RESEARCH PRPOSAL FOR GRANT

INTRODUCTION

Cardiovascular diseases (CVDs) and nonalcoholic fatty liver diseases (NAFLD) together are extreme manifestations of end-organ damage of the metabolic syndrome. NAFLD and CVD are interrelated to each other via various pathophysiologic processes. NAFLD increases the risk of CVD by Systemic inflammation, endothelial dysfunction, hepatic insulin resistance, oxidative stress, and altered lipid metabolism are some of the mechanisms [1]. Patients with NAFLD develop increased atherosclerosis, cardiomyopathy, and arrhythmia, which clinically result in cardiovascular morbidity and mortality. Increased blood hepcidin may be associated with the presence and promotion of atherosclerosis, the association of hepcidin with mortality among coronary artery disease (CAD) patients remains unknown. The iron hypothesis, which states that excessive iron would contribute to the pathogenesis of CAD, was first proposed by Sullivan in 1981 [2]. However, subsequent epidemiological studies about the association of circulating iron, non-iron binding capacity, transferrin receptor, and ferritin with atherosclerosis have shown conflicting results, with some studies confirming and others denying this possible deleterious effect [3,4]. Hepcidin (HAMP) has been recently recognized as the major regulatory protein in iron metabolism. This liver-produced peptide hormone (hepatokine) plays an important role in systemic regulation of iron homeostasis. It has been hypothesized that hepcidin may slow or prevent the mobilization of iron from macrophages and lead to an increased CVD/CAD risk, such as subclinical atherosclerosis, left ventricular hypertrophy, epicardial fat thickness, and valvular calcifications, functional cardiac changes, including diastolic dysfunction, cardiac arrhythmias and Kawasaki disease [7-9].

CLINICAL SIGNIFICANCE: Understanding and addressing the liver-heart axis is crucial for the comprehensive management of NAFLD and NAFLD-associated CAD/CVD. Clinicians need to consider the potential impact of liver dysfunction on cardiac health, particularly in patients with liver disease and heart failure. Studying the role of extrahepatic manifestations of NAFLD is crucial for a comprehensive understanding of the disease, improving clinical management, predicting disease progression, and identifying potential therapeutic targets. It allows clinicians to provide holistic care to patients with NAFLD and CAD/CVD.

OBJECTIVES

This study is aimed:

A. To investigate the relationships between hepcidin and the main features of Metabolic syndrome and NAFLD in Indian population.
B. To correlation serum/plasma Hepcidin level with existence of CAD/CVD in patients with metabolic syndrome and NAFLD.
C. To bring out a predictive biomarker signature for CAD/CVD in NAFLD/NASH patients.

BRIEF DESCRIPTION OF PILOT DATA

WDF dietary mouse model of metabolic syndrome and NAFLD: We have recently showed that mice fed on Fructose containing Western diet (WDF) for 0 to 30 weeks progressively gained body weight, fat mass and became glucose intolerant and insulin resistant, developed dyslipidaemia, hypertriglyceridemia, and had increased serum ALT, liver index, NAS Score, suggesting progression of metabolic syndrome with liver dysfunctions and NAFLD [10]. Further, we showed that WDF diet progressively increased expression of inflammatory and fibrotic genes in the liver and increased hepatic fibrosis confirming progressive development of early NASH at 16 weeks and severe NASH at 30 weeks WDF diet [10].

Association of NAFLD/NASH with progression of cardiac defects: Next, we compared the progressive changes in the liver with the heart function. Preliminary data of echocardiographic analysis

in WDF fed mice from 0 to 30 weeks showed significant decrease in ejection fraction (EF) and E/A (Figure Panel A and B).
HAMP expression was also gradually decreased in NASH liver and heart (Figure Panel C). Further, we observed significantly decreased hepcidin concentration in serum collected from WDF fed mice (Figure Panel D). Thus, our preliminary data strongly suggest that heart defects are associated with decreased circulating hepcidin during severe NASH.

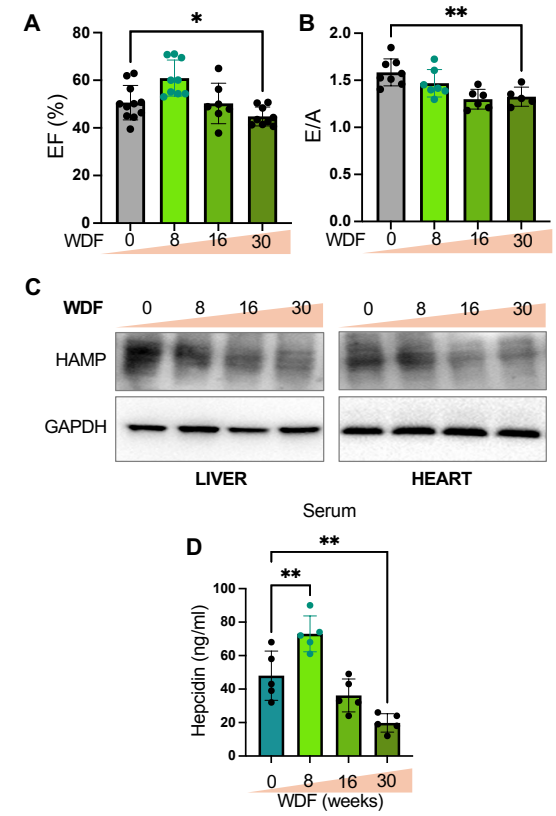

Figure: NAFLD-inducing WDF diet caused cardiac defects in mice

METHADOLOGY
Individuals aged 18 years or older, those are ready to give informed consent, will be recruited to participate in the study. Only subjects with available complete data allowing their classification according to established criteria for metabolic syndrome/NAFLD/NASH will be included (n=80 including male and female) from Gastroenterology and Hepatology department at AIIMS, Gorakhpur. Blood samples will be collected and CRP, ALT, AST, hepcidin, serum ferritin, lipid profile and blood sugar levels will be analysed in serum at Biochemistry department at AIIMS, Gorakhpur. Existence of CVD will be defined using CT Coronary Calcium scoring or Stress ECHO/TMT in Cardiology department at AIIMS, Gorakhpur. Subgroup analysis will be done using appropriate statistical methods to determine correlation and association between serum hepcidin and occurrence/severity of CAD/CVD. Further, performing association analysis using serum biochemical markers (CRP, ALT, AST, ferritin, LDL and blood sugar) with hepcidin and severity of CAD/CVD a signature will be created to predict the severity of CAD/CVD in the patients with metabolic syndrome/NAFLD/NASH.

EXPECTED OUTCOMES
With the proposed study, we expect to understand, if hepcidin level correlate significantly to metabolic syndrome/NAFLD/NASH patients. We also expect to see if hepcidin level correlate to CAD/CVD in NAFLD/NASH patients in Indian population. Moreover, we expect to answer if hepcidin level can be considered as a biomarker for establishing existence and/or severity of CAD/CVD in NASH/NAFLD patients in Indian population.

ALTERNATIVE STRATEGIES
In any unforeseen circumstances, when CT is not accessible, non-invasive Stress ECHO/TMT analysis will be performed for CAD/CVD assessment.

TIMELINE

Objectives	**Months**			
	Month 1-3	Month 4-6	Month 7-9	Month 10-12
A				
B				
C				

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