patients diagnosed according to The American Thoracic Society (ATS), European Respiratory Society (ERS), Japanese Respiratory Society (JRS), and Latin American Thoracic Society (ALAT){ ATS/ERS/JRS/ALAT} 2018 diagnostic approach.

They were selected from patients attending the Rheumatology and Rehabilitation Department and Chest Department, Faculty of Medicine, Minia University. Patients were informed about the trial and gave their

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verbal consent. Patients with other autoimmune illnesses such as SSCs and PM/DM were excluded from the study.

Patients were given a comprehensive examination. The disease activity score 28 (DAS28) was used to quantify disease activity, and the health assessment questionnaire was used to assess functional status (HAQ). C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were assessed. Rheumatoid factor (RF), anti-citrullinated peptide antibodies (ACPA), and HSP90a in serum were all tested immunologically.

Radiographic signs of ILD on HRCT; bilateral outlying reticular opacities or honeycombing with or without activity for ground-glass pattern were used to diagnose ILD. Chest assessment was done for RA-ILD through chest examination, chest X ray, pulmonary function test (PFT), and HRCT. HRCT patterns determine and classify according to American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. The statistical program SPSS version 22 was used for statistical analysis.

Results: In the period from August 2017 to October 2018, sixty RA and ten IPF patients were included in the present study. They were divided into three groups: **Group I,** which contain 43 patients with rheumatoid arthritis, 39 females (90.7%) and 4 males (9.3%), their age ranged from 28 - 63 years with a mean of 42.7 ± 9.1 years, and their disease duration ranged from 0.3 - 23 years with a mean of 7.8 ± 5.31 years, this group was further subdivided into two subgroups according to HRCT findings to RA without subclinical ILD Ia (n=18) and RA with subclinical ILD Ib (n = 25).**Group II,** 17 patients with rheumatoid arthritis and ILD, 16 females (94.1%) and 1 males (5.9%), their age ranged from 25 - 65 years with a mean of 49.7 ± 10.9 years, and their disease duration ranged from 0.6 - 21 years with a mean of 5.8 ± 6.42 years. **Group III,** 10 patients with IPF as control group, 7 females (70%) and 3 males (30%), their age ranged from 37-65 years with a mean of 52± 10.67 years.

No statistically significant difference was found between different groups regarding age, sex, family history and disease duration, while we found a statistically significant difference regarding smoking status (p= 0.01).

When we compare the laboratory finding in different groups we found a statistically high significant difference as regard ESR (p=0.005), RF (p=0.01), ACAPA (P=0.017) and HSP90a (p=0.001) as shown in table 1.

We found this significant mainly between RA patients without subclinical ILD (Ia) & RA patients with subclinical ILD (Ib) versus IPF patients (III) in HSP90a (p=0.03, p=0.001) as showen in figure 1.

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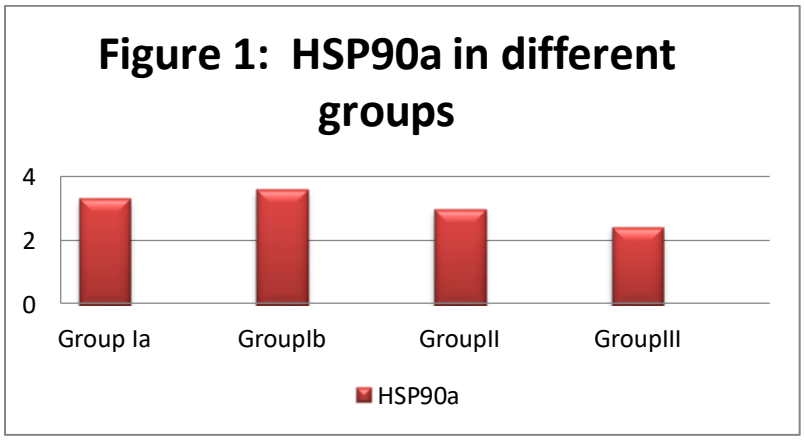
Table 1:Comparison between laboratory finding in different groups:

Laboratory finiding		**Group Ia** RA without subclinical ILD (n=18)	**GroupIb** RA with subclinical ILD (n=25)	**GroupII** RA with ILD (n=17)	**GroupIII** IPF (n=10)	**F**	**P value**
ESR 1st hr	**Range**	12-76	7-65	15-90	15-37	4.6	0.005**
	{ Mean ± SD }	34±18.5	39.8±14.7	47.9±18.7	25±7.6		
RF	**Range**	20-96	32-192	32-192	9-16	4.1	0.01*
	{ Mean ± SD }	48.5±22.6	91.7±56.3	96.3±50.6	12.5±4.9		
ACAPA	**Range**	120-321.9	244.3-1000	246.7-1600	-	4.6	0.017*
	{ Mean ± SD }	245.5±76.2	483.2±187.1	572.2±343.9	-		
CRP	**Range**	12-64	8-192	6-32	-	1.17	0.39
	{ Mean ± SD }	26.3±20.5	44.5±65.5	19.5±9.8	-		
HSP90a	**Range**	2.12-4.23	1.86-4.82	1.68-4.72	1.65-3.75	5.78	0.001**
	{ Mean ± SD }	3.3±0.7	3.6±0.8	2.97±0.75	2.4±0.7		



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In activity and functional assessment of articular manifestation we found a statistically significant difference as regards DAS 28 activity index (p=0.03) and HAQ disability index (p=0.02) mainly between RA patients without subclinical ILD (Ia) versus RA patients with ILD (II).

In our study, the frequency of ILD among RA patients was 70% and the patterns of ILD were in the form of UIP in 58.8%, NSIP in 17% and Combined (NSIP & UIP) (23.5 %) in RA-ILD. We found statistically high significant difference between different groups (p=0.000) as regard different ILD patterns. This significant mainly in NSIP pattern which was more evident in RA patients with subclinical ILD (Ib), when compared to RA patients with ILD (II) and IPF patients (III).

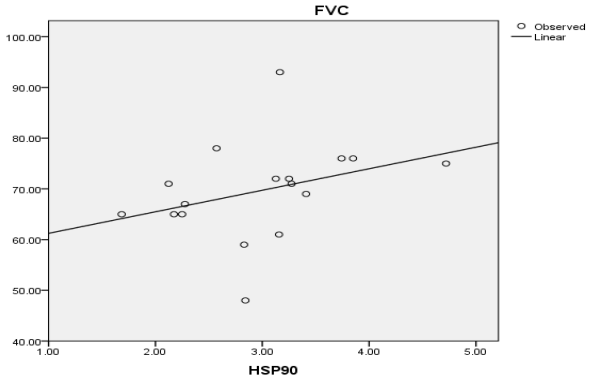
However, UIP pattern was more evident in RA patients with ILD (II), IPF patients (III) in comparative to RA patients with subclinical ILD (Ib). While combined pattern present only in RA with subclinical ILD group (Ib) and RA-ILD (II) by the following present consecutively (12%, 23.5%).

The correlation analysis in RA-ILD group (II) show a statistically significant correlation between ESR (r=0.85, p=0.01), ACAPA (r=-0.6, p=0.02), HSP90a (r=0.49, p=0.04) and FVC (figure 2) in these patients (table 2).

Table 2: Correlation between laboratory parameters & PFTs in group II:

Laboratory findings:		**FVC**	**DLCO**	**TLC**
ESR 1st hr	r	0.85	0.41	-0.17
	P value	0.01*	0.1	0.49
ACPA	r	-0.6	-0.3	-0.23
	P value	0.02*	0.28	0.41
HSP90	r	0.49	0.009	0.002
	P value	0.04*	0.97	0.99

Figure 2: Correlation between FVC & HSP90a in group II



There was statistically significant correlation between ACAPA (r=-0.61, p=0.02), HSP9a (r=-0.51, p=0.03) and chest x ray findings as shown in table 3.

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Table 3: Correlation between laboratory parameters & radiological findings in group II

Laboratory findings:		Chest x ray
ESR 1st hr	r	0.25
	P value	0.31
CRP	r	-0.003
	P value	0.99
RF	r	-0.05
	P value	0.86
ACPA	r	-0.61
	P value	0.02*
HSP90	r	-0.51
	P value	0.03*

No statistically significant correlation between clinical manifestations, X rays findings and PFTs. No statistically significant correlation between laboratory parameters and modified sharp score and no statistically significant correlation between test of 6MWD and ACPA, HSP90, PFTs nor kazarooni scores in group II.

Finally, we did a ROC curve study for HSP 90a in RA groups and found, HSP90 in RA group I (N=43) showed AUC 73% with sensitivity 65%, specificity 71% and cut off point 3.2 (figure 3). However in RA-ILD group (N=17) showed AUC 40% with sensitivity 23%, specificity 51% and cut off value 3.3 (figure 4).

Figure 3: ROC curve for HSP90a in group I:

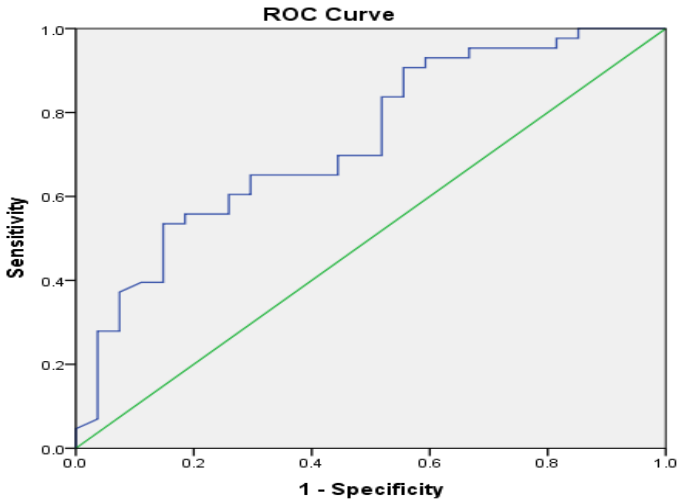

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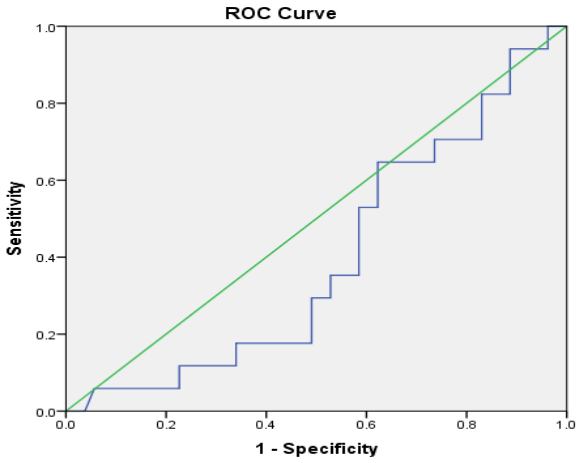
Figure 4: ROC curve for HSP90a in group II:



Discussion: One of the most prevalent extra articular consequences of RA is interstitial lung disease, which causes severe morbidity and mortality **(Kelly C, et al., 2014).** Because of the patient's low functional level as a result of long-term systemic and articular inflammation, the characteristic respiratory signs are often overlooked, resulting in a delayed diagnosis **(Zamora-Legoff J, et al., 2017).**

In the current study, there was a statistically high significant difference as regards HSP90a in RA patients with and without subclinical ILD compared to IPF patients (p=0.001, p=0.03) consecutively, also it was high in RA-ILD when compared with IPF patients but not reach statistically significance which reveals a high specificity of HSP90a in RA patients especially with ILD, this in agree with the findings of Harlow and colleagues where they found a statistical significant difference of anti citrullinated Hsp90 antibody responses between RA-ILD, RA alone and IPF cohorts (P = 0.002, P =0.006) **(Harlow L, et al., 2013).**

In another study where they assessed plasma Hsp90 in a total 136 patients, that were divided into groups according to the type of a rheumatic disease (SpA, RA, PsA), they found Plasma Hsp90 levels were significantly increased in axSpA and in RA patients compared to healthy control **(Storkanova H, et al., 2019).**

Also, **Dong H et al., (2020),** detected HSP90α in serum from IPF patients and healthy controls by ELISA and found that serum HSP90α levels were elevated in IPF patients compared with healthy controls. In contrast, **Procházková L et al., 2013,** analyzed the levels of HSP90 in peripheral blood of 58 patients with RA, 68 patients with AxSpA and 30 healthy volunteers and found that the serum levels of HSP90 were not different between the groups of patients and healthy controls.

In activity and functional assessment of articular manifestation we found a statistically significant difference as regards DAS 28 activity index (p=0.03) and HAQ disability index (p=0.02) mainly between RA patients without subclinical ILD (Ia) when compared to RA patients with ILD (II).

This was in harmony with, **Samy N et al., (2020),** where they studied 160 RA patients subdivided in to two groups, RA only and RA with ILD, RA-ILD patients were significantly higher DAS28 ($p < 0.001$). However, **Fadda S, et al., (2018),** found no significant difference in their patients as regards DAS28 when compare activity between RA-ILD patients and RA only.

As regard radiological finding in the current work, we found chest x ray abnormality – in form of reticlonodular appearance- in 36.7% (22) of RA patients and HRCT interstitial abnormality in 70% (42) of these patients which reinforce the more accuracy of HRCT in detection of ILD in RA patients.

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As a result, a normal chest examination, chest x-ray, and PFT findings cannot rule out ILD in RA patients, and HRCT is still required. This was in agree with, **Samy N et al., (2020),** where Chest x-ray showed abnormal findings in only 28 (17.5%) patients and HRCT abnormal findings were present in 102 (63.75%) patients including 22 (21.57%) who were asymptomatic.

On the other hand, a lower prevalence rate to fall in the range from 4 to 30% **(Ascherman D, 2010)** has been shown. The different prevalence rate may be attributed to different races and unequal detective procedures for ILD.

In the present study, ILD pattern was in the form of UIP 58.8%, NSIP 17% and combined in 23.5% of RA patients, this was in agreement with, **Fadda S, et al., (2018),** where they found Patterns of ILD were in the form of UIP in 62%, NSIP in 27% and mixed 11% in RA patients.
However, others, **Kim E, et al., (2010),** observed NISP in 50%, UIP and mixed patterns in 20% and 30% respectively. This different range depends mainly on the methods of detection and the design of the research.

When we do a correlation analysis in this work, we found that FVC in our study negatively correlated with ACPA (r=-0.6, p=0.02), while strongly correlated with ESR (r=0.85, p=0.01) and HSP90a (r=0.49, p=0.04). This is in disagree with others where they found no correlation between FVC change and other variables in their study **(Robles-Pérez A, et al., 2020).**This different results may due to different methods of detection.

Our results also showed a statistically significant correlation between DLCO and GGO score (r=-0.54, p=0.02) which went with others we they found that pulmonary function parameters (lower FEV1, FVC, TLC, and DLCO) in RA-ILD patients correlated with HRCT abnormalities **(Chen J, et al., 2013).**

In conclusion, HSP90a present in RA patients with increasing titre in RA with subclinical ILD and significant difference in RA with ILD but a more studies needed for better evaluation of its value in these patients. Because disease activity has a major impact on the extent and severity of ILD in RA patients, it is one of the most essential elements to regulate and restrict the progression of ILD in these individuals.

References

Kelly C, Saravanan V, Nisar M, Arthanari S, Woodhead F, Price-Forbes A, et al., (2014). Rheumatoid arthritis-related interstitial lung disease: associations, prognostic factors and physiological and radiological characteristics–a large multicentre UK study. Rheumatology ;53(9):1676–82.

Zamora-Legoff J, Krause M, Crowson C, Ryu J, Matteson E (2017). Progressive Decline of Lung Function in Rheumatoid Arthritis-Associated Interstitial Lung Disease. Arthritis Rheumatol;69(3):542–9.

Harlow L, Rosas I, Gochuico B, Mikuls T, Dellaripa P, Oddis C, et al.,(2013). Identification of citrullinated hsp90 isoforms as novel autoantigens in rheumatoid arthritis-associated interstitial lung disease. Arthritis Rheum.;65 (4):869–879.

Storkanova H, Bubova K, Oreska S, Spiritovic M, Hermankova B, Gregorova M, et al.,(2019). HSP90 in axial spondyloarthritis, psoriatic arthritis and rheumatoid arthritis. Annals of the Rheumatic Diseases. London**:** A76. p171.

Dong H, Zhong W, Zhang J, Liu Y, Chen W, Ye C, et al.,(2020). Extracellular HSP90a promotes cellular senescence in pulmonary fibrosis through TGFß signaling. European Respiratory Journal;56(suppl 64)**:** 451.

Procházková L, Hulejová J, Nemec P, Šenolt L, (2013). Circulating Heat Shock Protein 90 (HSP90) in patients with rheumatoid arthritis and axial spondylarthritis. Ceska Revmatologie;21(4):164-169.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

Samy N, Salah H, Hammoda R, (2020). Rheumatoid arthritis patients with interstitial lung disease: Clinical, radiological and laboratory characteristics.The Egyptian Rheumatologist.1110-1164.

Fadda S, Khairy N, Fayed H, Mousa H, Taha R, (2018). Interstitial lung disease in Egyptian patients with rheumatoid arthritis: Frequency, pattern and correlation with clinical manifestations and anti-citrullinated peptide antibodies level.The Egyptian Rheumatologist.Volume 40, Issue 3, Pages 155-160.

Ascherman D, (2010). Interstitial lung disease in rheumatoid arthritis. Curr Rheumatol Rep, 12 (5), pp. 363-369

Kim E, Elicker B, Maldonado F, Webb W, Ryu J, Van Uden J, et al., (2010). Usual interstitial pneumonia in rheumatoid arthritis-associated interstitial lung disease. Eur Respir J;35(6):1322–8.

Robles-Pérez A, Luburich P, Bolivar S, Dorca J, Nolla J, Molina-Molina M, et al., (2020). A prospective study of lung disease in a cohort of early rheumatoid arthritis patients. scientific reports,10**,** 15640.

Chen J, Shi Y, Wang X, Huang H, Ascherman D, 2013.Asymptomatic preclinical rheumatoid arthritis-associated interstitial lung disease. Clin Dev Immunol, p. 406927.

Vázquez N, Albaladejo L, Arija S, Barco C, Cano C, Garnica I, fernández-Nebro A, etal., (2019). Analysis of clinical–analytical characteristics in patients with rheumatoid arthritis and interstitial lung disease: Case–control study. Reumatología Clínica**,** Vol. 17. Issue 4,Pages 197-202.

Esposito A, Chu S, Madan R, Doyle T, Dellaripa P, 2019. Thoracic manifestations of rheumatoid arthritis. Clin Chest Med 40(3):545–560.

Shaw M, Collins B, Ho L, Raghu G (2015). Rheumatoid arthritis- associated lung disease. Eur Respir Rev 24(135):1–16.

Zhu J, Zhou Y, Chen X, Li J (2014). A metaanalysis of the increased risk of rheumatoid arthritis-related pulmonary disease as a result of serum anticitrullinated protein antibody positivity.J Rheumatol 41(7):1282–1289.

Hance W, Dole K, Gopal U, Bohonowych E, Jezierska-Drutel A, et al., (2012). Secreted Hsp90 is a novel regulator of the epithelial to mesenchymal transition (EMT) in prostate cancer. J Biol Chem; 287:37732–44.