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ORIGINAL ARTICLE

Integrated Assessment of Oxidative Stress, Inflammatory Biomarkers, and Gastrointestinal Motility Dysfunction in Patients with Non-Alcoholic Fatty Liver Disease (NAFLD)

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a growing metabolic disorder with systemic implications beyond the liver. Oxidative stress and low-grade inflammation are pivotal in its pathogenesis, but their association with gastrointestinal (GI) motility disturbances remains underexplored.

Objective: To evaluate oxidative stress markers, pro-inflammatory cytokines, and their correlation with gastrointestinal motility parameters in patients with NAFLD.

Methods: This cross-sectional study was conducted in the Department of Medicine, Lady Reading Hospital MTI, Peshawar, from May 2022 to May 2023. Ninety adult patients diagnosed with NAFLD via ultrasonography were included. Oxidative stress was assessed by measuring serum malondialdehyde (MDA) and reduced glutathione (GSH). Inflammatory markers, including TNF-α, IL-6, and IL-1β, were quantified using ELISA. Gastric emptying time was evaluated by radionuclide scintigraphy, while colonic transit time was assessed using the radiopaque marker method. Correlation analyses were conducted using Pearson's coefficient.

Results: NAFLD patients exhibited significantly elevated MDA (6.87 ± 1.52 nmol/mL) and inflammatory markers, with reduced GSH levels (2.03 ± 0.61 µmol/g protein). Gastric emptying time (99.56 ± 13.71 min) and colonic transit time (59.73 ± 8.62 h) were significantly delayed. Strong positive correlations were found between TNF-α and GI motility delays ($r > 0.67$, $p < 0.001$), while GSH showed inverse associations.

Conclusion: Patients with NAFLD experience significant oxidative stress, inflammation, and gastrointestinal motility impairment. These systemic abnormalities are interlinked and may serve as predictive markers for subclinical GI dysfunction. Integrating biochemical and motility assessments may enhance comprehensive care in NAFLD.

Keywords: NAFLD, oxidative stress, gastrointestinal motility, inflammation, TNF-α, glutathione

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as the most common chronic liver disorder worldwide, affecting approximately one-quarter of the global population. It encompasses a spectrum of hepatic abnormalities ranging from simple steatosis (fat accumulation without inflammation) to non-alcoholic steatohepatitis (NASH), which may progress to fibrosis, cirrhosis, and hepatocellular carcinoma [1]. Unlike alcoholic liver disease, NAFLD occurs in individuals with minimal or no alcohol consumption and is closely associated with metabolic syndrome components such as obesity, type 2 diabetes mellitus, dyslipidemia, and hypertension. In South Asian countries like Pakistan, the prevalence of NAFLD is rising sharply due to urbanization, dietary changes, sedentary behavior, and increasing rates of insulin resistance [2].

Although traditionally considered a hepatic condition, NAFLD is now recognized as a multisystem disease with extrahepatic manifestations. A growing body of evidence implicates oxidative stress and chronic systemic inflammation as key mediators in NAFLD pathogenesis and progression. Oxidative stress results from an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense system [3]. In NAFLD, mitochondrial dysfunction and excessive fatty acid oxidation generate free radicals, which in turn initiate lipid peroxidation, DNA damage, and cellular apoptosis. This oxidative insult not only aggravates hepatocellular injury but also contributes to inflammation through the activation of pro-inflammatory signaling cascades [4].

Inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) are upregulated in NAFLD and play a central role in hepatocellular inflammation and fibrosis. These cytokines are also known to exert systemic effects, potentially impacting other organ systems, including the gastrointestinal (GI) tract [5]. Recent studies have suggested a link between systemic inflammation and GI motility disturbances in patients with metabolic disorders. The mechanisms may involve direct effects of cytokines on enteric neurons, smooth muscle cells, or central autonomic regulation pathways [6].

Gastrointestinal motility disorders, such as delayed gastric emptying, slow colonic transit, bloating, constipation, and abdominal discomfort, are frequently reported in NAFLD patients but remain under-investigated [7]. These symptoms are often attributed to autonomic dysfunction or low-grade inflammation affecting the enteric nervous system. Despite these observations, few studies have concurrently evaluated the relationship between oxidative stress, systemic inflammatory burden, and GI motility abnormalities in NAFLD [8].

Given this background, the present study aims to comprehensively assess the levels of oxidative stress markers (such as malondialdehyde and glutathione), pro-inflammatory cytokines (TNF-α, IL-6, IL-1β), and gastrointestinal motility indices (gastric emptying time and colonic transit time) in patients with NAFLD. Understanding this integrated pathophysiological interplay may enhance the early identification of systemic manifestations of NAFLD and open avenues for targeted therapeutic strategies addressing not just hepatic, but also gastrointestinal dysfunctions associated with the disease [9].

MATERIALS AND METHODS

This descriptive cross-sectional study was conducted in the Department of Medicine at Lady Reading Hospital Medical Teaching Institution (MTI), Peshawar, Pakistan, over twelve months from May 2022 to May 2023. Lady Reading Hospital is a tertiary care referral center that receives a large and diverse patient population from across Khyber Pakhtunkhwa and

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neighboring regions, making it a suitable site for a representative clinical study. Institutional Review Board approval was obtained in the hospital, and all the participants provided written informed consent before participating in the study.

The recruitment of a non-probability consecutive sampling technique of 90 adult patients was carried out for this study. Abdominal ultrasound was used for confirmation of NAFLD, which showed increased hepatic echogenicity, vascular edge blurring, and posterior beam attenuation, which was reviewed by an expert radiologist. In addition, these patients were tested for increased levels of liver enzymes, especially alanine aminotransferase (ALT) and aspartate aminotransferase (AST), to confirm the diagnosis. The following were used as inclusion criteria for the study:<< Included were adults aged 30-65 years (regardless of gender) – with BMI ≥ 25 kg/m² and ultrasound evidence of hepatic steatosis, free of other chronic liver diseases. Inclusion was limited to patients who were provided with a non-alcoholic diet (men up to 20 g/day for men and women up to 10 g/day for women), clinically stable, and who had not used any medications within the last four weeks that influence oxidative stress and gastrointestinal motility.

Care was taken to apply exclusion criteria so that confounding variables would be excluded. The following patients with hepatitis B or C, autoimmune liver diseases, primary biliary cholangitis, Wilson's disease, hemochromatosis, and drug-induced liver injury were excluded, based on detailed patient history, serological results, and biochemical assessment. Patients who had received gastrointestinal surgery or had gastric diseases such as irritable bowel syndrome, peptic ulcer disease, or inflammatory bowel disease were excluded. The study also excluded pregnant and lactating women, persons under immunosuppressive therapy, and persons with confirmed malignancy or systemic inflammatory disease.

All participants in the study were well assessed using a clinical assessment, which included a full medical and physical examination. Age, gender, weight, height, and BMI were collected with the help of a structured questionnaire. Participants gave venous blood samples in the morning after going without eating for at least 10–12 hours overnight. The serum was stored at –80°C after separation and used for biochemical analysis whenever needed. Serum malondialdehyde (MDA) was assessed using the TBARS method to determine oxidative stress because this assay is known for lipid peroxidation measurement. Endogenous antioxidant reduced glutathione (GSH) was measured by the Ellman colorimetric method. The inflammatory response was established by measuring TNF-α, IL-6, and IL-1β levels in the serum using a high-sensitivity ELISA kit from BioTechne™, USA. All samples were measured twice, and thorough quality assurance measures were implemented to ensure the assay had precision.

Gastrointestinal motility was tested with two different, autonomous processes. The gold standard of functional gastric motility studies was used, using scintigraphy to measure gastric emptying time. A standardized low-fat solid test meal, consisting of a radiolabeled egg sandwich tagged with Technetium-99m sulfur colloid, was administered to fasting participants. Serial gamma camera images were obtained at 0, 30, 60, 90, and 120 minutes post-meal ingestion. The gastric emptying half-time (T½) was calculated using mono-exponential regression of gastric retention curves. A delayed gastric emptying was defined as a T½ exceeding 90 minutes. Colonic transit time was determined using the multiple radiopaque marker technique. Participants ingested a capsule containing 24 radiopaque markers each day for three consecutive days, and plain abdominal radiographs were taken on day four and day seven. The number of retained markers was counted, and total colonic transit time was calculated using the weighted average method. A colonic transit time exceeding 50 hours was considered prolonged.

All data were entered and analyzed using the Statistical Package for Social Sciences (SPSS) version 27.0. Continuous variables such as age, BMI, biomarker levels, and motility times were expressed as mean ± standard deviation. Frequencies and percentages were calculated for categorical variables such as gender and presence of comorbidities. Differences in mean values of oxidative and inflammatory markers, as well as gastrointestinal motility indices, were analyzed using independent samples t-tests. Pearson correlation coefficients were computed to evaluate the relationships between oxidative stress, pro-inflammatory cytokines, and gastric and colonic transit parameters. A p-value of less than 0.05 was considered statistically significant throughout the analysis.

RESULTS

A total of 90 patients with non-alcoholic fatty liver disease (NAFLD) were included in the study. The mean age of participants was 47.62 ± 8.94 years. There was a slight male predominance, with 51 males (56.7%) and 39 females (43.3%). The average body mass index (BMI) was 30.91 ± 3.45 kg/m², reflecting a predominance of overweight and obese individuals. Regarding comorbidities, 66 participants (73.3%) were known diabetics, 58 (64.4%) had hypertension, and 44 (48.9%) had dyslipidemia. These demographic and clinical features are summarized in Table 1.

Table 1: Demographic and Clinical Characteristics of Study Participants (n = 90)

Parameter	Mean ± SD / Frequency (%)
Age (years)	47.62 ± 8.94
Gender (Male/Female)	51 (56.7%) / 39 (43.3%)
BMI (kg/m²)	30.91 ± 3.45
Diabetes Mellitus	66 (73.3%)
Hypertension	58 (64.4%)
Dyslipidemia	44 (48.9%)

Assessment of oxidative stress revealed significantly elevated levels of malondialdehyde (MDA) with a mean of 6.87 ± 1.52 nmol/mL, indicating increased lipid peroxidation. Conversely, reduced glutathione (GSH), a key antioxidant marker, was significantly lower in NAFLD patients, with a mean of 2.03 ± 0.61 µmol/g protein. Inflammatory markers were also markedly raised. Mean tumor necrosis factor-alpha (TNF-α) was 84.65 ± 21.49 pg/mL, interleukin-6 (IL-6) was 61.87 ± 18.74 pg/mL, and interleukin-1 beta (IL-1β) was 45.22 ± 12.36 pg/mL. The elevations in these biomarkers were statistically significant when compared to standard physiological ranges ($p < 0.001$). These results confirm the presence of heightened oxidative stress and systemic inflammation in NAFLD patients, as shown in Table 2.

Table 2: Oxidative Stress and Inflammatory Markers in NAFLD Patients

Biomarker	Mean ± SD	Reference Range	p-value
Malondialdehyde (nmol/mL)	6.87 ± 1.52	<3.0 nmol/mL	<0.001
Reduced Glutathione (µmol/g protein)	2.03 ± 0.61	>4.0 µmol/g protein	<0.001
TNF-α (pg/mL)	84.65 ± 21.49	<30 pg/mL	<0.001
IL-6 (pg/mL)	61.87 ± 18.74	<10 pg/mL	<0.001
IL-1β (pg/mL)	45.22 ± 12.36	<5 pg/mL	<0.001

Gastrointestinal motility assessments showed that the mean gastric emptying half-time (T½) was 99.56 ± 13.71 minutes, significantly exceeding the upper physiological limit of 90 minutes, indicating delayed gastric motility in the majority of patients. Similarly, the mean colonic transit time (CTT) was 59.73 ± 8.62 hours, surpassing the normal limit of 50 hours. These findings provide evidence of global gastrointestinal motility disturbance in NAFLD patients and are presented in Table 3.

Table 3: Gastrointestinal Motility Parameters in NAFLD Patients

Parameter	Mean ± SD	Normal Range	p-value
Gastric Emptying Half-time (min)	99.56 ± 13.71	<90 min	<0.001
Colonic Transit Time (hours)	59.73 ± 8.62	<50 hours	<0.001

Correlation analysis revealed significant positive relationships between inflammatory markers and gastrointestinal motility delays. TNF-α demonstrated a strong correlation with gastric emptying time ($r = 0.742$, $p < 0.001$) and colonic transit time ($r = 0.678$, $p < 0.001$). Similarly, MDA levels showed a positive correlation with gastric T½ ($r = 0.697$) and CTT ($r = 0.649$), while GSH levels were inversely correlated with both motility parameters, suggesting that decreased antioxidant defense is linked to worsening motility. These correlations are summarized in Table 4.

Table 4: Correlation of Inflammatory and Oxidative Markers with GI Motility Parameters

Marker	Gastric T½ (r)	Colonic Transit Time (r)	p-value (both)
TNF-α	0.742	0.678	<0.001
MDA	0.697	0.649	<0.001
GSH	–0.705	–0.681	<0.001

Finally, this study demonstrates that patients with NAFLD have significant oxidative damage, ongoing inflammation, and diminished gastrointestinal motility. The positive correlations between inflammatory cytokines and motility delays observed point to a strong pathophysiological relationship, suggesting that these markers may be early markers of impairment of gastrointestinal function in NAFLD.

DISCUSSION

This examination rigorously evaluates oxidative stress, systemic inflammatory responses, and gastrointestinal motility abnormalities in a group of non-alcoholic fatty liver disease (NAFLD) patients visiting a tertiary hospital in Pakistan [10]. Further, the data also confirm that people with NAFLD have higher oxidative stress, as indicated by increased MDA and GSH loss, and higher inflammation with raised TNF-α, IL-6, and IL-1β values associated with significant gastric emptying and colonic transit delays. The current findings support the idea that more than just liver conditions are involved in NAFLD, and there are systemic disruptions in the gastrointestinal physiology that accompany it [11].

Findings support prior literature that accounts for the increase in malondialdehyde (MDA) in NAFLD patients to hepatic steatosis and then the increased generation of reactive oxygen species (ROS) via mitochondrial overload, beta-oxidation, and peroxisomal activity [12]. MDA, as a byproduct of lipid peroxidation, reflects oxidative injury at the cellular membrane level and has been previously linked to the progression from simple steatosis to steatohepatitis. Conversely, the significant reduction in glutathione (GSH), a key intracellular antioxidant, suggests impaired redox buffering, rendering hepatocytes and potentially enteric neurons more vulnerable to oxidative insult [13].

In parallel with oxidative stress, the significant upregulation of inflammatory markers such as TNF-α, IL-6, and IL-1β in our study population corroborates their established roles in the progression of liver injury and systemic metabolic disturbances [14]. These cytokines are not only involved in hepatic inflammation but have been implicated in altering smooth muscle contractility, affecting gut hormone secretion, and disrupting the enteric nervous system. TNF-α, in particular, is known to impair gastric myoelectric activity and delay gastrointestinal transit through both direct muscular effects and modulation of the vagal-cholinergic pathway [15].

The delayed gastric emptying time and prolonged colonic transit time observed in this study highlight a significant functional gastrointestinal impairment in NAFLD patients. These findings are in agreement with earlier reports that have demonstrated subclinical gastrointestinal dysmotility in individuals with metabolic syndrome and fatty liver disease. However, unlike previous studies that treated hepatic and GI features in isolation, our analysis provides a combined and integrated evaluation of biochemical and functional parameters, underscoring the tight interplay between hepatic inflammation, oxidative stress, and gastrointestinal regulation [16].

The strong positive correlations identified between TNF-α and gastric/colonic transit times, as well as the inverse relationship with GSH, support a mechanistic role for systemic inflammation and oxidative imbalance in driving GI motility disturbances. These associations suggest that gastrointestinal symptoms in NAFLD patients may be attributable not only to mechanical effects (such as hepatomegaly or intra-abdominal fat) but also to molecular and neurohormonal changes driven by inflammatory and oxidative processes [17].

The study has several strengths, including its prospective design, use of objective motility assessments, and detailed biochemical profiling. However, some limitations must be acknowledged. The study lacked a healthy control group for direct comparison of motility parameters. In addition, the cross-sectional nature of the study precludes any causal inference, and longitudinal studies are required to establish the temporal sequence of these abnormalities. Furthermore, lifestyle and dietary data, which can influence both liver status and GI motility, were not quantified [18].

Despite these limitations, this study adds to the growing body of evidence that NAFLD should be approached as a multisystem disorder with extrahepatic manifestations, including gastrointestinal dysfunction. The identification of easily measurable serum biomarkers that correlate with motility impairment offers a non-invasive approach to early detection and monitoring of systemic complications in NAFLD [19].

CONCLUSION

This study demonstrates that patients with non-alcoholic fatty liver disease experience significant systemic oxidative stress, elevated pro-inflammatory cytokines, and clinically evident gastrointestinal motility disturbances. The observed associations between biochemical markers (particularly TNF-α, MDA, and GSH) and gastric as well as colonic transit times suggest a pathophysiological link between hepatic inflammation and gastrointestinal dysregulation. These findings emphasize the importance of a holistic clinical evaluation in NAFLD, beyond liver-focused diagnostics, to include gastrointestinal symptoms and systemic inflammatory status. Early identification of such multisystem involvement may enable timely therapeutic interventions and improve patient outcomes. Future prospective studies should explore whether targeting oxidative stress and inflammation can improve gastrointestinal function in patients with NAFLD.

Conflict of Interest: The authors declare no conflict of interest related to this study.

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Authors' Contribution: H.U.K., S.K., and Z.F.Q. contributed to study conception, patient recruitment, and clinical data interpretation. T.H. supervised the methodology and statistical analysis and served as the corresponding author. S.A. assisted in manuscript drafting and critical review of public health implications. R.S.A. conducted the biochemical assays and contributed to pharmacological analysis. All authors reviewed and approved the final manuscript.

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