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Interplay of Heat Shock Proteins and Oxidative Stress in Modulating Neutrophil Activation in Cystic Fibrosis Inflammation

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ABSTRACT

Background: Cystic fibrosis, is an autosomal recessive disorder characterized by persistent inflammation, unregulated immune responses, and pneumonic complications. The central role of CF-associated inflammation is neutrophil activation triggered by oxidative stress and Heat Shock Proteins (HSPs). The present study seeks to explore the interaction of oxidative stress, HSPs, and neutrophil activation in CF patients.

Methods: A case-control study was conducted from January-2023 to June-2024 at the School of Pain Regenerative Medicine, The University of Lahore, including 100 CF patients and 100 age and gender-matched controls. A purposive sampling method was employed to recruit participants for this retrospective case-control study. Serum MDA, 4-HNE, 8-OHdG, and isoprostanes were quantified. The HSP27, HSP70, and HSP90, neutrophil activation was measured using neutrophil elastase, myeloperoxidase, and matrix metalloproteinase-9. Values are expressed as mean±SD with a significance threshold of $p < 0.05$.

Results: The MDA values were (5.89±1.38 nmol/mg), 4-HNE (18.3±4.63 mol/L). HSP27 levels were significantly increased (72.53±12.27 ng/mL), HSP70 (110.23±15.67 ng/mL), and HSP90 (88.6±14.21 ng/mL). Neutrophil activation markers, (250.88±35.16 g/L) and MPO (340.01 42.22 ng/mL) were significantly increased. Neutrophil elastase (200 g/L) has a high sensitivity (83%) (95 % CI: 4.36-14.25), which is a significant predictor of neutrophil-driven inflammation.

Conclusion: The findings suggest that neutrophil activation and sustained inflammation in CF are caused by HSPs and oxidative stress. Elevated HSPs and oxidative stress markers are associated with increased pneumonic inflammation. Novel curative measures to reduce CF-associated inflammation are likely to be developed through targeting HSPs and oxidative stress variables.

Keywords: Heat Shock Proteins, Malondialdehyde (MDA), Myeloperoxidase, Matrix metalloproteinase-9.

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INTRODUCTION

Cystic fibrosis is a systemic disease that has an autosomal recessive mode of transmission meaning that the abnormality is expressed only when the child inherits two bad genes of the same gene pool, the CFTR[1]. The dysfunction of the CFTR protein pursuing chloride ions transport across the epithelial cells leads to the successive migration of mucus in several organs. In the lungs, this mucus remains thick and forms a media for chronic infections and constant inflammation mainly due to neutrophils which are central in immune response[2,3]. Whereas healthy human subjects exhibit tightly controlled, short-term neutrophil responses, in CF, neutrophils are continuously activated and do not get cleared effectively, thus sustaining inflammation and progressive lung tissue destruction[3,4]. This immune dysregulation is more related to oxidative stress, the issue of excess production of ROS, and insufficient antioxidant capability. There is increasing evidence that points to the accumulation of products of lipid peroxidation, MDA, and 4-HNE, in CF patients due to inflammation and progression of the disease[5,6].

These proteins, including HSP 27, HSP 70, and HSP 90 can protect cells under stress through chaperon action and by preventing the formation of additional aggregates of non-degradable proteins[5,7]. Identified in CF, oxidative stress and chronic inflammation induce these HSPs involved in regulating neutrophil chemotaxis, aggregation, degranulation, and the synthesizing of various cytokines. This dysregulation then increases the inflammatory response and consequentially increases tissue damage in a continuous loop[8,9]. Although extensive work has investigated oxidative stress, HSPs, and neutrophil activation in inflammation, the exact mechanism of these factors in the context of the CF remains unclear[10]. Recent articles have pointed to the possibility of these pathways as being pro-inflammatory, opening up new possibilities for treatment. Nevertheless, the knowledge of these signaling events at the molecular level remains still fragmentary[11,12].

This work aimed at investigating the involvement of oxidative stress, HSPs, and neutrophil activation in inflammation associated with CF. Hence, this work seeks to assess the potential of oxidative stress indices as well as major HSPs to enhance the understanding of neutrophil-mediated tissue damage in CF at the molecular level. The results could help to establish new targets for treatment to reduce chronic inflammation and enhance CF patients' prognosis. Based on this assumption, it is proposed here that increased levels of, oxidative stress and HSP, leading to sustained activation of neutrophils and chronic inflammation represent a central pathway of CF disease. This work aimed at trying to understand these interactions, fill these gaps, and obtain information that may be useful in searching for ways of interrupting the inflammatory process in CF.

METHODS

A retrospective case-control study comprising 100 CF patients and 100 controls was conducted from January 2023 to June 2024. The study was conducted at the School and Regenerative Medicine, The University of Lahore. One hundred and ninety-six participants were recruited; 100 clinically diagnosed cystic fibrosis patients and 100 age- and gender-matched healthy controls. A purposive sampling method was employed to recruit participants for this retrospective case-control study. This research was approved by the institutional review board SPRM/UOL/MOCT/D01/0098. and the participants' informed consent was also obtained before participation was allowed.

Blood samples were collected from participants with CF and analyzed for immune function and inflammation. Oxidative stress markers like malondialdehyde, 4-hydroxynonenal, 8-hydroxy-2'-deoxyguanosine, and isoprostanes were measured in plasma using ELISA kits. Heat shock protein concentrations were assessed using commercially available ELISA kits. Plasma concentrations of neutrophil elastase, MPO, and MMP-9 were estimated using Neutrophil Elastase, Myeloperoxidase, and Matrix metalloproteinase-9 ELISA kits. The study aimed to compare immune function and inflammation in CF patients to healthy controls.

The sample size for comparing two groups can be calculated using the formula .

$$n=2(Z\alpha/2+Z\beta)^2\cdot\sigma 2/\Delta^2$$

$$n=2 \times (1.96+.84)^2 x 25.48/2^2$$

$$n=2 \times 7.84 \times 25.48/4$$

$$n=2 \times 199.76/4$$

n=2 x 49.94
n ≈100
Note (The estimated variance (σ2\sigma^2σ2) required for the study is approximately was (25.48)
Where:
• $Za/^2$: Z-value corresponding to the desired significance level
• Z_β: Z-value corresponding to the desired power
• σ^2: Estimated variance of the outcome variable.
• Δ: Minimum detectable difference between groups.
The statistical evaluation was performed using IBM SPSS Statistics Version 26 and GraphPad Prism Version 9.0. Descriptive statistics, together with continuous variables are expressed as mean standard deviation (SD), whereas categorical variables. Independent sample t-tests were used in the inferential study. To assess the sensitivity and specificity of the major biomarkers, Odds Ratios (OR) together with 95 % boldness intervals (CI) were calculated for risk assessment, and neutrophil activation is assessed by Pearson's correlation.

RESULTS

The study analyzed the relationship between oxidative stress markers, heat shock proteins (HSPs), and neutrophil activation in cystic fibrosis (CF) patients. The results showed a slight association between oxidative stress-induced DNA damage and lipid peroxidation in CF patients. The study also found a favorable relationship between 4-hydroxynonenal (4-HNE), HSP27, myeloperoxidase (MPO), and neutrophil elastase. However, no significant associations were found between 4-HNE and MMP-9, suggesting a narrow direct consequence of extracellular matrix degeneration. The study also revealed that 28.11% of patients with CF had a family history of cystic fibrosis. The hematological analysis indicated differences between CF patients and healthy controls. The CF group had higher WBC count, neutrophil ratio, lymphocyte ratio, mean hemoglobin levels, platelet counts, C-reactive protein (CRP) levels, and HSP expression. CF patients also showed higher MDA levels, 4-HNE levels, oxidative DNA lesion 8-hydroxy-2'-deoxyguanosine (8-OHdG), isoprostanes, and HSPs. HSP expression was increased in CF patients compared to a healthy population.

Serum HSP27 was significantly elevated in CF patients compared to the control group, while HSP70 and HSP90 levels were significantly elevated in CF patients. These findings indicate high-stress response in CF patients. Neutrophil elastase levels were also significantly elevated in CF patients due to neutrophil activation and increased tissue remodeling. The study found that MMP-9 demonstrated the highest sensitivity and accuracy (83%, supplementary material Fig. with some significant odds ratio (OR) of 8.50 (95% CI: 4.70-15.39). Neutrophil elastase also showed high sensitivity and a significant OR (7.89, 95% CI: 4.36-14.25). HSP70 had 81% sensitivity and 82% specificity, thus showing potential for diagnostic markers.

The study also found a strong relationship supplementary material, in different figures between 4-hydroxynonenal (4-HNE), HSP27, myeloperoxidase (MPO), and neutrophil elastase. However, no significant associations were found between 4-HNE and MMP-9, suggesting a narrow direct consequence of extracellular matrix degeneration. The study also found a nuanced interaction among oxidative stress markers, HSPs, and neutrophil activation in CF inflammation, demonstrating that oxidative stress modulates HSP expression and neutrophil activity, contributing to the inflammatory cascade observed in CF patients. Targeting these markers may provide a new curative avenue for controlling CF-related inflammation and neutrophil overactivation.

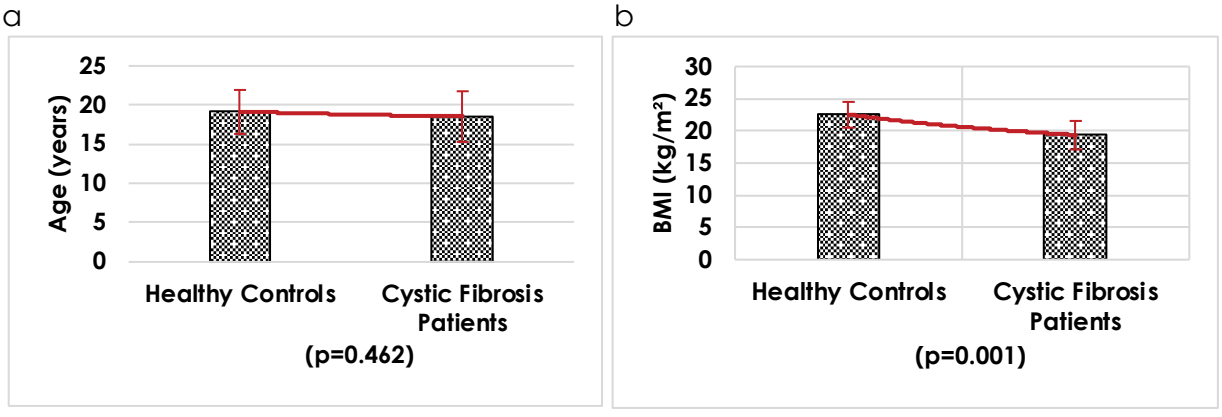

Figure 1: Baseline Characteristics of Study Population for The Interplay of Heat Shock Proteins and Oxidative Stress in Modulating Neutrophil Activation in Cystic Fibrosis Inflammation, where 'A and B' Representing Age and BMI Characteristics Respectively.

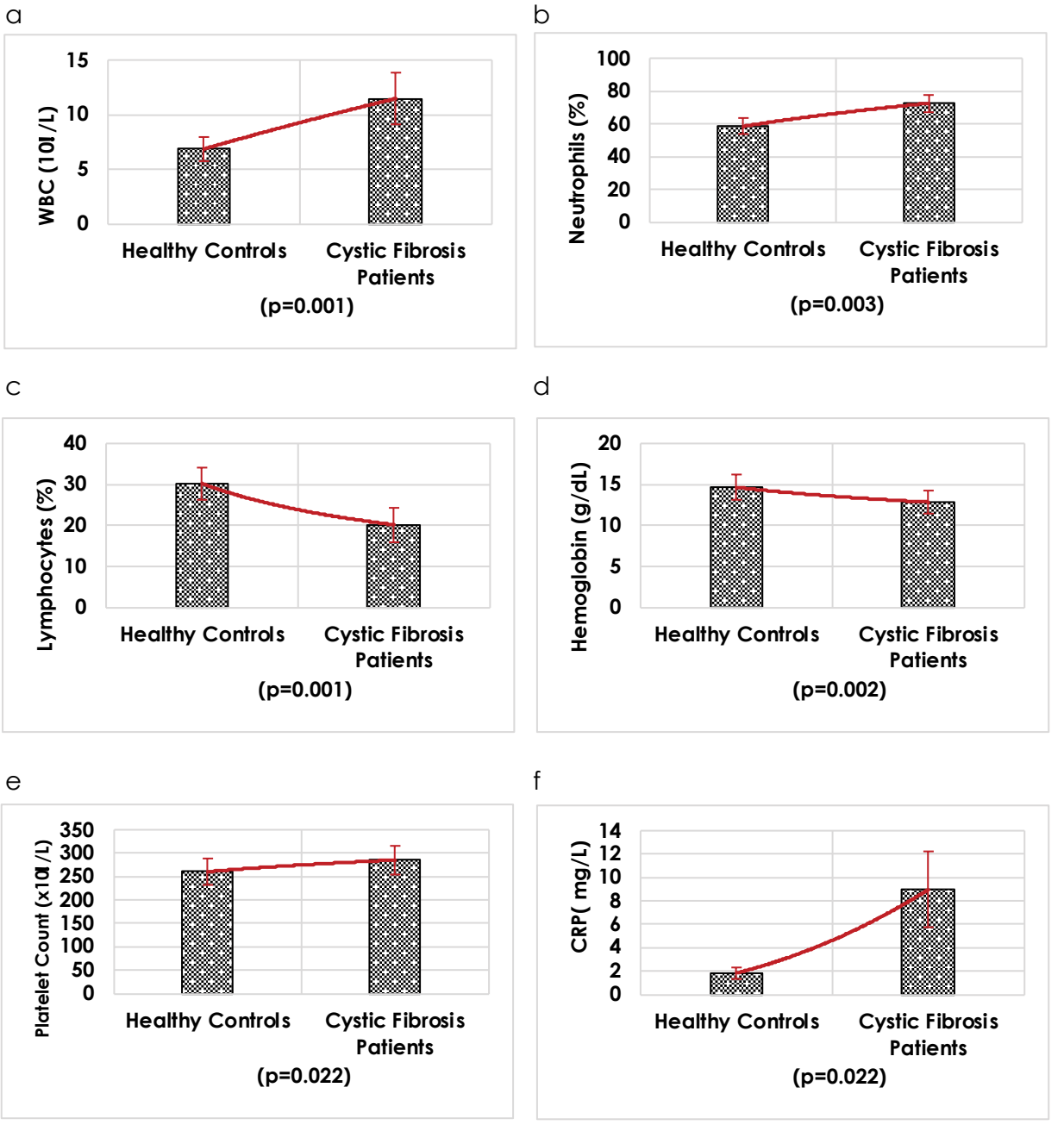

Figure 2: Hematological Profiling for The Interplay of Heat Shock Proteins and Oxidative Stress in Modulating Neutrophil Activation in Cystic Fibrosis Inflammation, where 'A, B, C, D, E and F' Representing WBCs, Neutrophils, Lymphocytes, Hemoglobin, Platelets, and CRP respectively.

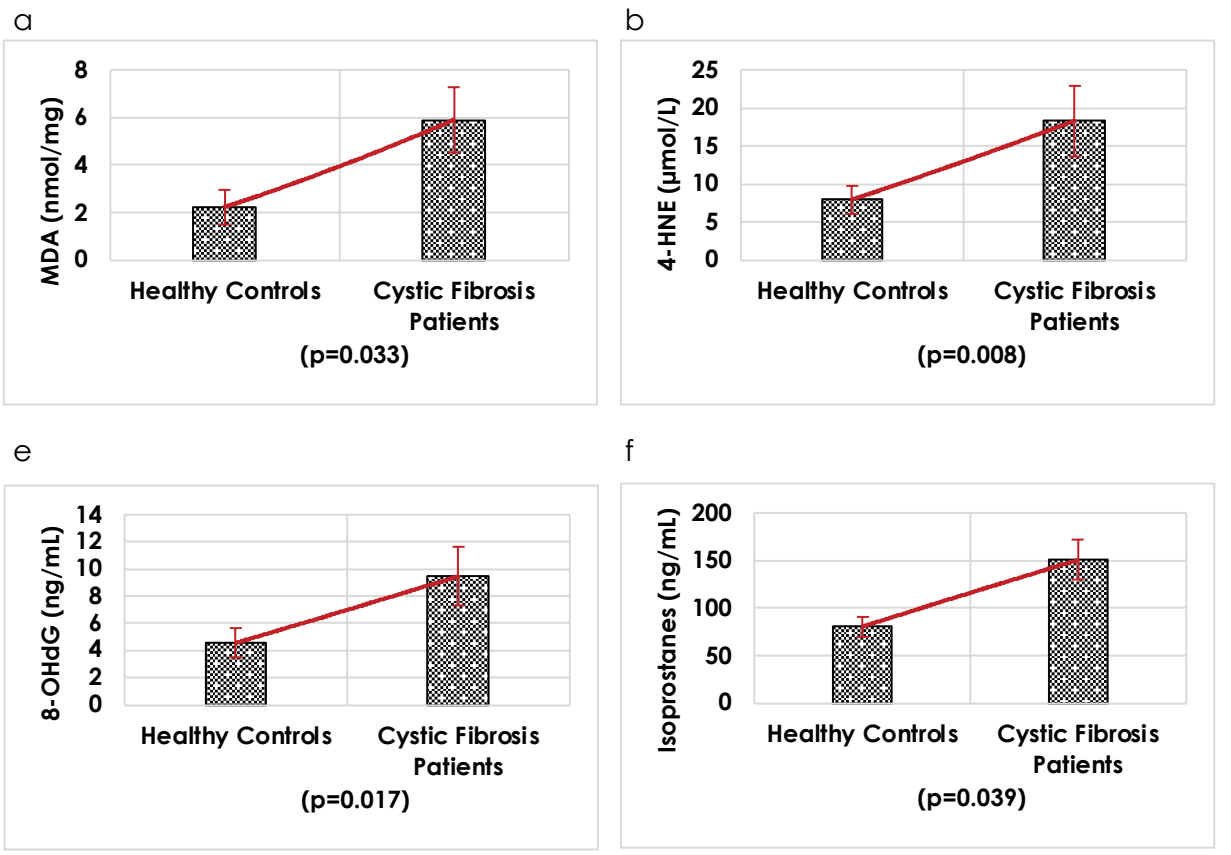

Figure 3: Oxidative Stress Markers for The Interplay of Heat Shock Proteins and Oxidative Stress in Modulating Neutrophil Activation in Cystic Fibrosis Inflammation, where 'A, B, C and D' Representing MDA, 4-HNE, 8-OHdG and Isoprostanes Characteristics Respectively

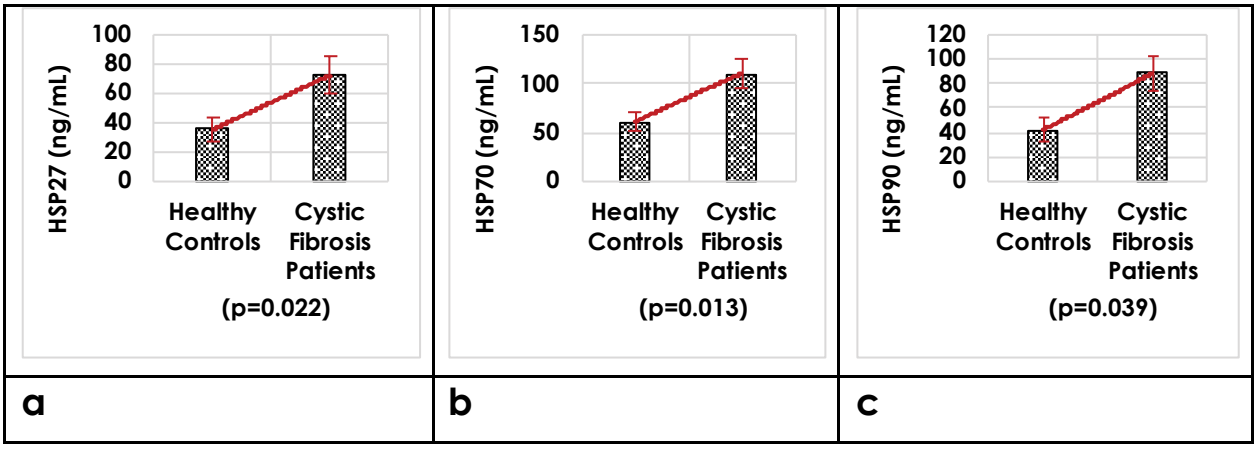

Figure 4: Interplay of Heat Shock Proteins (HSPs) Expression in Modulating Neutrophil Activation in Cystic Fibrosis Inflammation, where 'A, B, and C' represent different Characteristics

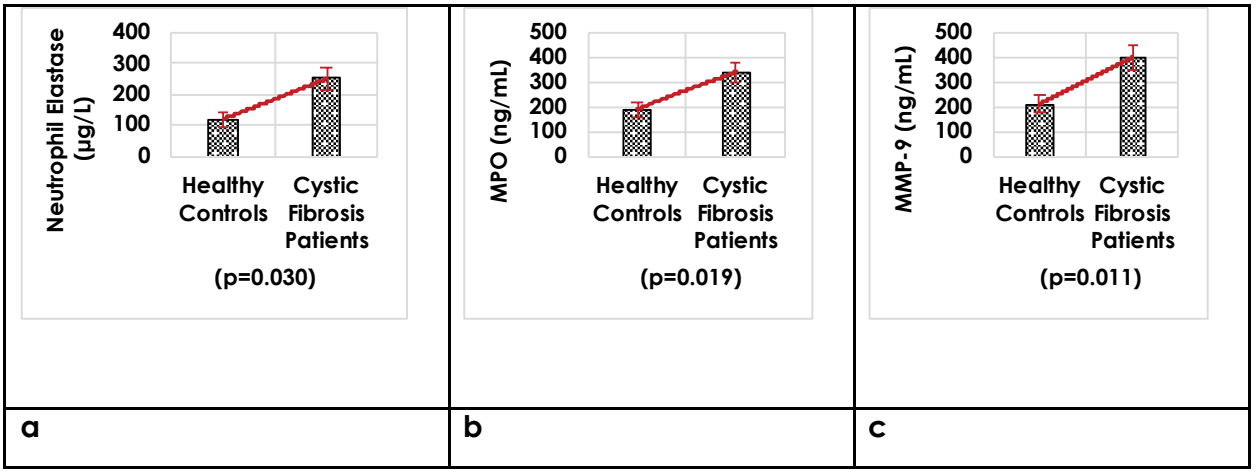

Figure 5: For the Interplay of Heat Shock Proteins and Oxidative Stress in Modulating Neutrophil Activation in Cystic Fibrosis Inflammation, where 'A, B, and C' Representing Characteristics of Neutrophils Activation Markers.

DISCUSSION

The study aimed to assess the impact of HSPs and oxidative stress on neutrophil activation in cystic fibrosis (CF) patients, focusing on the role of these proteins in modulating neutrophil-mediated immune response[14,15]. The results showed that there is increased oxidative stress and HSP proteins in CF patients, which is directly linked with neutrophil activation[15,16].The demographic analysis confirmed that the age and sex distribution between CF patients and healthy control subjects were fairly similar, but the BMI was significantly different. CF patients demonstrated an asthenic body build, mean BMI of 19.38±2.15 in contrast to 22.56±1.970 in healthy controls, p=0.001. Significantly higher levels of MDA, 4-HNE, 8-OHdG, and isoprostanes were demonstrated in CF patients as against the control, suggesting that these patients experienced higher membrane oxidation and increased overall systemic oxidative stress[17].

HSPs were found to be more abundant in CF patients, with HSP27, HSP70, and HSP90 being significantly more abundant than HSP90 in patients with CF. These increased HSP levels may represent a hepatic adaptation to chronic oxidative stress and inflammation characteristic of the CF lung disease process[18,19]. Likewise, MMP-9 levels were upregulated by 45%, and are involved in the degradation of extracellular matrix and airway remodeling[20]. These enzymes were tightly linked with lung damage, excessive mucus secretion, and inflammatory response in patients with cystic fibrosis[21]. The overall enhancement of HSP expression described in this study may correspondingly suggest their involvement of oxidative stress as well as excessive accumulation and toxicity of neutrophils within tissues.

It is known that fibrotic diseases are characterized by abnormal accumulation of ECM components that contain collagen and then chronic inflammatory events. Long -term pathological deposition of collagen in ECM disturbs the normal structure and function of the organs[22]. The essential mechanisms of fibrotic diseases have been widespread, but there is no ideal drug to prevent and treat it[23]. Due to the research and disturbance of the pathogens of fibrotic diseases, the HSP family and oxidative stress plays an important role in the process of fibrot-ic disease. This regulates the production and destruction of ECM in the process of fibrotic diseases and suppresses or thus calms the process of fibrotic diseases[24].

However, the specific pathways through which HSPs act as modulators of neutrophil activation are not straightforward. Further studies might be needed to define the exact mechanisms by which HSPs, oxidative stress, and inflammatory mediators are regulated in CF[25,26]. This is the first report to simultaneously analyze the redundant pathways involving oxidative stress and HSPs in CF, and demonstrate how those pathways lead to activation of neutrophils and inflammation. The analysis offers strong proof that HSPs, especially HSP70, rise to play a protective role in suppressing the negative effects of oxidative stress and abnormally high neutrophil activity[27]. This means that HSPs can serve as not only indicators of cellular stress but also as modulation of immune homeostasis, which has not been investigated in most previous CF studies in sufficient detail[28]. The current investigation will play a key role in understanding the interaction between oxidative stress, HSPs, and neutrophil activation in cystic fibrosis (CF)-related inflammation. The robust odds ratio shows a statistically significant association between the above biomarkers and CF-related inflammation processes. Understanding the role of HSPs in neutro-

phil activation paved the way for targeted therapy aimed at modulating HSP expression to reduce inflammation. This research provides a personalized strategy for managing CF, enabling an individualized care plan based on specific inflammatory and oxidative stress stages.

CONCLUSION

In cystic fibrosis inflammation, neutrophil activation alters heat shock protein and oxidative stress levels. Oxidative stress, heat shock proteins (HSPs), and activated neutrophils rise in cystic fibrosis (CF) patients, creating a vicious cycle that worsens inflammation and disease. These aims may assist find cystic fibrosis-friendly anti-inflammatory therapies.

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None

CONFLICT OF INTEREST

None

PATIENT CONSENT

Detailed and informed consent was taken from all patients before they participated in the study.

ETHICAL APPROVAL

The study received ethical approval from the Institutional Review Board (IRB) of The School of Pain and Regenerative Medicine under reference number SPRM/UOL/MOCT/D01/0098.

AUTHORS' CONTRIBUTIONS

Conception and designing of work, analysis, and data interpretation done by **AM, AQ** and **HS**. Drafting of work, revising critically for important intellectual content done by **GZ**. Drafting the work and reviewing critically and proofreading done by **AZ** and **HS**. Agreement to be accountable for all accepts of the work ensuring that question-related accuracy is done by **GZ, JI.** Final approval of the version to be published is done by all authors.

REFERENCES

1. Laucirica DR, Garratt LW, Kicic A. Progress in model systems of cystic fibrosis mucosal inflammation to understand aberrant neutrophil activity. Frontiers in immunology. 2020 Apr 7;11:595. https://-doi.org/10.3389/fimmu.2020.00595.

2. Wang G, Nauseef WM. Neutrophil dysfunction in the pathogenesis of cystic fibrosis. Blood, The Journal of the American Society of Hematology. 2022 Apr 28;139(17):2622-31. https://-doi.org/10.1182/blood.2021014699.

3. Keown K, Brown R, Doherty DF, Houston C, McKelvey MC, Creane S, Linden D, McAuley DF, Kidney JC, Weldon S, Downey DG. Airway inflammation and host responses in the era of CFTR modulators. International journal of molecular sciences. 2020 Sep 2;21(17):6379. https://doi.org/10.3390/ijms21176379.

4. Yonker LM, Marand A, Muldur S, Hopke A, Leung HM, De La Flor D, Park G, Pinsky H, Guthrie LB, Tearney GJ, Irimia D. Neutrophil dysfunction in cystic fibrosis. Journal of Cystic Fibrosis. 2021 Nov 1;20(6):1062-71. https://doi.org/10.1016/j.-jcf.2021.01.012.

5. Abd El-Fattah EE, Zakaria AY. Targeting HSP47 and HSP70: promising therapeutic approaches in liver fibrosis management. Journal of Translational Medicine. 2022 Nov 26;20(1):544. https://-doi.org/10.1186/s12967-022-03759-z.

6. Moliteo E, Sciacca M, Palmeri A, Papale M, Manti S, Parisi GF, Leonardi S. Cystic fibrosis and oxidative stress: the role of CFTR. Molecules. 2022 Aug 21;27(16):5324. https://doi.org/10.3390/molecules27165324.

7. Simon S, Aissat A, Degrugillier F, Simonneau B, Fanen P, Arrigo AP. Small Hsps as Therapeutic Targets of Cystic Fibrosis Transmembrane Conductance Regulator Protein. International Journal of Molecular Sciences. 2021 Apr 20;22(8):4252. https://-doi.org/10.3390/ijms22084252.

8. Soares VE, do Carmo TI, Dos Anjos F, Wruck J, de Oliveira Maciel SF, Bagatini MD, de Resende E Silva DT. Role of inflammation and oxidative stress in tissue damage associated with cystic fibrosis: CAPE as a future therapeutic strategy. Molecular and Cellular Biochemistry. 2022 Jan;477(1):39-51. https://-doi.org/10.1007/s11010-021-04263-6.

9. McKelvey MC, Weldon S, McAuley DF, Mall MA, Taggart CC. Targeting proteases in cystic fibrosis lung disease. Paradigms, progress, and potential. American Journal of Respiratory and Critical Care Medicine. 2020 Jan 15;201(2):141-7. https://-doi.org/10.1164/rccm.201906-1190PP.

10. Voynow JA, Shinbashi M. Neutrophil elastase and chronic lung disease. Biomolecules. 2021 Jul 21;11(8):1065. https://doi.org/10.3390/biom11081065.

11. Causer AJ, Shute JK, Cummings MH, Shepherd AI, Gruet M, Costello JT, Bailey S, Lindley M, Pearson C, Connett G, Allenby MI. Circulating biomarkers of antioxidant status and oxidative stress in people with cystic fibrosis: A systematic review and meta-analysis. Redox biology. 2020 May 1;32:101436. https://-doi.org/10.1016/j.redox.2020.101436.

12. Patergnani S, Vitto VA, Pinton P, Rimessi A. Mitochondrial stress responses and "Mito-Inflammation" in cystic fibrosis. Frontiers in pharmacology. 2020 Sep 30;11:581114. https://doi.org/10.3389/f-phar.2020.581114.

13. Charan J, Biswas T. How to calculate sample size

for different study designs in medical research? Indian J Psychol Med. 2013 Apr;35(2):121-6. doi: 10.4103/0253-7176.116232.
14. Frey DL, Boutin S, Dittrich SA, Graeber SY, Stahl M, Wege S, Herth FJ, Sommerburg O, Schultz C, Mall MA, Dalpke AH. Relationship between airway dysbiosis, inflammation and lung function in adults with cystic fibrosis. Journal of Cystic Fibrosis. 2021 Sep 1;20(5):754-60. https://doi.org/10.1016/j.jcf.2020.12.022.
15. Zhu J, Liu L, Ma X, Cao X, Chen Y, Qu X, Ji M, Liu H, Liu C, Qin X, Xiang Y. The role of DNA damage and repair in idiopathic pulmonary fibrosis. Antioxidants. 2022 Nov 19;11(11):2292. https://doi.org/10.3390/antiox11112292.
16. Tucker SL, Sarr D, Rada B. Neutrophil extracellular traps are present in the airways of ENaC-overexpressing mice with cystic fibrosis-like lung disease. BMC immunology. 2021 Dec;22:1-1. https://doi.org/10.1186/s12865-021-00397-w.
17. Almughem FA, Aldossary AM, Tawfik EA, Alomary MN, Alharbi WS, Alshahrani MY, Alshehri AA. Cystic fibrosis: overview of the current development trends and innovative therapeutic strategies. Pharmaceutics. 2020 Jul 2;12(7):616. https://doi.org/10.3390/pharmaceutics12070616.
18. Galiniak S, Mołoń M, Rachel M. Links between disease severity, bacterial infections and oxidative stress in cystic fibrosis. Antioxidants. 2022 Apr 29;11(5):887. https://doi.org/10.3390/antiox11050887.
19. Kapnadak SG, Dimango E, Hadjiliadis D, Hempstead SE, Tallarico E, Pilewski JM, Faro A, Albright J, Benden C, Blair S, Dellon EP. Cystic Fibrosis Foundation consensus guidelines for the care of individuals with advanced cystic fibrosis lung disease. Journal of Cystic Fibrosis. 2020 May 1;19(3):344-54. https://doi.org/10.1016/j.jcf.2020.02.015.
20. Esposito R, Mirra D, Spaziano G, Panico F, Gallelli L, D'Agostino B. The Role of MMPs in the Era of CFTR Modulators: An Additional Target for Cystic Fibrosis Patients?. Biomolecules. 2023 Feb 10;13(2):350. https://doi.org/10.3390/biom13020350.
21. Cameli P, Carleo A, Bergantini L, Landi C, Prasse A, Bargagli E. Oxidant/antioxidant disequilibrium in idiopathic pulmonary fibrosis pathogenesis. Inflammation. 2020 Feb;43:1-7. https://doi.org/10.1007/s10753-019-01059-1.
22. Regard L., Martin C., Chassagnon G., Burgel P.-R. Acute and Chronic Non-Pulmonary Complications in Adults with Cystic Fibrosis. Expert Rev. Respir. Med. 2019;13:23–38. doi: 10.1080/17476348.2019.1552832.
23. Yamada A., Komaki Y., Komaki F., Micic D., Zullow S., Sakuraba A. Risk of Gastrointestinal Cancers in Patients with Cystic Fibrosis: A Systematic Review and Meta-Analysis. Lancet Oncol. 2018;19:758–767. doi: 10.1016/S1470-2045(18)30188-8.
24. Bell S.C., Mall M.A., Gutierrez H., Macek M., Madge S., Davies J.C., Burgel P.-R., Tullis E., Castaños C., Castellani C., et al. The Future of Cystic Fibrosis Care: A Global Perspective. Lancet Respir. Med. 2020;8:65–124. doi: 10.1016/S2213-2600(19)30337-6.
25. Zhang X, Zhang X, Huang W, Ge X. The role of heat shock proteins in the regulation of fibrotic diseases. Biomedicine & Pharmacotherapy. 2021 Mar 1;135:111067. https://doi.org/10.1016/j.biopha.2020.111067.
26. Cabrini G, Rimessi A, Borgatti M, Lampronti I, Finotti A, Pinton P, Gambari R. Role of cystic fibrosis bronchial epithelium in neutrophil chemotaxis. Frontiers in immunology. 2020 Aug 4;11:1438. https://doi.org/10.3389/fimmu.2020.01438.
27. Ong T, Ramsey BW. Cystic fibrosis: a review. Jama. 2023 Jun 6;329(21):1859-71. https://doi.org/10.1001/jama.2023.8120.
28. Kumar V, Roy S, Behera BK, Das BK. Heat shock proteins (Hsps) in cellular homeostasis: a promising tool for health management in crustacean aquaculture. Life. 2022 Nov 3;12(11):1777. https://doi.org/10.3390/life12111777