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Immunological Evaluation of 8-Isoprostane and Periostin of Idiopathic Pulmonary Fibrotic patients in Al-Diwaniyah province

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Abstract

Background: Idiopathic pulmonary fibrosis (IPF) is a hetero-geneous sickness considered via an perversely stimulated immunity system. Existing study aimed to gauge of 8-Isoprostane and Periostin of IPF sick in Al-Diwaniyah province. **Methods**: the existing training stayed a case-control training comprised 70 sick who were diagnosed (Clinically, and X-ray) with IPF and 70 healthy control groups. The samples collected from individuals visited Al-Diwaniya teaching hospital in Al-Diwaniyah city. ELISA was used in this study for quantitative determination of 8-Isoprostane and Periostin in serum samples of participants and done it according to company instruction (BT-LAB). **Results**: The ELISA test showed important rise in concentration mean of 8-Isoprostane and Periostin in the sick (3876.7± 927 ng/ml and 42.65 ± 12.3 respectively) compared to the control (1443.67 ± 306 ng/ml and 16.25 ± 11.6 ng/ml separately) (P< 0.05). The absorption of 8-Isoprostane increased in females (3688.3± 953 ng/ml) while concentration mean of Periostin increased in males (4199.6± 900 ng/ml). Moreover, the concentration mean of 8-Isoprostane and Periostin augmented in patients in age group3 and group1 (4182.7 ± 903 ng/ml and 50.83 ± 13.4 ng/ml respectively).We found a higher mean of 8-Isoprostane and periostin concentration in smoking patients (4037.25 ± 955 ng/ml and 54.57 ± 12.6 ng/ml) compared to non-smokers (3771.2 ± 912 ng/ml and40.83 ± 11.5 ng/ml respectively). In conclusion: 8-Isoprostane and periostin are clearly associated with IPF and their concentrations may be affected by age and gender. Therefore, these indicators can be relied upon as markers in the analysis of IPF.

Keywords: IPF, 8-Isoprostane, periostin, ELISA, Al- Diwaniyah,

Introduction

Idiopathic pulmonary fibrosis is a long-lasting, developing lung illness noticeable via ongoing fibrosis in the lungs and histological traits typical of normal interstitial pneumonia. [1]. Symptoms such as heightened coughing and dyspnea contribute to a diminished quality of life. On average, sick diagnosed with IPF have a median persistence of 3–5 years. [1,2]. The immune system is garnering more attention in the context of interstitial lung illnesses, as our

understanding of its involvement in fibrosis progress and reply to treatments continues to grow. [3]. Dysregulated immune responses and imbalanced processes of injury, inflammation, and repair are key factors in initiating and advancing idiopathic pulmonary fibrosis. The guiding arm of the immunity system productions vital parts in managing harmful immune reactions, regulating inflammation, and influencing the shift from inflammation to fibrosis. [4]. It's believed that persistent alveolar-micro injuries result in a sustained and disordered wound healing process, contributing to the progression of IPF. [5]. Fibrogenesis is characterized via a significant buildup of extracellular matrix (ECM) generated via myofibroblasts, which includes substances like collagen, laminin,elastin, fibronectin, hyaluronan, and glycoproteins. This accumulation leads to the irreparable congealing of alveolar walls, impairing the altercation of oxygen and carbon dioxide among the bloodstream and alveolar air. [6,7].

F2-isoP and other isoprostanes possess significant biological properties across various organs. Within the lung, these compounds play a regulatory role in cellular processes that influence airway smooth muscle tone, neural secretion, epithelial ion flow, endothelial cell adhesion and permeability, as well as macrophage adhesion and function. [8] **.**

Isoprostanes are substances similar to prostaglandins formed through the oxidative breakdown of arachidonic acid by free radicals. Elevated levels of F2-isoprostanes in biological samples have long been acknowledged as an indicator of heightened oxidative stress [9]. Increased concentrations of F2-isoprostanes have been detected in the blood, bronchoalveolar lavage (BAL) fluid, and exhaled breath condensate of patients with idiopathic pulmonary fibrosis (IPF). Furthermore, their ranks are raised in the bronchoalveolar lavage liquid of persons distress from interstitial lung illnesses. **[10].**

Periostin, an extracellular matrix protein fitting to the fasciclin family, productions a vital role in the pathogenesis of illnesses considered via raised inflammation and fibrosis levels. Research designates that periostin is considerably upregulated in the lungs of sick with idiopathic pulmonary fibrosis (IPF), mainly in fibroblasts within vigorously fibrotic parts [11]. The mixture of periostin in fibroblasts is encouraged by numerous features, comprising TGF-β and IL-4/IL-13. [12].

The protein periostin is coded through the osteoblast-specific factor-2 gene, which is its authorized name. In humans, this gene is likewise denoted to as POSTN, PN, OSF-2, and PDLPOSTN (Gene ID: 10631). [13].

According to Naik et al. (2012), Periostin promotes deposition of extracellular matrix (ECM), proliferation of mesenchymal cells, and fibrosis in the lung and other organs. Elevated levels of periostin have been observed in idiopathic pulmonary fibrosis (IPF) compared to control groups. [14].

Regrettably, there is a lack of comprehensive studies on the immune mechanism of lung fibrosis globally. Many immune indicators, whether cellular or mediators, remain inadequately researched. [13-15]. In Iraq, particularly in Diwaniyah province, there is a dearth of studies examining the part of 8-Isoprotane and Periostin in patients with IPF. This prompted the initiation of our current research on this subject.

Material and methods

Patients and Blood Sample Collection: the existing training was a case-control study comprised 70 patients who were diagnosed (Clinically, and X-ray) with IPF, in addition, 70 healthy control groups who had no times past or medical indication of PF or asthma. The patients were seen to Al-Diwaniyah teaching hospital in Al-Diwaniyah city from the dated of January, 2023 until the end of November 2023. All information about recovery patients and control group was noted in questionnaire forma during direct meeting with patients and control individuals, the questionnaire forma, which contain name, age, gender, and smoking or not. Three ml of blood placed in a plane tube which left to clotted at room temperature (20- 25 ºC) for 30 minutes, at 2500 rpm centrifuged for 10 minutes, and formerly separated the serum into four parts in a ependroff tubes, stored at -20ºC for immunological assays.

Immunological assays: In this study, ELISA was employed to quantitatively determine levels of 8-isoprostane and periostin in serum samples from patients and healthy controls, following the manufacturer's instructions (BT-LAB). Optical density (OD) readings were measured by means of a microplate reader set to 450 nm, immediately afterward addition the stop solution, within a 10-minute timeframe. The ELISA results were calculated based on the OD readings obtained for both standards and tasters. A standard curve was then generated through secrecy the mean OD values for each standard on the X-axis alongside their respective concentrations on the Y-axis, and a best-fit curve was tired concluded the points on the graph.

Ethical Approval: The training stayed accepted out after gaining the agreements of the sick and the Iraqi Ministry of Health.

Statistical analysis: The statistical test stood prepared by means of the Statistical Set for the Social Sciences, style 22, with Microsoft Excel 2010. The outcomes stood measured statistically different while the probability (P value) was less than 0.05 [16]**.**

Results

The ages of the sick extended as of 30 to 90 years, with an mean age of 59.5 ± 17.92 years, and the mutual of them stayed female (44%), as most of these females were within the age grouping of 30 – 45 years, as in Table (1 and 2). Moreover, we found the majority of sick in the third age grouping (60-75 years), at a rate of 36%. On the other hand, the average age of healthy people was 51.7 ± 13.41 years, and the percentage of females was also more than that of males (42%) as shown in table (3). Statistically, we did not invention important variances (P >0.05) when comparing the genders and ages of patients with the control.

To conclude the character of smoking in the occurrence and progress of the disease, we conducted a questionnaire about the percentage of smoking among patients. We found that the number of smokers were 7 individuals (10%) out of 70 patients (P = 0.002), as in Figure (1).

Table (1): The case-control alteration in mean age.

Age (years)	Healthy controlling	Cases	*P* value
Range	31- 88	30 – 90	
Mean ± SD	51.7 ± 13.41	59.5 ± 17.92	0.114 [NS]

SE	**1.60**	**2.14**	
N	**70**	**70**	

Table (2) : Dissemination of sick with IPF over age grouping and sex

Age groups	Age range	Men	Women	N (%)	*P* value
Group 1	**30 -45**	1	13	14 (20)	0.001 [S]
Group 2	**45- 60**	1	10	11 (16)	0.046 [S]
Group 3	**60-75**	16	8	24 (34)	0.042 [S]
Group 4	**≥ 75**	8	13	21 (30)	0.057 [NS]
Total	**30 – 90**	26	44	70 (100)	0.039 [S]

Table (3) : The case-control alteration in sex dissemination.

Gender	Healthy controlling		Cases		*P* value
	N.	%	N.	%	
Female	**42**	**60**	**44**	**63**	**0.537 [NS]**
Male	**28**	**40**	**26**	**37**	**0.536 [NS]**
Total	**70**	**100**	**70**	**100**	

*** NS= No Significant Association (*P*>0.05)**

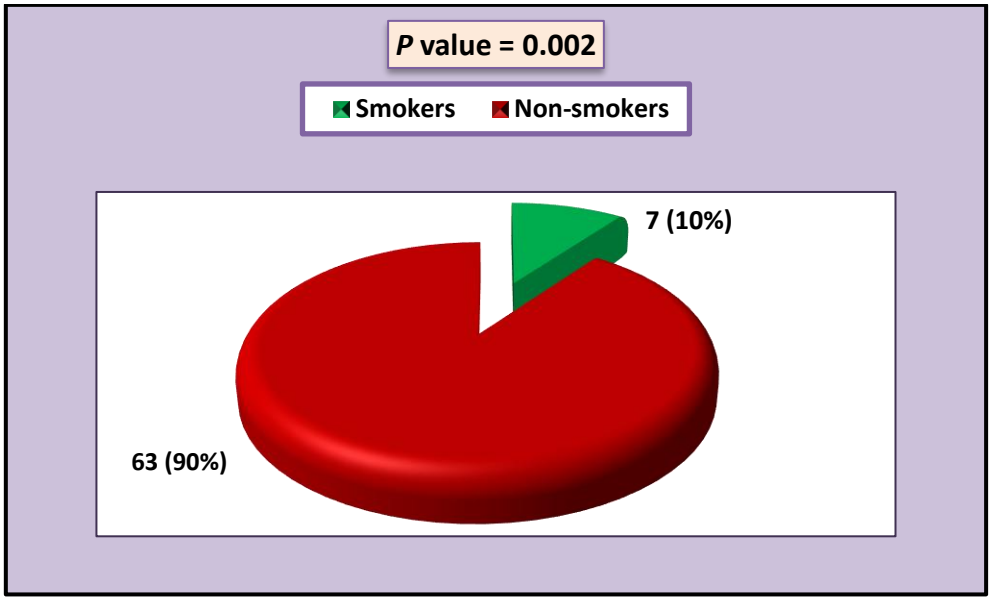

Figure (1): Smoking rate among sick with idiopathic pulmonary fibrosis

The outcomes of the ELISA test showed significant increase in concentration mean of 8-isoprotane and Periostin in the sick serum (3876.7 ± 927 ng/ml and 42.65 ± 12.3 respectively) compared to the control (1443.67 ± 306 ng/ml and 16.25 ± 11.6 ng/ml separately) (P< 0.05), as in Table (4). When distributing the concentration of these indicators according to the genders of the patients, we found that the concentration of 8-isoprotane increased in females (3688.3± 953ng/ml) while concentration mean of Periostin increased in males (40.49 ± 12.8 ng/ml), as

in the table (5). Moreover, the absorption mean of 8-Isoprostane and Periostin augmented in sick in age group3 and group1 (4182.7 ± 903 ng/ml and 50.83 ± 13.4 ng/ml respectively), as in table (6).

In Table (7) We found a higher mean of 8-Isoprostane and periostin concentration in smoking patients (4037.25 ± 955 ng/ml and 54.57 ± 12.6 ng/ml) compared to non-smokers (3771.2 ± 912 ng/ml and 40.83 ± 11.5 ng/ml respectively).

Table (4): Appraisal of some immune indicators between patients and controls

Immune markers	Cases	Control	T test	95 %CI	P value
	Mean ± SD	Mean ± SD			
8-isoprotane (ng/ml)	3876.7 ± 927	1443.67 ± 306	20.88	2202.59 to 2663.41	0.003 [S]
Periostin (ng/ml)	42.65 ± 12.3	16.25 ± 11.6	13.36	22.15 to 29.85	0.039 [S]

Table (5): Distribution studied immunologic markers according to patients' gender

Immune markers	Females	Males	T test	95 %CI	P value
	Mean ± SD	Mean ± SD			
8-isoprotane (ng/ml)	3688.3± 953	4199.6± 900	3.262	-820.79 to -201.21	0.014 [S]
Periostin (ng/ml)	43.92± 11.5	40.49 ± 12.8	1.965	-0.02 to 8.02	0.377 [NS]

Table (6): Distribution of 8-Isoprotane and Periostin according to patients' age groups

Immune markers	Grouping 1	Grouping 2	Grouping 3	Grouping 4	P value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
8-Isoprotane (ng/ml)	4113 ± 965	3159.83 ± 888	4182.7 ± 903	3583.4 ± 886	0.033 [S]
Periostin (ng/ml)	50.83 ± 13.4	35.40 ± 9.55	40.17 ± 11.6	42.23 ± 10.3	0.075 [NS]

Table (7): Distribution studied immunologic markers according to cigarettes smoking

Immune markers	Smokers	Non-smokers	T test	95 %CI	P value

	Mean ± SD	Mean ± SD			
8-Isoprotane (ng/ml)	**4037.25 ± 955**	**3771.2 ± 912**	**1.6857**	**-46.03 to 578.13**	**0.039 [S]**
Periostin (ng/ml)	**54.57 ± 12.6**	**40.83 ± 11.5**	**7.062**	**9.893 to 17.587**	**0.369[NS]**

Discussion

In our results, we found that IPF increases with age (over 60 years) especially in males while it appears less commonly under 60 years especially in females. However this result agreed with number of results of previous studies [17,18]. According to Leuschner *et al.* study, Out of the total 1,009 patients, 350 (34.7%) were aged 75 years or older. Compared to younger patients, elderly IPF patients exhibited a greater number of co-morbidities (3.6 ± 2.5 vs. 2.8 ± 2.3; $p < 0.001$). [18]. The reason of increasing IPF with age discussed by Raghu *et al.*, (2018) who showed that IPF predominantly affects individuals aged over 60 years, attributed to biological changes that render aging lungs more susceptible to the disease. [19]. Lately, genetic changes have been pinpointed that correlate with a heightened risk of developing IPF. However, these alterations may also elevate the likelihood of other age-related lung conditions like long-lasting obstructive pulmonary disease (COPD) or lung tumor [20]. While the precise link among aging and the onset of IPF remains indistinct, various processes associated with the disease have been identified, such as telomerase limitation, mitochondrial dysfunction, cellular aging, and dysregulation of the extracellular matrix. [18]. A recent study from a US registry exposed a negative association among age and the utilization of antifibrotic therapy (pirfenidone and nintedanib). [21]. Conversely, Leuschner et al.'s study revealed that more than half of both ageing and nonelderly sick stood prescribed anti-fibrotic treatment when they joined the study, indicating no discrepancies based on age. [18] Recent findings indicate that patients aged 75 years and above with IPF are more prone to discontinuing pirfenidone over a one-year follow-up period and experiencing a greater occurrence of gastrointestinal disorders. [22].

In recent times, there has been a growing recognition of the gender dimension in diseases like COPD or lung cancer. While it's established that IPF is further prevailing in men than women, there's scant knowledge regarding potential gender-based variations in the disease's manifestation and its accompanying comorbidities. [23]. Our study showed that most IPF patients were females (63%) in contact to study of Zaman *et al.,* in 2020. In research of Zaman *et al.*, Among the pooled cohort of 1,263 patients with development information, around 71% stayed males. Next altering for age, FVC % forecast, and Dlco % foretold, man gender stayed autonomously linked to a more danger of loss or lung transplantation (danger proportion for males, 1.4; 95% CI, 1.2-1.7; $P < 0.001$). [24]. Previous research by Han et al. has proposed that women might undergo a gentler illness development, as indicated by the desaturation part on a 6-minute walk examination, in comparison to males. [25]. Additional research has indicated that men diagnosed with IPF face a advanced danger of humanity paralleled to women with the condition. However, the extent of the contrast in survival duration has not been previously

documented. [26,27]. The underlying reasons for these outcome variations based on sex—whether they stem from differing risks, disease severity, distinct phenotypes, or inherent biological factors related to sex—remain uncertain. [24].

In study, smoking detected in small numbers of older males and not determine in females and this may be related to earlier respiratory problems of females which prevent cigarette smoking or may inadequate samples that involved in our research. Despite studies indicating a robust correlation among cigarette smoking and the progress of IPF, the apparatus concluded which smoking might contribute to the IPF pathogenesis remains unknown. [25]. Due to IPF being acknowledged as an age-related ailment, there's speculation that smoking could potentially play a role in the onset of interstitial lung illness in an age-dependent fashion. Additionally, there's a notion that heightened oxidative tension in both existing and previous smokers could accelerate illness advancement. [26].

Immunologically, present study determined high serum concentration of 8-Isoprostane in IPF likened to control in our city, 8-Isoprostane raised in sick with IPF, this agreement with the study of Malli *et al.*,(2013) who found that serum 8-isoprostane ranks stood augmented in entirely sick groups vs controls ($p<0.001$) [27]. Also, the results of Montuschi *et al.,* study showed that oxidative stress is augmented in cystic fibrosis and might be counted through calculating 8-isoprostane absorptions in breath condensate of IPF patients **[28].** Ogawa et al. (2006) discovered that elevated levels of 8-isoprostane connected with the strictness of pulmonary fibrosis, the range of renal vascular destruction, and immunological anomalies in systemic sclerosis. This correlation proposes that increased oxidative stress may contribute to the progression of systemic sclerosis. **[29].**

Current results showed increase serum concentration of periostin in IPF especially in patients in age group1 and in smokers. So this immunological marker appeared comparable in men and women. Okamoto *et al.,* remember Periostin's involvement in fibrogenesis suggests it could serve as a valuable biomarker for forecasting illness development and assessing therapeutic effectiveness in sick with IPF. **[30].**

Yoshihara et al. (31) discovered that exposure to periostin or its overexpression does not enhance propagation or the appearance of cell cycle-related genes in lung fibroblasts. These findings propose that the cell cycle in lung fibroblasts is tightly controlled, unlike in cancer cells, and that excessive periostin does not promote proliferation of lung fibroblasts in vitro (32). However, it has been reported that the negative regulatory instrument of the cell cycle is weakened in patients with IPF (33). In such cases, periostin stimulation may improve the proliferation of lung fibroblasts. Moreover, the observation that lung construction remains intact in periostin-deficient mice suggests that periostin may production a dispensable role in the proliferation of lung fibroblasts under normal conditions. **[34].**

Present outcomes found significant increase of 8-isoprotane and Periostin in IPF smokers compared with non- smokers. Researchers found that revelation of fibroblasts to cigarette smoke displays a quick and constant stimulation of prostaglandin-endoperoxide synthase, cyclooxygenase2. Also, increased prostaglandin-endoperoxide synthase, cyclooxygenase2 mRNA ranks are institute in peripheral blood mononuclear cells of smokers. Finally, immune

cells for instance leucocytes and neutrophils are important bases of 8-Isoprostane and periostin **[35,36].**

Conclusion

The outcomes of our training displayed a upper rate of 8-Isoprotane and Periostin concentrations in patients compared to healthy people, and the concentrations of these indicators varied when distributed according to gender. Our outcomes designate that 8-Isoprotane and Periostin production a part in mediating lung fibrosis. These results could aid researchers in understanding the recent advancements in IPF pathogenesis associated with 8-Isoprotane and Periostin, offering new goals and a theoretic foundation for the progress of medical medicines for IPF.

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