

Research Article

Assessment of Serum Glutathione as an Antioxidant Marker in Non-Alcoholic Fatty Liver Disease (NAFLD) Patients

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ABSTRACT

Background: Non-Alcoholic Fatty Liver Disease (NAFLD) represents a major cause of chronic liver disease worldwide, associated with obesity, insulin resistance, and dyslipidemia. Oxidative stress plays a crucial role in its pathogenesis. Glutathione (GSH), a key intracellular antioxidant, neutralizes reactive oxygen species (ROS) and maintains redox balance. This study aimed to assess serum glutathione levels in NAFLD patients and compare them with healthy controls to evaluate its potential as an antioxidant marker.

Methods: A case-control study was conducted on 100 participants—60 patients clinically and radiologically diagnosed with NAFLD and 40 age- and sex-matched healthy controls. Serum glutathione levels were estimated by the colorimetric method using Ellman's reagent (DTNB). Anthropometric and biochemical parameters such as BMI, fasting blood glucose, lipid profile, and liver function tests were also recorded.

Results: Mean serum glutathione levels were significantly lower in NAFLD patients (2.41 ± 0.52 $\mu\text{mol/L}$) compared to controls (4.73 ± 0.68 $\mu\text{mol/L}$; $p < 0.001$). A progressive decline in glutathione concentration was observed with increasing severity of hepatic steatosis as assessed by ultrasonography. Serum GSH levels negatively correlated with BMI and serum triglycerides, indicating an association between oxidative stress and metabolic derangements in NAFLD.

Conclusion: Reduced serum glutathione levels reflect compromised antioxidant defense and increased oxidative stress in NAFLD. GSH estimation may serve as a simple, non-invasive biomarker for disease assessment and therapeutic monitoring.

Keywords: Non-Alcoholic Fatty Liver Disease, Glutathione, Oxidative Stress, Antioxidant Status, Liver Steatosis

Introduction:

Non-Alcoholic Fatty Liver Disease (NAFLD) is one of the most prevalent chronic liver disorders, affecting up to one-third of the adult population globally.^{1,2} It encompasses a spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma.³ The

pathogenesis of NAFLD is multifactorial, involving insulin resistance, lipid accumulation, mitochondrial dysfunction, and oxidative stress.⁴

Oxidative stress plays a pivotal role in hepatocellular injury by promoting lipid peroxidation and inflammatory cascades. Glutathione (GSH), a tripeptide composed of

glutamate, cysteine, and glycine, acts as a major intracellular antioxidant that detoxifies free radicals and maintains redox homeostasis. Depletion of GSH predisposes hepatocytes to oxidative damage, contributing to disease progression^{5,6}

Despite growing interest in redox imbalance in NAFLD, few studies have focused solely on serum glutathione as a potential biomarker. This study therefore aims to assess serum glutathione levels in NAFLD patients and evaluate its clinical significance independent of other oxidative markers.⁷ Previous studies have demonstrated significant alterations in glutathione metabolism and antioxidant defense in patients with NAFLD. Reduced glutathione levels have been associated with increased oxidative stress and progression of hepatic injury.^{8,9} Experimental studies have also shown that depletion of intracellular glutathione can increase oxidative damage in fatty liver models.¹⁰ Oxidative stress is considered an important mechanism linking metabolic dysfunction to hepatic inflammation, steatosis, and disease progression in NAFLD.¹¹

Interventional evidence has further suggested a potential role for glutathione in NAFLD, with improvement in certain biochemical and metabolic parameters following glutathione supplementation.¹² Studies specifically assessing glutathione levels in NAFLD patients have also reported alterations in glutathione status compared with healthy individuals.¹³ These findings support the potential utility of glutathione as a marker of altered antioxidant capacity in NAFLD. In addition, increased oxidative stress and lipid peroxidation have been demonstrated in patients with NAFLD, further supporting the role of impaired antioxidant defenses in disease pathogenesis.¹⁴

Aim and Objectives

Aim:

To evaluate the serum glutathione levels in patients with Non-Alcoholic Fatty Liver Disease (NAFLD) and assess its role as an antioxidant marker reflecting oxidative stress and disease severity.

Objectives:

1. To estimate serum glutathione levels in clinically and radiologically diagnosed NAFLD patients.
2. To compare serum glutathione levels between NAFLD patients and age- and

sex-matched healthy controls.

3. To analyze the association of serum glutathione levels with clinical and biochemical parameters such as BMI, fasting blood glucose, and lipid profile.

MATERIALS AND METHODS

Study Design and Population

A hospital-based case-control study was conducted in the Department of Biochemistry, Rama Medical College Hospital & Research Centre, Kanpur.

A total of 100 subjects were enrolled:

- Group I (Cases): 60 clinically and ultrasonographically diagnosed NAFLD patients.
- Group II (Controls): 40 age- and sex-matched healthy individuals without evidence of liver disease.

Inclusion Criteria

- Adults aged 25–60 years
- Clinically and sonographically confirmed NAFLD
- Non-alcoholic individuals (alcohol intake < 20 g/day for men, < 10 g/day for women)

Exclusion Criteria

- Viral hepatitis, autoimmune or drug-induced liver disease
- Chronic alcohol consumption
- Renal, cardiac, or systemic inflammatory disorders
- Antioxidant or hepatoprotective drug intake within 3 months

Sample Collection and Biochemical Estimation

Fasting venous blood samples (5 mL) were collected and centrifuged to obtain serum.

Serum glutathione concentration was determined by the Ellman's method using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), measuring absorbance at 412 nm.

Routine biochemical parameters—liver function tests (ALT, AST, ALP, bilirubin), fasting glucose, and lipid profile—were analyzed using standard enzymatic methods.

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD). Statistical comparisons were performed using the t-test and Pearson's correlation coefficient (r). A p-value < 0.05

was considered statistically significant.

RESULTS

Table 1. Demographic and Anthropometric Characteristics

Parameter	Controls (n=40)	NAFLD (n=50)	p-value
Age (years)	41.2 ± 8.5	43.6 ± 9.1	0.18
BMI (kg/m²)	23.1 ± 2.9	28.7 ± 3.6	<0.001
Male/Female	24/16	30/20	0.97

The NAFLD and control groups were comparable in terms of age (p = 0.18) and sex distribution (p = 0.97). However, BMI was significantly higher in NAFLD patients than controls (p < 0.001), suggesting an association between increased BMI and NAFLD.

Table 2. Comparison of Glycemic and Lipid Profile Parameters Between Controls and NAFLD Patients

Parameter	Controls (n=40)	NAFLD (n=50)	p-value
Fasting glucose (mg/dL)	92.4 ± 10.8	108.3 ± 14.2	<0.001
Total cholesterol (mg/dL)	178.6 ± 25.4	207.8 ± 31.6	<0.001
Triglycerides (mg/dL)	132.5 ± 24.1	182.7 ± 35.9	<0.001
HDL-C (mg/dL)	48.2 ± 6.7	40.1 ± 7.2	<0.001
LDL-C (mg/dL)	103.9 ± 21.8	131.2 ± 27.4	<0.001

NAFLD patients showed significantly higher fasting glucose, total cholesterol, triglycerides, and LDL-C, while HDL-C was significantly lower compared with controls (p < 0.001 for all parameters), indicating significant metabolic and lipid abnormalities in NAFLD.

Table 3. Comparison of Liver Function Tests Between Controls and NAFLD Patients.

Parameter	Controls (n=40)	NAFLD (n=50)	p-value
ALT (U/L)	25.4 ± 6.2	48.7 ± 18.5	<0.001
AST (U/L)	23.1 ± 5.4	38.6 ± 14.2	<0.001
ALP (U/L)	91.5 ± 17.3	112.4 ± 24.8	<0.001
Total bilirubin (mg/dL)	0.72 ± 0.18	0.84 ± 0.21	0.006

NAFLD patients had significantly higher ALT, AST, ALP, and total bilirubin levels compared with controls, indicating biochemical evidence of hepatic dysfunction (p < 0.05).

Table 6. Distribution of NAFLD Patients and Serum GSH According to Ultrasonographic Grade of Hepatic Steatosis.

Grade of Steatosis	Number of Patients (n)	Percentage (%)	Serum GSH (μmol/L), Mean ± SD
Grade I	24	48%	2.72 ± 0.39
Grade II	18	36%	2.32 ± 0.35
Grade III	8	16%	1.84 ± 0.31
Total	50	100%	2.41 ± 0.52

Serum GSH levels showed a progressive decline with increasing grade of hepatic steatosis, from 2.72 ± 0.39 μmol/L in Grade I to 1.84 ± 0.31 μmol/L in Grade III, suggesting reduced antioxidant status with increasing disease severity.

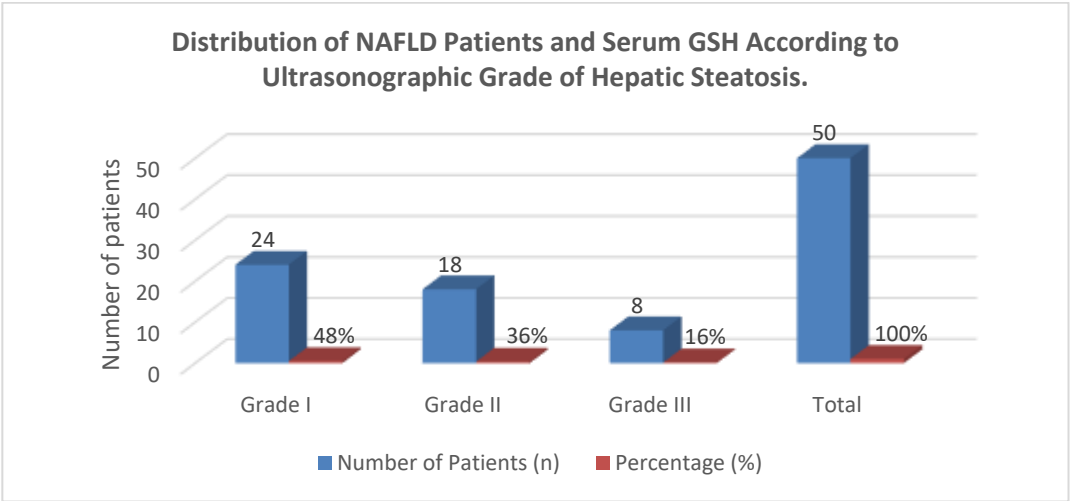


Fig: 1 Graphical represents Distribution of NAFLD Patients and Serum GSH According to Ultrasonographic Grade of Hepatic Steatosis.

Table 4. Comparison of Serum Glutathione Levels Between Controls and NAFLD Patients

Parameter	Controls (n=40)	NAFLD (n=50)	p-value
Serum GSH (μmol/L)	4.73 ± 0.68	2.41 ± 0.52	<0.001

Serum GSH levels were significantly lower in NAFLD patients (2.41 ± 0.52 μmol/L) compared with controls (4.73 ± 0.68 μmol/L), indicating reduced antioxidant capacity in NAFLD (p < 0.001).

Table 5. Correlation of Serum GSH with Metabolic and Liver Parameters in NAFLD Patients

Variable	R-value	p-value
BMI	−0.61	<0.001
Triglycerides	−0.47	0.001
Fasting glucose	−0.38	0.006
ALT	−0.43	0.002
AST	−0.36	0.010

Serum GSH showed a significant negative correlation with BMI, triglycerides, fasting glucose, ALT, and AST, indicating that reduced GSH levels were associated with increased metabolic and hepatic abnormalities in NAFLD.

Serum GSH levels showed:

- Negative correlation with BMI (r = −0.61, p < 0.001)
- Negative correlation with serum triglycerides (r = −0.47, p < 0.01)

A trend of decreasing GSH with increasing grades of hepatic steatosis was observed.

DISCUSSION

This study demonstrated significantly lower serum glutathione levels in NAFLD patients compared to healthy controls, confirming impaired antioxidant defense and increased oxidative stress in these patients. The decline

in GSH concentration paralleled disease severity and metabolic risk factors such as obesity and hypertriglyceridemia.

Glutathione serves as a frontline antioxidant that protects hepatocytes from lipid peroxidation and mitochondrial damage. Its depletion compromises detoxification capacity, leading to oxidative injury and progression from simple steatosis to NASH. The inverse correlation between GSH and BMI further supports the role of obesity-induced oxidative stress in hepatic dysfunction.

Previous studies have also reported reduced hepatic and plasma glutathione levels in metabolic and fatty liver disorders, reinforcing the findings of the present study. However, unlike multi-marker studies that evaluate GSH alongside MDA or GGT, this work isolates the diagnostic potential of GSH as a single, cost-effective oxidative stress biomarker.

CONCLUSION

Serum glutathione levels were significantly lower in NAFLD patients compared with healthy controls and showed a progressive decline with increasing severity of hepatic steatosis. The significant negative correlations of GSH with BMI, triglycerides, fasting glucose, ALT, and AST suggest an association between reduced antioxidant status and metabolic and hepatic abnormalities. Serum GSH may therefore serve as a simple and economical marker of impaired antioxidant defense in NAFLD. However, further studies with larger sample sizes are required to establish its clinical utility for disease assessment and therapeutic monitoring.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this study.

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