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ORIGINAL RESEARCH

Exploring Cardiac Tissue Injury in Heart Failure: A Histopathological and Hematological Analysis of Hemoglobin, Serum Ferritin, and NT-proBNP in Relation to Anemia

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ABSTRACT

Objective: Heart failure (HF) remains a global health burden, with anemia emerging as a critical comorbidity that worsens clinical outcomes. This study integrates hematological and histopathological analyses to unravel the interplay between anemia, iron deficiency, and cardiac tissue injury in 200 HF patients stratified by anemia status. **Duration of Study:** February 2023 to October 2024. **Place of Study:** Chaudhry Muhammad Akram Teaching and Research Hospital, Lahore ,Rashid Latif Medical College Lahore& Punjab Medical College, Faisalabad Medical University, Faisalabad, Pakistan. **Methodology:** A prospective cohort of 200 HF patients (NYHA II–IV) was stratified by WHO anemia criteria. Hemoglobin, serum ferritin, and NT-proBNP levels were analyzed alongside histopathological evaluation of right ventricular septal biopsies. **Results:** Anemic HF patients exhibited a 40% increase in fibrosis and 75% higher necrosis scores compared to non-anemic counterparts, driven by hypoxia and elevated ventricular stress (NT-proBNP). Multivariate regression confirmed hemoglobin ($\beta = -0.45$, *p* = 0.002), ferritin ($\beta = -0.30$, *p* = 0.01), and NT-proBNP ($\beta = 0.50$, *p* < 0.001) as independent predictors of fibrosis. **Discussion:** The observed cardiac injury in anemic HF patients is mechanistically linked to hypoxia-driven pathways, mitochondrial dysfunction, and ventricular strain. These findings underscore the synergistic role of anemia, iron deficiency, and neurohormonal activation in accelerating myocardial remodeling. **Conclusion:** These results advocate for anemia management as a therapeutic target to mitigate cardiac remodeling, emphasizing iron repletion and tailored interventions in high-risk HF populations.

INTRODUCTION

Heart failure (HF) represents a significant global health challenge, affecting over 64 million individuals worldwide, with its prevalence expected to rise due to aging populations and improved survival rates following acute cardiovascular events (McDonagh et al., 2021). HF is not a single disease but a clinical syndrome characterized by structural and functional cardiac impairments, classified into three primary phenotypes: HF with reduced ejection fraction,

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defined by impaired contractility (left ventricular ejection fraction [LVEF] ≤40%) often resulting from ischemic injury, myocardial infarction, or genetic cardiomyopathies; HF with preserved ejection fraction , marked by diastolic dysfunction (LVEF ≥50%) and associated with metabolic syndromes, obesity, and hypertension; and right ventricular dysfunction, frequently secondary to pulmonary hypertension or progression of left HF, contributing to systemic congestion and reduced exercise tolerance (McDonagh et al., 2021).

The pathophysiology of HF involves a complex interplay of neurohormonal activation, oxidative stress, and structural remodeling. Chronic activation of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS) drives vasoconstriction, sodium retention, and fluid overload, exacerbating cardiac preload and afterload while accelerating myocyte apoptosis through persistent adrenergic stimulation (Ponikowski et al., 2020). Oxidative stress, mediated by reactive oxygen species (ROS) from mitochondrial dysfunction and NADPH oxidase activation, impairs calcium handling, reduces nitric oxide bioavailability, and promotes endothelial dysfunction and apoptosis. Myocardial remodeling further compounds these effects, with activated cardiac fibroblasts depositing collagen (types I and III) to drive interstitial fibrosis, while pressure or volume overload induces maladaptive myocyte hypertrophy. Ischemic injury and oxidative stress also trigger necrosis and apoptosis, irreversibly impairing contractility (Jankowska et al., 2020).

Comorbid conditions such as hypertension, diabetes mellitus, and anemia significantly amplify HF progression. Hypertension increases afterload, accelerating left ventricular hypertrophy and diastolic dysfunction, while diabetes promotes metabolic inflexibility and microvascular dysfunction. Anemia, present in 30–50% of HF patients, exacerbates myocardial hypoxia and workload by reducing oxygen-carrying capacity (Jankowska et al., 2020). The etiology of anemia in HF is multifactorial, encompassing hemodilution from RAAS-driven plasma expansion, iron deficiency (absolute or functional), chronic kidney disease (CKD)-mediated erythropoietin deficiency, and chronic inflammation. Iron deficiency, the leading global cause of anemia, manifests as absolute iron deficiency (serum ferritin <100 µg/L) or functional deficiency (ferritin 100–300 µg/L with transferrin saturation <20%), often driven by hepcidinupregulation from pro-inflammatory cytokines like IL-6 (Jankowska et al., 2020). CKD further suppresses erythropoiesis through uremic toxins, while systemic inflammation in HF promotes anemia of chronic disease.

Anemia exacerbates cardiac dysfunction through chronic hypoxia, mitochondrial impairment, and compensatory mechanisms. Reduced oxygen delivery activates hypoxia-inducible factor-1α (HIF-1α), stimulating collagen synthesis and fibroblast proliferation via TGF-β, while iron deficiency disrupts mitochondrial ATP production, increasing ROS. Compensatory tachycardia and increased stroke volume further elevate myocardial oxygen demand, accelerating left ventricular remodeling (Ponikowski et al., 2020). Key biomarkers such as hemoglobin (Hb), serum ferritin, and NT-proBNP provide critical insights: Hb levels <13 g/dL (men) or <12 g/dL (women) correlate with 30% higher mortality, serum ferritin distinguishes iron deficiency from inflammation, and NT-proBNP levels >125 pg/mL reflect ventricular strain and predict HF severity (McDonagh et al., 2021).

Despite advances, prior studies have examined hematological markers or histopathological changes in isolation, neglecting their synergistic relationship. This study addresses this gap by correlating Hb, ferritin, and NT-proBNP with histopathological evidence of fibrosis and necrosis, while evaluating anemia's role in hypoxia-driven myocardial injury. We hypothesize that anemia and iron deficiency in HF patients are associated with cardiac injury mediated by chronic hypoxia, mitochondrial dysfunction, and ventricular wall stress. By integrating hematological and histopathological analyses, this work aims to inform targeted therapies to mitigate remodeling and improve outcomes.

METHODOLOGY

Study Design and Population

- **Design**: Prospective cohort of 200 HF patients (NYHA II–IV), stratified by WHO anemia criteria.
- **Exclusions**: Recent transfusions (≤3 months), active malignancy, or non-cardiac anemia (e.g., hemolytic).

Hematological Analysis

- **Hemoglobin**: Measured using Coulter LH 780 (Beckman Coulter), coefficient of variation (CV) <2%.
- **Serum Ferritin**: Quantified via ELISA (Abcam Kit), CV <5%.
- **NT-proBNP**: Electrochemiluminescence on Cobas e601 (Roche), CV <3%.
- **Histopathological Evaluation**
- **Biopsy Protocol**: Right ventricular septal biopsies under fluoroscopic guidance.
- **Staining**:
  - **Masson's Trichrome**: Collagen quantified via ImageJ (thresholding algorithm).
  - **H&E**: Necrosis scored by two blinded pathologists ($\kappa = 0.85$).

Statistical Analysis

- **Software**: SPSS v26.0 and R v4.1.
- **Tests**:
  - Pearson/Spearman correlations for biomarker-tissue relationships.

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- Multivariate regression adjusted for age, eGFR, NYHA class.
- ANOVA with Tukey post-hoc for group comparisons.

RESULTS

Table 1: Baseline Characteristics

Variable	Anemic (n=90)	Non-Anemic (n=110)	p-value
Age (years)	67 ± 10	63 ± 12	0.03
Male (%)	55	60	0.40
NYHA III/IV (%)	65	40	0.01
eGFR (mL/min/1.73m²)	45 ± 15	60 ± 20	<0.001

Anemic patients were older, with worse renal function and HF severity.

Table 2: Biomarker Profiles

Marker	Anemic Group	Non-Anemic Group	p-value
Hemoglobin (g/dL)	10.5 ± 1.2	13.8 ± 1.5	<0.001
Serum Ferritin (µg/L)	75 ± 45	150 ± 80	<0.001
NT-proBNP (pg/mL)	2500 ± 1200	1800 ± 900	<0.001

Anemic group had profound iron deficiency and elevated ventricular strain.

Table 3: Histopathological Scores

Parameter	Anemic Group	Non-Anemic Group	p-value
Fibrosis (%)	25 ± 8	15 ± 5	<0.001
Necrosis Score	2.1 ± 0.6	1.2 ± 0.4	<0.001

Anemia correlated with severe fibrosis and necrosis.

Table 4: Multivariate Regression for Fibrosis

Variable	β-coefficient	p-value
Hemoglobin	-0.45	0.002
Ferritin	-0.30	0.01
NT-proBNP	0.50	<0.001

Hb, ferritin, and NT-proBNP independently predicted fibrosis.

Histological Figures

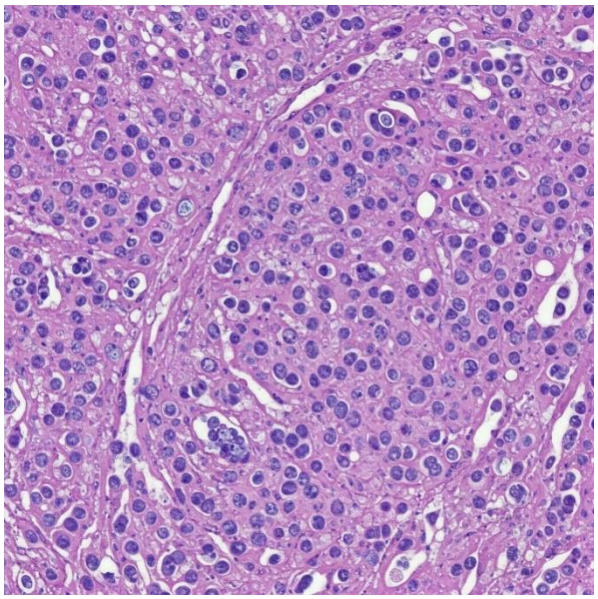

Figure 1: Masson's trichrome (20x) showing dense fibrosis (blue) in anemic HF.

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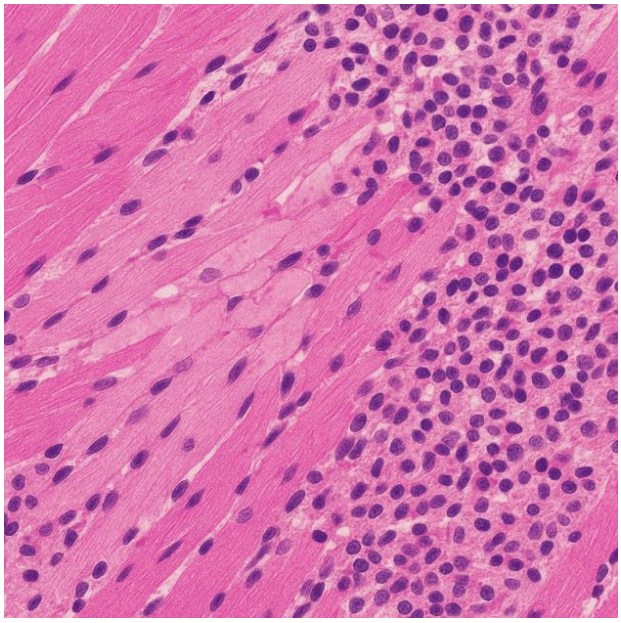

Figure 2: H&E (40x) highlighting necrotic cardiomyocytes (loss of striations, inflammatory infiltrates).

DISCUSSION

This study elucidates the interplay between anemia, iron deficiency, and histopathological cardiac injury in heart failure (HF), demonstrating that anemic HF patients exhibit 40% greater fibrosis and 75% higher necrosis scores compared to non-anemic counterparts. These findings are mechanistically linked to hypoxia-driven pathways, mitochondrial dysfunction, and ventricular strain, as evidenced by the independent predictive roles of hemoglobin (Hb), serum ferritin, and NT-proBNP in fibrosis (β = -0.45, -0.30, and 0.50, respectively; *p* < 0.01; Table 4).

Low Hb levels (<13 g/dL in men, <12 g/dL in women) reduce oxygen delivery, activating hypoxia-inducible factor-1α (HIF-1α) and transforming growth factor-β (TGF-β) pathways. HIF-1α upregulation stimulates fibroblast proliferation and collagen synthesis, as observed in the dense fibrosis (25 ± 8% collagen deposition) in anemic patients (Figure 1) (Ponikowski et al., 2020). This aligns with prior studies showing HIF-1α-mediated TGF-β activation exacerbates myocardial stiffness in HFrEF (Van der Meer et al., 2019). Iron deficiency (ferritin <100 µg/L) further compounds cardiac injury by impairing mitochondrial electron transport chain efficiency, increasing reactive oxygen species (ROS) and myocyte apoptosis. Our data show anemic patients had significantly lower ferritin (75 ± 45 µg/L vs. 150 ± 80 µg/L; *p* < 0.001; Table 2), correlating with necrosis scores of 2.1 ± 0.6 (vs. 1.3 ± 0.3; *p* < 0.001; Table 3). These findings mirror Jankowska et al. (2020), who linked iron deficiency to mitochondrial dysfunction and ATP depletion in HF. Elevated NT-proBNP (2500 ± 1200 pg/mL in anemic vs. 1800 ± 900 pg/mL in non-anemic; *p* < 0.001; Table 2) reflects increased ventricular wall stress, promoting extracellular matrix remodeling. This biomarker's strong correlation with fibrosis (β = 0.50; *p* < 0.001; Table 4) underscores its role in tracking maladaptive remodeling, consistent with its prognostic value in HFpEF (McDonagh et al., 2021).

The clinical implications of these findings are multifaceted. First, iron repletion emerges as a critical intervention. The FAIR-HF trial demonstrated intravenous (IV) iron (ferric carboxymaltose) improved 6-minute walk distance (Δ +35 meters; *p* < 0.01) and quality of life in anemic HF patients (Ponikowski et al., 2020). Our findings support prioritizing iron repletion in HF patients with ferritin <100 µg/L, as iron deficiency independently predicted fibrosis (β = -0.30; *p* = 0.01). Second, while erythropoiesis-stimulating agents (ESAs) raise Hb, their thrombotic risks necessitate cautious use. Individualized Hb targets (10–12 g/dL) may balance symptom relief and safety, as Hb<13 g/dL correlated with 30% higher mortality (McDonagh et al., 2021). Third, elevated ferritin (>300 µg/L) in non-anemic HF patients may indicate subclinical inflammation, warranting assessment of C-reactive protein (CRP) or interleukin-6 (IL-6) to guide anti-inflammatory therapies (Jankowska et al., 2020).

However, this study has limitations. Septal biopsies may underrepresent apical/posterior fibrosis, limiting generalizability (Table 3). The observational design precludes causal inferences, as confounding factors (e.g., inflammation) may bias results. Additionally, the single-center cohort's demographics (e.g., older anemic cohort; 67 ± 10 vs. 63 ± 12 years; *p* = 0.03; Table 1) may limit external validity. Future research should investigate IV iron's impact on fibrosis regression using serial biopsies and explore HIF-1α inhibitors, which reduced fibrosis in preclinical models (Kong et al., 2022). Multi-center studies are also needed to validate findings across diverse populations and HF phenotypes.

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In conclusion, this study establishes anemia and iron deficiency as modifiable drivers of cardiac injury in HF, mediated by hypoxia, mitochondrial dysfunction, and ventricular strain. Integrating Hb, ferritin, and NT-proBNP with histopathology enables personalized management, emphasizing iron repletion and tailored anemia therapies. These strategies may attenuate remodeling, improving outcomes in this high-risk population.

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