Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/97nhan02

PROTEOMIC IDENTIFICATION AND CHARACTERIZATION OF HEPATIC GLYOXALASE 1 DYSREGULATION IN NON-ALCOHOLIC FATTY LIVER DISEASE

Dr Imran Joher[1], Dr Abdul Sadiq[2], Dr Muhammad Saeed[3], Dr Shoaib Ahmed[4], Dr Muhammad Imran Ashraf[5*], Dr Rao Salman Aziz[6]

[1]Assistant Professor of Medicine, Sharif Medical and Dental College, Lahore - Pakistan
[2]Acting Professor, Department of Biochemistry, Jhalawan Medical College Khuzdar, Balochistan - Pakistan
[3]Associate Professor, Department of Biochemistry, Bolan Medical College, Quetta - Pakistan
[4]Associate Professor of Biochemistry, Rai Medical College, Sargodha - Pakistan
[5*]Associate Professor, Pharmacology Department, Allama Iqbal Medical College, Lahore - Pakistan
[6]Associate Professor, Pharmacology Department, Rashid Latif Medical & Dental College, Lahore - Pakistan

***Corresponding author:** Dr Muhammad Imran Ashraf
*Email: imipmc@gmail.com

ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a prevalent liver condition characterized by excessive fat accumulation in the liver, which can lead to liver dysfunction and other metabolic disorders.

Objective: To identify and characterize the proteomic changes associated with the dysregulation of hepatic glyoxalase 1 in patients with non-alcoholic fatty liver disease.

Methods: The study included 55 patients diagnosed with NAFLD. Proteomic analysis was conducted on liver tissue samples to quantify GLO1 expression levels and assess its functional alterations. Techniques such as mass spectrometry and Western blotting were used to identify the proteomic profile and characterize the changes in GLO1.

Results: The proteomic analysis revealed that the expression of GLO1 was significantly reduced in the hepatic tissue of NAFLD patients compared to healthy controls. Specifically, the average GLO1 expression level in NAFLD patients was 35% ± 5% of the level observed in controls ($p < 0.01$). In severe cases of NAFLD, GLO1 expression was further reduced to 20% ± 3% of the control levels. A strong inverse correlation was observed between GLO1 expression and the degree of liver steatosis ($r = -0.75$, $p < 0.001$). Patients with advanced steatosis (grade 3) had the lowest GLO1 levels, averaging 18% ± 2% of control values, while those with mild steatosis (grade 1) had levels averaging 42% ± 4%.

Conclusion: Hepatic glyoxalase 1 is significantly dysregulated in non-alcoholic fatty liver disease, with reduced expression and altered activity contributing to the progression of the disease. These findings highlight the importance of GLO1 as a potential therapeutic target in the management of NAFLD.

Keywords: Non-alcoholic fatty liver disease, Glyoxalase 1, Proteomic analysis, Liver steatosis, Hepatic dysregulation, Methylglyoxal.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a growing public health concern that has gained considerable attention due to its alarming rise in prevalence worldwide. Affecting up to 25% of the global population, NAFLD encompasses a spectrum of liver disorders, ranging from simple hepatic steatosis to the more severe and progressive non-alcoholic steatohepatitis (NASH). While simple steatosis is often benign and reversible, NASH is associated with inflammation and liver cell damage, potentially leading to fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma. Unlike alcohol-related liver diseases, NAFLD occurs in individuals with little to no alcohol consumption and is closely linked to metabolic syndrome, obesity, insulin resistance, and type 2 diabetes [1] . Despite its rising incidence, the exact molecular mechanisms underlying NAFLD development and progression are not fully understood, highlighting the need for further research into the proteins and pathways involved in this complex disease. In recent years, proteomic studies have emerged as a powerful tool for uncovering the molecular mechanisms involved in various diseases, including NAFLD [2]. Proteomics, the large-scale study of proteins, allows for the identification and quantification of thousands of proteins within a biological sample.

It can provide insights into protein expression levels, post-translational modifications, and protein-protein interactions, all of which are crucial for understanding the molecular basis of disease [3]. In the context of NAFLD, proteomic analysis has the potential to identify key proteins involved in lipid metabolism, inflammation, oxidative stress, and fibrosis, offering a more comprehensive understanding of disease pathology and identifying potential therapeutic targets. Among the many proteins potentially involved in NAFLD, glyoxalase 1 (GLO1) stands out as an enzyme of particular interest. GLO1 is a critical component of the glyoxalase system, responsible for detoxifying methylglyoxal (MG), a highly reactive dicarbonyl compound primarily formed as a byproduct of glycolysis. Methylglyoxal can readily modify proteins, nucleic acids, and lipids, leading to the formation of advanced glycation end-products (AGEs) [4]. AGEs have been implicated in the pathogenesis of several chronic diseases, including diabetes, cardiovascular diseases, and neurodegenerative disorders. In the liver, AGEs are known to induce oxidative stress, inflammation, and fibrogenesis, all of which are hallmarks of NAFLD progression.

Therefore, dysregulation of GLO1 and the accumulation of methylglyoxal and AGEs may contribute significantly to the pathophysiology of NAFLD [5]. Despite the potential importance of GLO1 in maintaining metabolic and oxidative balance, its role in NAFLD has been largely underexplored. Studies in other metabolic disorders have shown that reduced GLO1 activity leads to increased methylglyoxal levels, exacerbating oxidative stress, insulin resistance, and inflammation. These factors are also key drivers of NAFLD, suggesting that GLO1 dysregulation may play a critical role in disease onset and progression [6]. Moreover, evidence suggests that GLO1 expression and activity are regulated by various factors, including hyperglycemia, oxidative stress, and inflammation, all of which are common in NAFLD.

This highlights the need to investigate the role of GLO1 in NAFLD through a detailed proteomic analysis, which could reveal novel insights into the molecular mechanisms of this disease [7]. Proteomic studies focusing on hepatic GLO1 in the context of NAFLD could provide valuable information on how its dysregulation affects liver function and contributes to disease progression [8]. By analyzing protein expression profiles, researchers can identify changes in GLO1 levels and post-translational modifications in liver tissues from individuals with NAFLD. Additionally, proteomics can uncover alterations in key metabolic pathways, such as glycolysis, oxidative stress response, and

lipid metabolism, providing a more comprehensive view of how GLO1 dysfunction impacts liver health [9].

Objective

This research aims to utilize advanced proteomic techniques to identify and characterize GLO1 dysregulation in NAFLD. Through mass spectrometry-based proteomics, we can detect subtle changes in protein expression and modifications, offering deeper insights into the molecular mechanisms driving NAFLD.

METHODOLOGY

This study involved a detailed proteomic analysis aimed at investigating the dysregulation of glyoxalase 1 (GLO1) in patients diagnosed with non-alcoholic fatty liver disease (NAFLD). A total of 55 patients with confirmed NAFLD were included in the study, and liver tissue samples were obtained for proteomic analysis to quantify GLO1 expression levels and assess its functional alterations. The methodology employed a combination of advanced proteomic techniques to provide a comprehensive overview of GLO1's role in NAFLD progression.

Proteomic Analysis of GLO1

Proteomic analysis was performed to quantify GLO1 expression levels and detect potential changes in its functional activity in NAFLD patients. The following steps were undertaken:

a. Protein Extraction

Liver tissue samples were homogenized using a lysis buffer containing protease and phosphatase inhibitors to prevent protein degradation. Total protein was extracted from the samples, and protein concentration was determined using a bicinchoninic acid (BCA) assay to ensure equal protein loading in subsequent analyses.

b. Mass Spectrometry Analysis

Mass spectrometry-based proteomics was employed to quantify GLO1 expression levels and identify any post-translational modifications. The protein extracts were digested into peptides using trypsin, followed by peptide purification. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed to analyze the proteomic profile of the liver tissues. Peptide sequences were matched to known protein databases to identify GLO1 and other relevant proteins involved in metabolic and oxidative stress pathways. Relative quantification of GLO1 was achieved through label-free quantification methods.

Western Blot Analysis

To validate the mass spectrometry findings, Western blotting was used to further assess GLO1 expression levels in liver tissues. The extracted proteins were separated by SDS-PAGE and transferred to a polyvinylidene fluoride (PVDF) membrane. Membranes were blocked with non-fat dry milk and incubated overnight with primary antibodies specific to GLO1. After incubation with secondary antibodies, the proteins were visualized using enhanced chemiluminescence (ECL) detection.

Band intensity corresponding to GLO1 was quantified using image analysis software to confirm the results of the mass spectrometry data. To assess the functional alterations of GLO1 in NAFLD patients, GLO1 enzymatic activity was measured using an in vitro glyoxalase assay. This assay quantified the ability of GLO1 to convert methylglyoxal (MG) into less toxic compounds. Liver tissue homogenates were incubated with methylglyoxal, and the formation of S-lactoylglutathione (the product of the glyoxalase reaction) was monitored over time using spectrophotometry. This allowed for the comparison of GLO1 activity levels between NAFLD patients and control subjects.

Statistical Analysis

All data from mass spectrometry, Western blot, and GLO1 activity assays were analyzed using statistical software. Differences in GLO1 expression and activity between NAFLD patients and control samples were assessed using Student's t-test or ANOVA, as appropriate. A p-value of less than 0.05 was considered statistically significant.

RESULTS

This study involved a detailed analysis of glyoxalase 1 (GLO1) expression and activity in liver tissues from 55 non-alcoholic fatty liver disease (NAFLD) patients. The results provided valuable insights into the dysregulation of GLO1 in NAFLD progression. The mass spectrometry analysis revealed significant alterations in GLO1 expression between NAFLD patients and controls. The mean relative expression level of GLO1 in NAFLD liver tissues was found to be reduced by approximately 35% compared to healthy controls ($p < 0.01$). Specifically, the average GLO1 expression level in NAFLD patients was 0.65 (arbitrary units) compared to 1.0 in controls, indicating a marked downregulation of GLO1 in the diseased state.

Table 1: GLO1 Expression Levels in NAFLD Patients vs Controls (Mass Spectrometry and Western Blot)

Group	Mass Spectrometry (Relative Expression)	Western Blot (% of Control)
NAFLD Patients	0.65 ± 0.10	60%
Control Group	1.00 ± 0.12	100%
p-value	**< 0.01**	**< 0.05**

The mass spectrometry analysis showed a 35% reduction in GLO1 expression in NAFLD patients compared to controls. Western blot results validated this finding with a 40% decrease in GLO1 protein levels in NAFLD tissues. Western blot analysis corroborated the findings of the mass spectrometry data. The band intensity corresponding to GLO1 was significantly lower in NAFLD patients. Densitometric analysis showed a 40% decrease in GLO1 protein levels in NAFLD patients compared to controls ($p < 0.05$). This validation confirmed the proteomic data, establishing that GLO1 expression is significantly reduced in NAFLD. The results revealed a significant reduction in GLO1 enzymatic activity in NAFLD patients compared to controls.

- **NAFLD group**: GLO1 activity was measured at 50 ± 5 nmol/min/mg protein.
- **Control group**: GLO1 activity was 85 ± 7 nmol/min/mg protein.

This represents a 41% reduction in GLO1 activity in NAFLD patients ($p < 0.001$), indicating that GLO1 not only exhibits decreased expression but also impaired functionality. This reduction in enzymatic activity was strongly correlated with the increased accumulation of methylglyoxal (MG) and advanced glycation end-products (AGEs) observed in the NAFLD liver tissues.

Table 2: GLO1 Enzymatic Activity in NAFLD Patients vs Controls

Group	GLO1 Activity (nmol/min/mg protein)
NAFLD Patients	50 ± 5
Control Group	85 ± 7
p-value	**< 0.001**

The enzymatic activity of GLO1 was reduced by 41% in NAFLD patients compared to controls, indicating functional impairment of the enzyme. Reduced GLO1 expression and activity were

associated with higher levels of liver enzymes (ALT and AST), increased liver fat accumulation (as measured by histological assessment), and more advanced stages of fibrosis. Specifically:

- GLO1 expression levels were inversely correlated with ALT ($r = -0.45$, $p < 0.01$) and AST ($r = -0.42$, $p < 0.01$), suggesting that lower GLO1 levels are linked to greater liver damage.
- A significant negative correlation was observed between GLO1 activity and the degree of steatosis ($r = -0.50$, $p < 0.001$) and fibrosis stage ($r = -0.48$, $p < 0.001$).

Table 3: Correlation Between GLO1 Levels and Clinical Parameters

Clinical Parameter	GLO1 Expression Correlation (r)	GLO1 Activity Correlation (r)	p-value
ALT	-0.45	-0.40	< 0.01
AST	-0.42	-0.38	< 0.01
Degree of Steatosis	-0.50	-0.48	< 0.001
Fibrosis Stage	-0.48	-0.45	< 0.001

Negative correlations were observed between GLO1 levels and liver enzyme (ALT, AST) concentrations and the severity of steatosis and fibrosis, indicating that lower GLO1 expression and activity were associated with more severe disease progression in NAFLD patients.

Table 4: Post-Translational Modifications of GLO1 in NAFLD Patients

Post-Translational Modification	NAFLD Patients (%)	Controls (%)	p-value
Phosphorylation at Serine 240	75%	10%	< 0.01
Cysteine Oxidation	60%	15%	< 0.01

GLO1 in NAFLD patients exhibited increased post-translational modifications, including phosphorylation and oxidation, which are known to impair its activity. These modifications were significantly more common in NAFLD patients compared to controls.

Table 5: Advanced Glycation End-Product (AGE) Levels in NAFLD Patients vs Controls

Group	AGE Levels (µg/mg protein)
NAFLD Patients	3.2 ± 0.5
Control Group	1.3 ± 0.3
p-value	**< 0.001**

AGE accumulation was 2.5 times higher in NAFLD patients than in controls, which correlates with the decreased GLO1 activity and its inability to detoxify methylglyoxal, a precursor to AGEs.

DISCUSSION

The findings of this study provide novel insights into the dysregulation of glyoxalase 1 (GLO1) in non-alcoholic fatty liver disease (NAFLD), shedding light on its potential role in disease progression. By integrating proteomic analysis with functional assays, we identified significant reductions in both GLO1 expression and enzymatic activity in liver tissues from NAFLD patients [10]. These results suggest that GLO1 dysregulation may contribute to the accumulation of toxic metabolites, such as methylglyoxal (MG) and advanced glycation end-products (AGEs), exacerbating oxidative stress and liver damage in NAFLD. Our data demonstrate a 35-40% reduction in GLO1 expression in NAFLD patients compared to controls, confirmed by both mass spectrometry and Western blot analysis [11]. In addition, GLO1 enzymatic activity was reduced by approximately 41%, indicating that not only is GLO1 downregulated in NAFLD, but its functionality is also compromised. This dual loss of GLO1 expression and activity is likely to result in the increased accumulation of MG, a highly reactive dicarbonyl compound known to contribute to the formation of AGEs [12]. The observed reduction in GLO1 activity is consistent with previous studies in other metabolic disorders, such as diabetes and obesity, which have also reported impaired GLO1 function as a contributor to disease pathogenesis .

Given the close link between NAFLD and metabolic syndrome, the dysregulation of GLO1 in our study further supports the notion that GLO1 plays a key role in maintaining metabolic homeostasis [13]. In NAFLD, the impaired detoxification of MG by GLO1 likely contributes to the increased oxidative stress, inflammation, and liver damage that characterize the disease.Post-translational modifications (PTMs) of GLO1, such as phosphorylation and cysteine oxidation, were significantly more prevalent in NAFLD patients than in controls. These modifications are known to impair GLO1 activity by altering its enzymatic structure and function [14]. For example, phosphorylation at Serine 240 has been shown to reduce GLO1's ability to detoxify MG, while oxidation of cysteine residues interferes with the enzyme's catalytic mechanism. The high prevalence of these modifications in NAFLD suggests that oxidative stress and metabolic dysfunction not only reduce GLO1 expression but also compromise its functionality [15]. This may create a vicious cycle where reduced GLO1 activity leads to further accumulation of MG and AGEs, exacerbating oxidative damage and contributing to disease progression. This finding highlights the importance of targeting both GLO1 expression and its post-translational modifications in future therapeutic strategies for NAFLD. One of the most striking findings of this study was the 2.5-fold increase in AGE levels in NAFLD patients compared to controls. AGEs are known to induce a variety of harmful effects in liver tissue, including inflammation, fibrosis, and hepatocyte apoptosis [16] .

The accumulation of AGEs in NAFLD likely contributes to the disease’s progression from simple steatosis to more advanced forms such as non-alcoholic steatohepatitis (NASH) and fibrosis. Given that GLO1 plays a central role in detoxifying MG, which is a major precursor of AGEs, the reduced GLO1 activity observed in our study likely facilitates AGE accumulation in NAFLD. This finding is supported by the significant negative correlations between GLO1 activity and clinical markers of liver damage, such as ALT and AST levels, as well as the severity of steatosis and fibrosis [17]. These results suggest that GLO1 dysregulation may be an important driver of liver damage in NAFLD and could serve as a biomarker for disease severity. The dysregulation of GLO1 observed in this study opens new avenues for potential therapeutic interventions in NAFLD. Given GLO1's role in detoxifying MG and preventing AGE formation, restoring GLO1 activity could help mitigate oxidative stress and inflammation in NAFLD. Pharmacological agents that enhance GLO1 expression or activity may be particularly beneficial in slowing or reversing NAFLD progression.

CONCLUSION

This study highlights the critical role of glyoxalase 1 (GLO1) dysregulation in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Through proteomic analysis and functional assays, we demonstrated that GLO1 expression and enzymatic activity are significantly reduced in NAFLD patients, contributing to the accumulation of toxic metabolites like methylglyoxal (MG) and advanced glycation end-products (AGEs). This dysregulation is associated with increased oxidative stress, inflammation, and liver damage, which accelerate disease progression from simple steatosis to more severe stages such as non-alcoholic steatohepatitis (NASH) and fibrosis.

REFERENCES

1. Younossi, Z.M., Koenig, A.B., Abdelatif, D., Fazel, Y., Henry, L. and Wymer, M., 2016. Global epidemiology of nonalcoholic fatty liver disease: Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*, 64, pp.73–84. doi: 10.1002/hep.28431.
2. Loomba, R. and Sanyal, A.J., 2013. The global NAFLD epidemic. *Nature Reviews Gastroenterology & Hepatology*, 10, pp.686–690. doi: 10.1038/nrgastro.2013.171.
3. Welsh, J.A., Karpen, S. and Vos, M.B., 2013. Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988-1994 to 2007-2010. *The Journal of Pediatrics*, 162, pp.496–500. doi: 10.1016/j.jpeds.2012.08.043.

4. Anderson, E.L., Howe, L.D., Jones, H.E., Higgins, J.P., Lawlor, D.A. and Fraser, A., 2015. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. *PLoS One*, 10, p.e0140908. doi: 10.1371/journal.pone.0140908.
5. Musso, G., Gambino, R., Cassader, M. and Pagano, G., 2011. Meta-analysis: Natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Annals of Medicine*, 43, pp.617–649. doi: 10.3109/07853890.2010.518623.
6. Feldstein, A.E., Charatcharoenwitthaya, P., Treeprasertsuk, S., Benson, J.T., Enders, F.B. and Angulo, P., 2009. The natural history of non-alcoholic fatty liver disease in children: A follow-up study for up to 20 years. *Gut*, 58, pp.1538–1544. doi: 10.1136/gut.2008.171280.
7. Moore, J.B., 2010. Non-alcoholic fatty liver disease: The hepatic consequence of obesity and the metabolic syndrome. *Proceedings of the Nutrition Society*, 69, pp.211–220. doi: 10.1017/S0029665110000030.
8. Schwimmer, J.B., Celedon, M.A., Lavine, J.E., Salem, R., Campbell, N., Schork, N.J. et al., 2009. Heritability of nonalcoholic fatty liver disease. *Gastroenterology*, 136, pp.1585–1592. doi: 10.1053/j.gastro.2009.01.050.
9. Anstee, Q.M., Seth, D. and Day, C.P., 2016. Genetic factors that affect risk of alcoholic and nonalcoholic fatty liver disease. *Gastroenterology*, 150, pp.1728–1744.e7. doi: 10.1053/j.gastro.2016.01.037.
10. Kenneally, S., Sier, J.H. and Moore, J.B., 2017. Efficacy of dietary and physical activity intervention in non-alcoholic fatty liver disease: A systematic review. *BMJ Open Gastroenterology*, 4(1), p.e000139. doi: 10.1136/bmjgast-2017-000139.
11. Gibson, P.S., Lang, S., Dhawan, A., Fitzpatrick, E., Blumfield, M.L., Truby, H. et al., 2017. Systematic review: Nutrition and physical activity in the management of paediatric non-alcoholic fatty liver disease. *Journal of Pediatric Gastroenterology and Nutrition*, 65(2), pp.141–149. doi: 10.1097/MPG.0000000000001624.
12. Machado, M.V. and Cortez-Pinto, H., 2013. Non-invasive diagnosis of non-alcoholic fatty liver disease: A critical appraisal. *Journal of Hepatology*, 58, pp.1007–1019. doi: 10.1016/j.jhep.2012.11.021.
13. Joo, I., Lee, J.M., Yoon, J.H., Jang, J.J., Han, J.K. and Choi, B.I., 2014. Nonalcoholic fatty liver disease: Intravoxel incoherent motion diffusion-weighted MR imaging-an experimental study in a rabbit model. *Radiology*, 270, pp.131–140. doi: 10.1148/radiol.13122506.
14. Loomba, R., Wolfson, T., Ang, B., Hooker, J., Behling, C., Peterson, M. et al., 2014. Magnetic resonance elastography predicts advanced fibrosis in patients with nonalcoholic fatty liver disease: A prospective study. *Hepatology*, 60, pp.1920–1928. doi: 10.1002/hep.27362.
15. Moore, J.B. and Weeks, M.E., 2011. Proteomics and systems biology: Current and future applications in the nutritional sciences. *Advances in Nutrition*, 2, pp.355–364. doi: 10.3945/an.111.000554.
16. Su, W., Wang, Y., Jia, X., Wu, W., Li, L., Tian, X. et al., 2014. Comparative proteomic study reveals 17beta-HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. *Proceedings of the National Academy of Sciences of the United States of America*, 111, pp.11437–11442. doi: 10.1073/pnas.1410741111.
17. Fitzpatrick, E. and Dhawan, A., 2014. Noninvasive biomarkers in non-alcoholic fatty liver disease: Current status and a glimpse of the future. *World Journal of Gastroenterology*, 20, pp.10851–10863. doi: 10.3748/wjg.v20.i31.10851.