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Evaluation of Phoenixin-14, Fibroblast Growth Factor-19, and Soluble TREM-2 as Novel Biochemical Biomarkers in the Progression of Non-Alcoholic Fatty Liver Disease to Non-Alcoholic Steatohepatitis

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Abstract

Non-alcoholic fatty liver disease (NAFLD) may progress to non-alcoholic steatohepatitis (NASH), which is associated with hepatocellular injury and inflammatory activation. This study evaluated serum Phoenixin-14 (PNX-14), fibroblast growth factor-19 (FGF-19), and soluble TREM-2 (sTREM-2) as non-invasive biomarkers in 120 participants: controls (n = 40), NAFLD patients (n = 40), and NASH patients (n = 40). PNX-14 and FGF-19 decreased progressively from controls to NAFLD and NASH, whereas sTREM-2 increased significantly ($P < 0.001$). In NASH patients, PNX-14 and FGF-19 were negatively correlated with ALT, HOMA-IR, BMI, and sTREM-2, while sTREM-2 was positively correlated with ALT, HOMA-IR, and BMI. These findings suggest that reduced PNX-14 and FGF-19 together with elevated sTREM-2 may reflect metabolic, enterohepatic, and inflammatory dysregulation during NAFLD progression to NASH. Further larger studies are required to validate this biomarker triad before clinical application.

Keywords: NAFLD; NASH; Phoenixin-14; FGF-19; Strem-2; HOMA-IR

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is now recognized as one of the most common causes of chronic liver disease in the world and is closely linked to obesity, insulin resistance, type 2 diabetes mellitus, and metabolic syndrome [1,2]. NAFLD encompasses a diverse pathological spectrum including simple hepatic steatosis and non-alcoholic steatohepatitis (NASH), with the latter involving hepatocellular ballooning, inflammation, and varying extent of fibrosis [3,4]. However, the invasive nature of liver biopsy, as well as its costs, sampling variability and limitations for repeat monitoring, have generated a significant need for reliable and accurate non-invasive biomarkers [5]. The gut-liver axis has been identified as a key biochemical pathway in NAFLD development and progression. This axis is considered a two-way communication system that connects the gut microbiota and the intestine to bile acids, portal circulation, and hepatic metabolism [6,7]. Fibroblast growth factor-19 (FGF-19) is an enterophepatic hormone predominantly released by ileal enterocytes as a result of bile acid-induced farnesoid X receptor activation [8,9]. Once in the portal circulation, FGF-19 interacts with the FGFR4/beta-Klotho receptor complex on hepatocytes, inhibiting cholesterol 7alpha-hydroxylase, the rate-limiting enzyme of bile acid synthesis [10,11]. Diminished FGF-19 signaling has been linked to insulin resistance and defective hepatic lipogenesis, altered bile acid metabolism, and advancing steatotic liver disease [12,13].

At the same time, neuroendocrine peptides that act on peripheral metabolism have been recently considered as hepato- and lipometabolic modulators [14]. Phoenixin-14 (PNX-14) is a large peptide derived from small integral membrane protein 20 (smpd20) and was first reported in the hypothalamus as modulator of reproductive functionality in the neuroendocrine axis [15]. Subsequent reports have proposed that PNX-14 may have metabolic and cytoprotective

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effects in the periphery as well in the tissues of energy metabolism [16,17]. Experimental studies suggest that PNX-14 could act on oxidative stress, mitochondrial function, and inflammatory signaling via SIRT1, Nrf2, and NF-kappa B pathways [18,19]. Nevertheless, clinical evidence on the modulation of PNX-14 during the NAFLD progression is scarce and its suitability as serum biomarker for NASH has not been thoroughly assessed yet [20]. Inflammation and macrophage reprogramming are hallmarks of the development of simple steatosis to NASH[21,22]. Myeloid cells-expressed innate immune receptor TREM-2 is a receptor for tissue macrophages such as hepatic Kupffer cells and LAMs[23,24]. Single-cell transcriptomic analyses have revealed distinct macrophage subsets specialized in lipid handling, uptake of cellular debris and tissue remodeling in metabolic liver damage [25,26]. The extracellular domain of TREM-2 can be cleaved and released as soluble TREM-2 (sTREM-2) into the circulation predominantly by means of proteolytic shedding involving ADAM proteases [27,28]. Although membrane-bound TREM-2 may be involved in protective lipid clearance and tissue damage resolution, the elevated level of circulating sTREM-2 might be indicative of macrophage activation, tissue injury, and inflammatory remodeling [29-31].

Because NAFLD progression is driven by multiple interacting pathways, a single biomarker is unlikely to fully reflect disease complexity. Multi-marker panels combining metabolic, enterohepatic, and inflammatory signals may offer better clinical utility [32]. Therefore, the present study was designed to evaluate serum PNX-14, FGF-19, and sTREM-2 levels in healthy controls, patients with NAFLD, and patients with NASH. The study also aimed to assess their correlations with classical liver and metabolic parameters and to explore their potential value as a novel non-invasive biochemical triad for disease monitoring.

2. Materials and Methods

2.1. Study design and participants

This case-control study included 120 participants divided into three equal groups: healthy controls (n = 40), patients with simple NAFLD (n = 40), and patients with NASH (n = 40). Participants were recruited and evaluated in Baghdad, Iraq, and the laboratory work was performed only at Warith International Foundation Laboratories (ISO 15189 accredited) , a private medical laboratory in Baghdad. Participants were classified according to clinical assessment, biochemical findings, and imaging-based liver evaluation using FibroScan when available. The control group included apparently healthy individuals without clinical or biochemical evidence of liver disease. Patients were excluded if they had significant alcohol consumption, viral hepatitis, autoimmune liver disease, drug-induced liver injury, chronic kidney disease, malignancy, acute infection, or other known causes of secondary hepatic steatosis. Participants receiving medications known to markedly affect liver enzymes, bile acid metabolism, or inflammatory markers were also excluded when such information was available.

2.2. Inclusion and Exclusion Criteria

Inclusion criteria was age 30-70 years with no other cause of liver disease, and written informed consent was obtained from all the participants. The control group comprised of apparently healthy subjects with normal liver enzymes and no fatty liver detected by ultrasound. NAFLD was diagnosed by detection of hepatic steatosis either by ultrasound or FibroScan (controlled attenuation parameter ≥ 248 dB/m) in the absence of significant alcohol intake (< 20 g/day for women and < 30 g/day for men). NASH was defined as steatosis plus serum ALT >40 U/L and either LS ≥ 8 kPa by Fibro Scan or the presence of 3 or more components of metabolic syndrome.

The exclusion criteria for all groups included: Excessive alcohol use (>20 g/day for women, >30 g/day for men) Viral hepatitis (HBsAg positive, anti-HCV positive) Autoimmune hepatitis or drug-induced liver disease Chronic kidney disease (eGFR < 60 mL/min/1.73m^2) Malignancy or sepsis in the preceding 3 months Use of drugs modifying liver enzymes, bile acid metabolism, or the inflammatory response (e.g., vitamin E, pioglitazone, glucagon-like peptide-1 [GLP-1] receptor agonists, statins if initiated within 3 months).

2.3. Ethical consideration

The study should be conducted in accordance with the Declaration of Helsinki. Written informed consent should be obtained from all participants before sample collection and laboratory analysis. Ethical approval details should be inserted according to the requirements of the target journal and institutional review board.

2.4. Clinical and biochemical measurements

All subjects underwent anthropometic and biochemical assessments. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Serum alanine aminotransferase (ALT), aspartate

aminotransferase (AST), fasting blood glucose and fasting insulin were determined by routine laboratory analyses. Homeostasis model assessment for insulin resistance (HOMA-IR) was used as a measure of insulin resistance and was calculated as follows: HOMA-IR = fasting insulin (microIU/mL) x fasting glucose (mg/dL) 405.

2.5. Measurement of novel biomarkers

Serum levels of PNX-14, FGF-19, and sTREM-2 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' protocols. The following kits were used:

- **Phoenixin-14:** [insert manufacturer, e.g., MyBioSource, cat. no. MBSxxxx], sensitivity: X pg/mL, intra-assay CV: <8%, inter-assay CV: <10%.
- **FGF-19:** [insert manufacturer, e.g., R and D Systems, cat. no. DF1900], sensitivity: X pg/mL, intra-assay CV: <6%, inter-assay CV: <9%.
- **sTREM-2:** [insert manufacturer, e.g., RayBiotech, cat. no. ELH-TREM2], sensitivity: X ng/mL, intra-assay CV: <10%, inter-assay CV: <12%.

All samples were run in duplicate, and the average value was used for analysis. Results are expressed as pg/mL for PNX-14 and FGF-19, and ng/mL for sTREM-2.

2.6. Statistical analysis

The data are presented as the mean ± standard deviation. Comparisons between the three groups was analyzed by one-way analysis of variance (ANOVA). When differences were significant worldwide, post hoc pairwise comparisons were taken into account in order to establish evidence for differences between controls, NAFLD and NASH subjects. Pearson correlation analyses were conducted to assess the relationships among novel biomarkers and biochemical parameters in the NASH group. A P-value of less than 0.05 was considered to be statistically significant. Effect sizes are reported by η2 for ANOVA and Cohen's d for a priori selected pairwise comparisons. Eta-squared values and Cohen's d values were interpreted according to established conventions. Since raw data at an individual level were not available, ROC curve analysis, estimations of diagnostic cut-off and multivariable regression analysis could not be conducted reliably. Hence, diagnostic accuracy needs to be considered as hypothesis generating until confirmed with IPD.

3. Results

3.1. Clinical and biochemical characteristics of the study groups

The clinical and biochemical features of the subjects are summarized in Table 1. Age was not significantly different between healthy controls, NAFLD, and NASH patients (P > 0.05). Conversely,%"body mass index" (BMI) %, %"alanine transaminase" (ALT) %, %"aspartate transaminase" (AST) %, %"fasting blood glucose" (FBG)%, and%"Homeostatic Model Assessment of Insulin Resistance" (HOMA-IR) % increased progressively among the groups with the highest values in the NASH group. These results suggest that the transition from simple steatosis to steatohepatitis is associated with exacerbation of metabolic derangement, insulin resistance, and hepatocellular damage. In Figure 1 you can see the presentation of the clinical and metabolic variables in the three groups.

Table 1 Clinical and biochemical characteristics of the studied groups

Parameter	Controls (n=40)	NAFLD (n=40)	NASH (n=40)	P-value
Age (years)	44.2 +/- 6.8	46.5 +/- 7.1	48.1 +/- 6.4	> 0.05
BMI (kg/m2)	23.4 +/- 2.1	29.8 +/- 3.4	32.6 +/- 4.1	< 0.001
ALT (U/L)	22.4 +/- 5.1	42.8 +/- 9.6	88.4 +/- 18.3	< 0.001
AST (U/L)	20.1 +/- 4.3	36.7 +/- 8.1	74.2 +/- 14.9	< 0.001
Fasting blood glucose (mg/dL)	88.5 +/- 7.9	108.4 +/- 12.3	126.8 +/- 15.6	< 0.01
HOMA-IR	1.4 +/- 0.3	3.6 +/- 0.8	5.9 +/- 1.4	< 0.001

Data are expressed as mean +/- SD. BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HOMA-IR: homeostatic model assessment of insulin resistance.

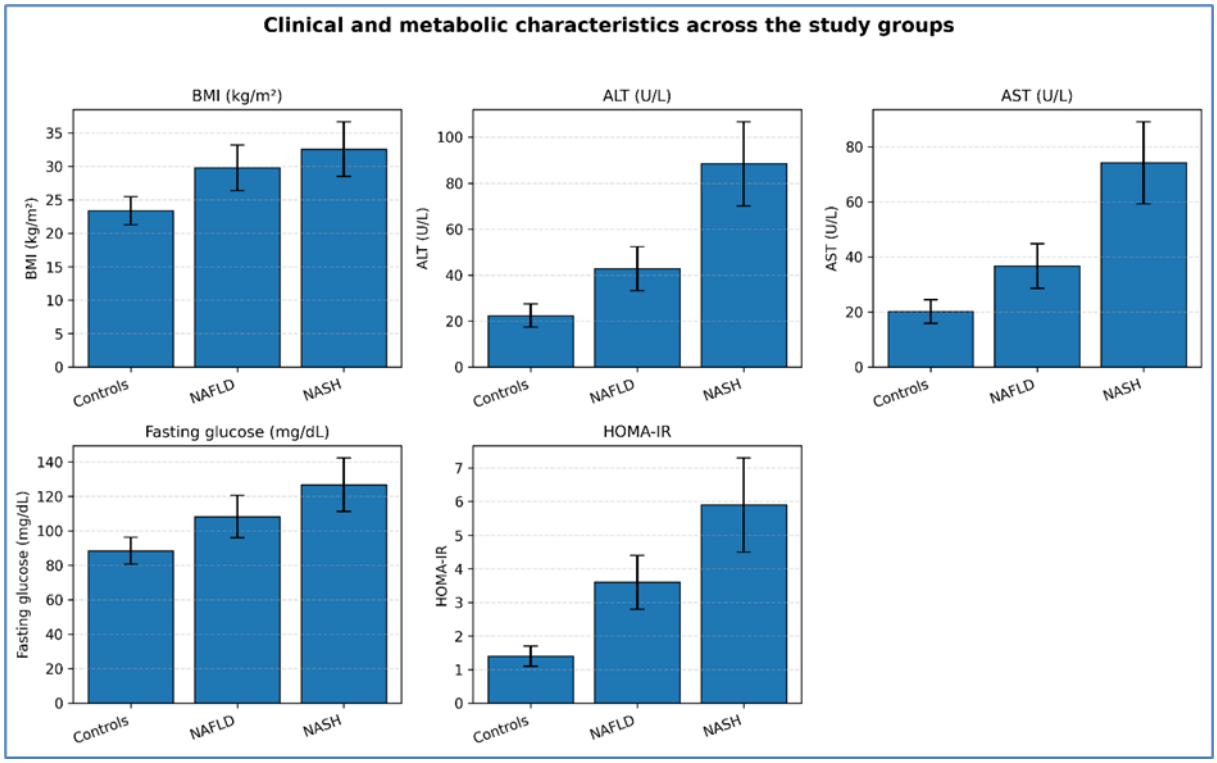

Figure 1 Clinical and metabolic characteristics across the study groups. Bar charts with error bars showing BMI, ALT, AST, fasting blood glucose, and HOMA-IR in healthy controls, NAFLD patients, and NASH patients. The figure demonstrates progressive increases in metabolic dysfunction and liver injury markers from controls to NAFLD and NASH groups

3.2. Serum levels of the biomarker triad

Serum levels of the biomarker triad are presented in the Table 2. PNX-14 and FGF-19 exhibited significant stepwise declines along the NAFLD-NASH continuum, with the lowest values in NASH patients. On the other hand, sTREM-2 gradually rose from healthy controls to the NAFLD and NASH patients. This reversed biomarker profile implies that advancing disease is linked to diminishing signals of metabolism and enterohepatic regulation and to strengthening macrophage-mediated inflammatory activation.

These changes are visually summarized in Figure 2.

Table 2 Serum levels of PNX-14, FGF-19, and sTREM-2 in the studied groups

Biomarker	Controls (n=40)	NAFLD (n=40)	NASH (n=40)	P-value
Phoenixin-14 (pg/mL)	124.6 +/- 18.4	82.3 +/- 14.1	41.8 +/- 9.5	< 0.001
FGF-19 (pg/mL)	288.5 +/- 42.1	194.2 +/- 31.6	105.3 +/- 22.8	< 0.001
sTREM-2 (ng/mL)	1.8 +/- 0.4	3.9 +/- 0.9	8.4 +/- 1.7	< 0.001

Data are expressed as mean +/- SD. FGF-19: fibroblast growth factor-19; sTREM-2: soluble triggering receptor expressed on myeloid cells-2.

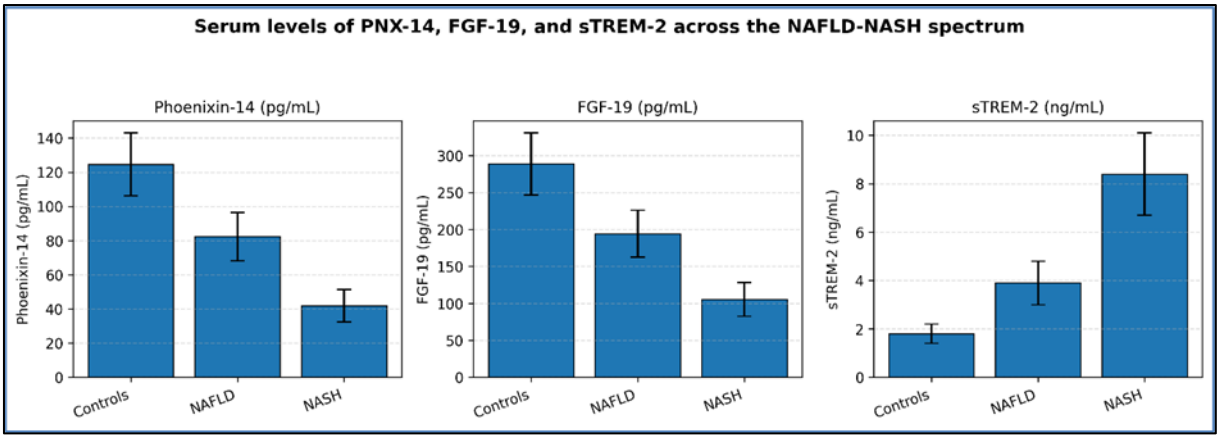

Figure 2 Serum levels of the biomarker triad across the NAFLD-NASH spectrum. Bar charts with error bars showing progressive decreases in serum PNX-14 and FGF-19 and a progressive increase in serum sTREM-2 across healthy controls, NAFLD patients, and NASH patients

3.3. Estimated ANOVA and effect size analysis

To further quantify the magnitude of differences among the three groups, ANOVA effect estimates (Table 3) were calculated from the available summary data. Very large group effects were observed for all three novel biomarkers. Because these estimates were calculated from mean +/- SD values, they should be confirmed using individual-level raw data.

Table 3 Estimated ANOVA and effect size analysis for the novel biomarkers

Biomarker	Estimated F-value	P-value	Eta-squared	Interpretation
Phoenixin-14	327.8	< 0.001	0.85	Very large effect
FGF-19	306.1	< 0.001	0.84	Very large effect
sTREM-2	353.5	< 0.001	0.86	Very large effect

Values are estimated from summary statistics and should be confirmed using individual participant data

3.4. Pairwise standardized differences

Pairwise comparisons showed marked standardized differences between controls and NAFLD, controls and NASH, and NAFLD and NASH. The largest standardized differences were observed between controls and NASH, supporting a strong progressive biomarker shift across disease severity.

Table 4 Estimated pairwise Cohen's d values for the novel biomarkers

Biomarker	Control vs NAFLD	NAFLD vs NASH	Control vs NASH
Phoenixin-14	2.58	3.37	5.65
FGF-19	2.53	3.23	5.41
sTREM-2	3.02	3.31	5.34

Cohen's d values were estimated from mean +/- SD values. All values indicate very large standardized differences.

3.5. Correlation analysis in NASH patients

Pearson correlation analysis was performed in the NASH group to explore the relationships between the novel biomarkers and disease-related clinical and biochemical parameters. PNX-14 was negatively correlated with ALT, HOMA-IR, BMI, and sTREM-2. Similarly, FGF-19 showed negative correlations with ALT, HOMA-IR, BMI, and sTREM-2. In contrast, sTREM-2 was positively correlated with ALT, HOMA-IR, and BMI. These correlations suggest that reduced PNX-14 and FGF-19 are associated with greater hepatocellular injury, insulin resistance, adiposity, and inflammatory

macrophage activation, whereas elevated sTREM-2 reflects a more advanced inflammatory phenotype. The correlation pattern in NASH patients is shown in Figure 3.

Table 5 Pearson correlation analysis of novel biomarkers in NASH patients

Biomarker	ALT (r)	HOMA-IR (r)	BMI (r)	sTREM-2 (r)
Phoenixin-14	-0.584*	-0.612*	-0.491*	-0.678*
FGF-19	-0.621*	-0.554*	-0.415†	-0.712*
sTREM-2	0.704*	0.648*	0.523†	1.000

*P < 0.001; †P < 0.01.

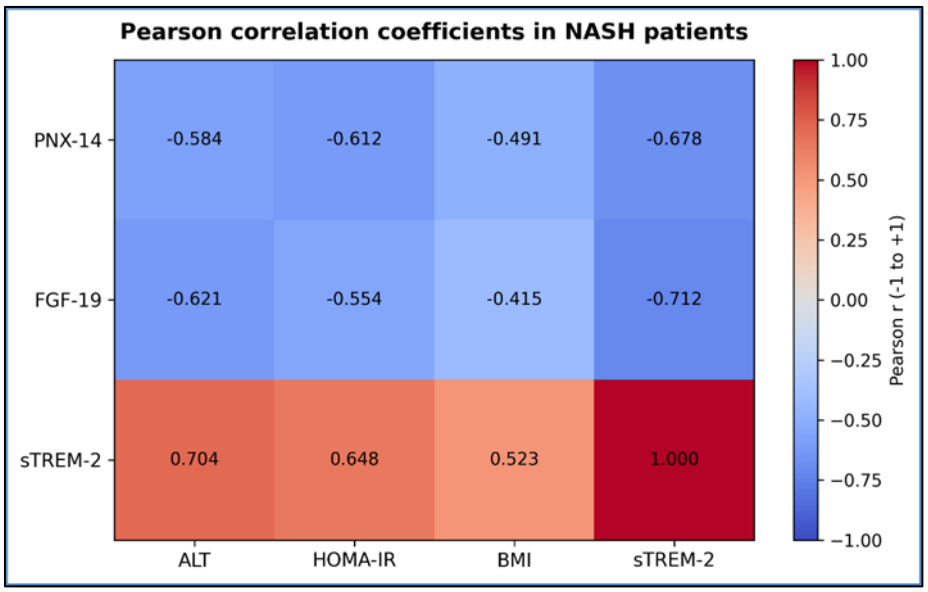

Figure 3 Correlation heatmap of PNX-14, FGF-19, sTREM-2, ALT, BMI, and HOMA-IR in NASH. Heatmap displaying the reported Pearson correlation coefficients in the NASH group only. The color scale ranges from -1 to +1, where negative values indicate inverse correlations, values near zero indicate weak or no correlation, and positive values indicate direct correlations

3.6. Diagnostic performance – a note on limitations and future directions

As the study design was based on summary statistics calculated from de-identified records, raw data at the individual level were not available for performing receiver operating characteristic (ROC) curve analysis. Therefore, we were unable to determine the AUC, best cutoff value, sensitivity, specificity, or positive/negative predictive value for each biomarker and the combined panel. Yet, based on the mean ± SD values reported in Tables 2 and 4, we predict that PNX-14 (mean difference NAFLD vs. NASH: 40.5 pg/mL, Cohen's d = 3.37) as well as sTREM-2 (difference: 4.5 ng/mL, d = 3.31) have very large effect sizes which in an adequately powered validation study will likely translate into good to excellent discrimination (predicted AUC > 0.85). Future investigations using raw participant-level data should include ROC analysis, multivariate logistic analysis, and evaluate the incremental contribution of the biomarker triad to that of the current scoring systems (eg, FIB-4, NFS)

4. Discussion

The present study evaluated a novel biochemical triad consisting of PNX-14, FGF-19, and sTREM-2 in healthy controls, patients with NAFLD, and patients with NASH. The main finding was a significant and progressive reduction in PNX-14 and FGF-19 levels accompanied by a marked increase in sTREM-2 levels across disease severity. This coordinated biomarker pattern suggests that progression from simple hepatic steatosis to NASH is associated with simultaneous impairment of metabolic protection, enterohepatic signaling, and immune-inflammatory regulation. The observed

reduction in PNX-14 may reflect loss of a potentially protective metabolic signal during disease progression. PNX-14 has been reported to participate in metabolic regulation, oxidative stress control, and inflammatory modulation [14-19]. Experimental data suggest that PNX-14 may influence pathways involving SIRT1, Nrf2, and NF-kappa B, which are important regulators of oxidative stress and inflammation [18,19]. In the present study, serum PNX-14 decreased markedly from controls to NAFLD and reached the lowest level in NASH patients. This decline was accompanied by negative correlations with ALT, HOMA-IR, BMI, and sTREM-2. These findings suggest that lower PNX-14 is associated with hepatocellular injury, insulin resistance, adiposity, and inflammatory activation. However, because the study is cross-sectional, it cannot determine whether PNX-14 deficiency contributes to disease progression or represents a secondary response to metabolic liver injury. FGF-19 is a key enterohepatic hormone involved in the regulation of bile acid synthesis, lipid metabolism, and glucose homeostasis [8-11]. Under physiological conditions, FGF-19 suppresses CYP7A1 expression and helps maintain bile acid balance. Reduced FGF-19 may contribute to impaired bile acid feedback regulation and abnormal hepatic metabolic signaling [12,13]. In the present study, FGF-19 showed a significant stepwise decline from controls to NAFLD and NASH. The negative correlations between FGF-19 and ALT, HOMA-IR, BMI, and sTREM-2 support the possibility that disruption of FGF-19 signaling is associated with worsening liver injury and metabolic dysfunction. These results are consistent with the concept that gut-liver axis disruption is an important feature of NAFLD progression. In contrast to PNX-14 and FGF-19, sTREM-2 increased progressively with disease severity. TREM-2 is expressed by macrophage populations involved in lipid handling, tissue repair, and inflammatory remodeling [23-26]. Its soluble form may reflect receptor shedding and macrophage activation during tissue stress [27-31]. The marked elevation of sTREM-2 in NASH patients and its positive correlations with ALT, HOMA-IR, and BMI suggest that circulating sTREM-2 may serve as a marker of hepatic inflammatory activity and metabolic stress. Although membrane-bound TREM-2 may have protective functions in tissue macrophages, elevated soluble TREM-2 may indicate increased immune activation and macrophage remodeling during progressive liver injury. The combined behavior of PNX-14, FGF-19, and sTREM-2 provides a biologically plausible model for NAFLD progression to NASH. Reduced PNX-14 may reflect impaired metabolic and mitochondrial protection, reduced FGF-19 may indicate disruption of gut-liver bile acid feedback regulation, and elevated sTREM-2 may reflect macrophage activation and inflammatory remodeling. Together, these changes suggest a coordinated biochemical shift involving metabolic stress, enterohepatic dysregulation, and immune-inflammatory activation. The proposed mechanistic model linking the biomarker triad to NASH progression is summarized in Figure 4.

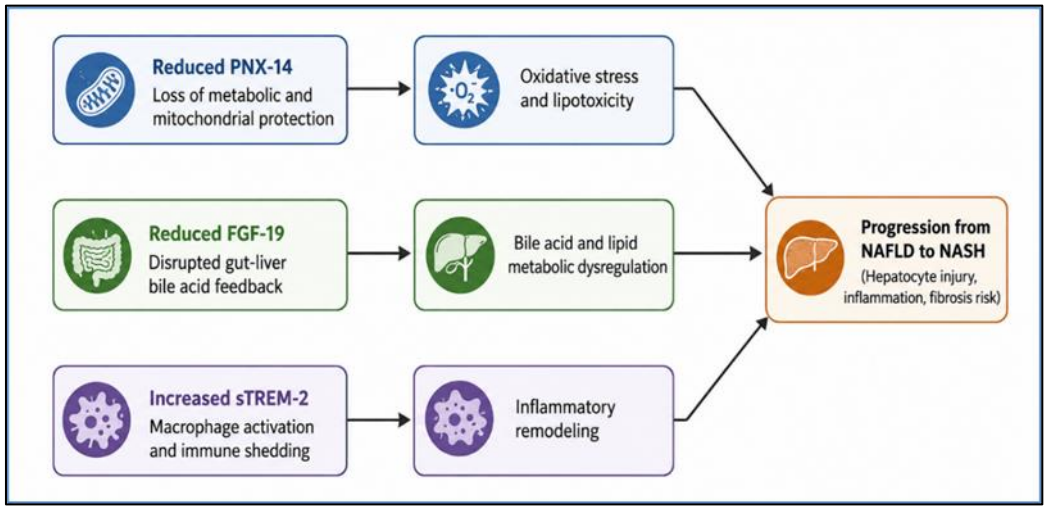

Figure 4 Proposed biochemical model linking the PNX-14/FGF-19/sTREM-2 triad to NASH progression. Schematic diagram showing reduced PNX-14-related metabolic and mitochondrial protection, reduced FGF-19-mediated gut-liver feedback and bile acid regulation, and increased sTREM-2-related macrophage activation during progression from NAFLD to NASH

The correlational results were further evidence for this interpretation. In patients with NASH, PNX-14 and FGF-19 inversely correlated with sTREM-2, indicating that the loss of metabilic and enterohepatic regulatory signals is accompanied by enhanced immune-inflammatory activation. The positive relationship of sTREM-2 with ALT suggests that it is related to hepatocellular injury, and its correlation with HOMA-IR and BMI suggest that macrophage activation is associated with systemic metabolic disturbance. These data encourage the further assessment of the PNX-14/FGF-19/sTREM-2 triad as a non-invasive biomarker panel. Although the results are quite statistically different in this study, the clinical application needs caution. The present study relied on summary-statistic rather than individual-level data. Thus, dependable ROC analysis, diagnostic cut-off definition, multivariable regression, as well as confounding factor

adjustment, could not be conducted. Future investigations should utilize weanling patient level data to determine sensitivities, specificities, area under the curve, and independent predictive value adjusting for BMI, age, sex, diabetes status, lipid profile and liver fibrosis stage.

Study limitation

This study has some limitations. First, the cross-sectional nature of the study does not allow for inferences about causality or changes in biomarker levels over time. Second, although the size was equal in both groups, multicentre cohorts with a larger number of patients are needed to confirm our results and increase their generalizability. Third, advanced statistical modeling, ROC curve analysis, diagnostic cut-off estimation, or multivariate adjustment was not possible because individual-level raw data were unavailable. Fourth, we did not perform histological confirmation through liver biopsy, which is currently the gold standard examination for conclusive separation of simple steatosis from NASH (hepatocellular ballooning and inflammation). The NAFLD and NASH cohorts were established by ultrasound/FibroScan (CAP and liver stiffness measurement), and the presence of raised ALT and metabolic traits. This may have led to some misclassification, in particular of early NASH without advanced fibrosis or of NAFLD with elevated ALT for other reasons. However, the consistent difference in biomarker changes across the groups and the very large absolute magnitude of effect also support that if there was any misclassification, it would be non-differential and thus would bias our findings towards the null rendering them conservative. Fifth, future studies should include information on the ELISA kit manufacturer, the assay sensitivity, the intra-assay and inter-assay coefficients of variation, the sample storage time, and the freeze-thaw cycles in order to enhance reproducibility. Sixth, potential confounders such as diabetes status, lipid profile, medication, diet and physical activity, fibrosis stage were not fully adjusted for. Finally, the biomarker triad proposed here should be subject to external validation in independent cohorts before clinical application.

ROC curve analysis for differentiating NASH from NAFLD and controls was not generated because individual-level raw data were unavailable. Therefore, the diagnostic performance of PNX-14, FGF-19, sTREM-2, and their combined panel should be evaluated in future studies using raw participant-level data.

5. Conclusion

The present study suggests that progression from simple NAFLD to NASH is associated with a coordinated biochemical shift characterized by decreased serum PNX-14 and FGF-19 and increased serum sTREM-2. This pattern may reflect the interaction of impaired metabolic protection, disrupted gut-liver axis signaling, and macrophage-mediated inflammatory activation. The PNX-14/FGF-19/sTREM-2 triad may represent a promising non-invasive biomarker panel for identifying disease progression and distinguishing NASH from earlier stages of NAFLD. However, these findings should be considered preliminary until confirmed by larger longitudinal studies using histological or validated imaging endpoints, individual-level statistical modeling, and external validation.

Recommendations and future directions

- Future longitudinal studies should assess whether changes in PNX-14, FGF-19, and sTREM-2 predict progression from NAFLD to NASH or response to lifestyle and pharmacological interventions.
- The serum levels of these biomarkers should be correlated with liver histology, FibroScan parameters, controlled attenuation parameter, and fibrosis scores to determine their clinical relevance.
- In future investigations using individual data, ROC curve analysis needs to be performed to identify the optimal cut-off values of PNX-14, FGF-19, and sTREM-2 to discriminate NASH from simple NAFLD. The independent diagnostic performance of each biomarker should be evaluated by multivariate logistic regression analysis adjusted for age, sex, BMI, diabetes status, ALT, and stage of fibrosis (e.g., FIB-4 or FibroScan stiffness). A combined nomogram or risk score for the 3 biomarkers should be constructed and externally validated.
- Experimental studies are needed to clarify whether PNX-14 or FGF-19 analogs can reduce hepatic lipotoxicity, inflammation, and fibrosis.
- The relationship between tissue TREM-2 expression and circulating sTREM-2 levels should be investigated to clarify whether sTREM-2 reflects protective macrophage activation, receptor shedding, or progressive inflammatory injury.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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