Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 03, 2024

ASSOCIATION OF OXIDATIVE STRESS AND INFLAMMATORY MARKERS IN DIABETIC NON ALCOHOLIC FATTY LIVER DISEASE- A COMPARATIVE BIOCHEMICAL STUDY

Md. Faisal Iqubal[1], Savita Rathore[2], Mushir Ahmad[3], Afreena Nasir[4]*, Gauhar Husain[5]

[1]Department of Biochemistry, Index Medical College Hospital & Research Centre, Indore M.P.

[2]Department of Biochemistry, Amaltas Institute of Medical Sciences Dewas M.P.

[3]Department of Biochemistry, Venkateshwara Institute of Medical Sciences, Gajarula, Amroha U.P.

[4*]Department of Biochemistry, Government Medical College, Jalaun (Orai) U.P.

[5]Department of Biochemistry, Integral Institute of Medical Sciences & Research, Kursi Road, Lucknow, U.P.

Corresponding Author: Dr. Afreena Nasir

Department of Biochemistry, Government Medical College, Jalaun (Orai), Uttar Pradesh, India.

Abstract: Association of oxidative stress and inflammatory markers in diabetic Non alcoholic fatty liver disease and compare with control group. This descriptive study done with 200 patients, 100 patients clinical and laboratory diagnosed with Non alcoholic fatty liver diseases and 100 controls. In which Oxidative stress biomarkers (LPO, MDA, SOD, Catalase) and Inflammatory markers (CRP, IL6, TNF α) was compared and obtained significant result concluded as the most important problem for oxidative stress in NAFLD remains the interpretation and correlation of some results obtained through experimental and clinical studies. A lot of these are difficult to translate into routine clinical practice, aimed at restoring a "healthy" reinforcing the antioxidant status and reduced the inflammatory and oxidative stress, because of a loss of strong evidences that support their application in the therapy of this disease. Reading was tabulated for statistical analysis

Keywords: Oxidative stress markers, inflammatory markers, Non-alcoholic fatty liver disease.

INTRODUCTION

Fatty liver disease is a broad term for the buildup of triglyceride fats in the liver. This condition is common, and most people show no signs or symptoms and do not experience any adverse effects. However, fatty liver is a progressive disease and as it advances in severity it can cause irritation, inflammation and scarring known as fibrosis, when fat content of the liver increases to greater than 5-10%, its function can become significantly impaired**.** Non alcoholic fatty liver disease is increasingly being recognized as a major cause of liver related morbidity and mortality, because of its potential to progress to cirrhosis and liver failure. NAFLD is deposition of fat in the liver of a non-alcoholic subject, a condition which may progress to end stage liver disease.[1]

NAFLD is a very common disorder that has been increasing in prevalence worldwide. A population based analysis through the United States National Health and Nutrition Examination Survey

indicates that the percentage of the US population with NAFLD has steadily increased over the past 20 years[2]. The numbers vary across epidemiologic studies, but the median prevalence in the US and worldwide is in the range of 20% and likely even higher in Asia[3]. Hepatic steatosis and steato-hepatitis can occur in association with multiple diseases affecting the liver, including hepatitis A, B and C, autoimmune hepatitis, hemochromatosis, and hypothyroidism. However, much of the increase in prevalence of NAFLD is driven by its epidemiologic and pathophysiologic links to type 2 DM (T2DM) and obesity. The prevalence of NAFLD in obese adults with T2DM has been estimated to be greater than 70%.[4] Alanine aminotransferase has been noted to be more than twice normal in 20% of children with T2DM, and this is attributed in most cases to NAFLD.[5]

AIMS AND OBJECTIVE

Association of oxidative stress and inflammatory markers in diabetic Non alcoholic fatty liver disease and compare with control group.

MATERIALS AND METHODS

This descriptive study was conducted in Department of Biochemistry, Index Medical College & Hospital, and Research centre. In this study 200 samples selected from OPD, clinical and laboratory diagnosed with Non alcoholic fatty liver disease with the age group of 30 to 60 years who were attending the Medicine OPD & IPD at Index Medical College & Hospital. In this study sample divided into two groups, Group 1consist 100 patients had NAFLD and group 2 100 healthy individual act as a control group. In this study those patients were excluded who were suffering from infectious disease, malignancy, congenital liver disease; drug induced hepatitis and pregnant patients. After completion of history and followed all precaution, 5 ml of venous blood drawn using disposable syringe and collected blood in sterile clot activator vial and proceed for laboratory test according to study in which included Blood sugar level that's estimated by GOP-POD end point colorimetric method (Fasting and PP), Oxidative stress biomarkers (LPO, MDA, SOD, Catalase) and Inflammatory markers (CRP, IL6, TNF α) and reading was tabulated for statistical analysis.

RESULTS AND STATISTICAL ANALYSIS

In this study 200 patients taken, group 1 (NAFLD) has 71 male and 29 female, mean age group 50.05, minimum age 30 and maximum age 70 years considered. In group 2 (control group) has 70 male and 30 female with mean age 49.18, minimum age 28 years and maximum age 78 years found as result on the basis of age and gender criteria as shows in table 1. group 1 consist study group (NAFLD) and group 2 consist control group and comparison and association statistical analysis perform on the basis of oxidative stress (LPO, MDA, SOD, Catalase) as showing in table 4, 5,6, and 7 and inflammatory marker (CRP,IL6, TNF-α)as showing in table 8, 9, 10.

Study group (NAFLD) n=100				Control Group, n=100			
Male	Female	Min	Max	Male	Female	Min	Max
71	29	30	70	70	30	28	78
Mean age =50.05 years				Mean age 49.18 years			

Table 1: gender distribution and age group

Parameter	Group	Mean ±SD	Median (IQR)	Kruskalwallis H test value	p-value
F Blood Sugar	NAFLD	102.64±13.59	171.0 (180.8-150.0)	227.53	
	Control	105.07±24.17	108.0 (117.5-85.2)		

Table 2: Fasting Blood sugar levels between the two groups of study subjects

Parameter	Group	Mean ±SD	Median (IQR)	Kruskalwallis H test value	p-value
PP Blood Sugar	NAFLD	141.68±19.13	159.0(175.0-122.0)	211.796	Not Significant
	Control	148.02±16.35	165.0(174.7-142.0)		

Table 3: PP Blood sugar levels between the two groups of study subjects

Parameter	Group	Mean ±SD	Median (IQR)	Kruskalwallis H test value	p-value
LPO	NAFLD	5.90±0.77	5.8 (6.9-5.5)	254.904	<0.0001
	Control	4.31±0.26	5.4 (5.7-4.2)		

Table-4: LPO levels between the two groups of study subjects

Parameter	Group	Mean ±SD	Median (IQR)	Kruskalwallis H test value	p-value
MDA	NAFLD	3.81±0.51	3.9 (4.1-3.5)	273.907	<0.0001
	Control	2.32±0.25	2.4 (2.6-2.2)		

Table-5: MDA levels between the two groups of study subjects

Parameter	Group	Mean ±SD	Median (IQR)	Kruskalwall H test value	p-value
SOD	NAFLD	198.05±25.51	191.0(221.0-170.0)	173.055	<0.0001
	Control	150.64±18.53	155.5 (1733.5-106.0)		

Table-6: SOD levels among the four groups of study subjects

Parameter	Group	Mean ±SD	Median (IQR)	Kruskalwallis H test value	p-value
Catalase	NAFLD	174.29±22.87	179.0 (189.0-167.0)	224.928	<0.0001
	Control	116.56±15.84	117.0 (128.0-102.2)		

Table-7: Catalase levels among the four groups of study subjects

Parameter	Group	Mean ±SD	Median (IQR)	Kruskalwallis H test value	p-value
CRP	NAFLD	2.39±0.75	1.9 (2.8-1.9)	243.959	<0.0001
	Control	1.30±0.47	1.3 (1.7-0.92)		

Table-8: CRP levels among the four groups of study subjects

Parameter	Group	Mean ±SD	Median (IQR)	Kruskalwallis H test value	p-value
IL6	NAFLD	47.85±22..67	37.0 (71.0-32.0)	268.005	<0.0001
	Control	15.31±3.98	16.0 (17.0-13.0)		

Table-9: IL6 levels among the four groups of study subjects

Parameter	Group	Mean ±SD	Median (IQR)	Kruskalwallis H test value	p-value
TNFα	NAFLD	39.00±11.11	39.0 (48.0-29.0)	183.028	<0.0001
	Control	24.56±10.10	21.0 (19.0-18.0)		

Table-10: TNFα levels among the four groups of study subjects

DISCUSSION

Non-alcoholic Fatty liver, have inflammation and liver cell damage, as well as fat in the liver. Inflammation and liver cell damage can cause fibrosis, or steatosis of the liver. Later may lead to cirrhosis or liver cancer. The study is a comparative descriptive study of 200 patients with Non Alcoholic Fatty liver disease with type-2 Diabetes Mellitus conducted over a period of more than 2 years at Index Medical College & Hospital, Research center Indore. Several studies have been done on Fatty liver diseases since its initial description by Ludwig et al in 1980[6]. Very few studies have been done in India. No comparative descriptive study has yet examined the association of oxidative stress and inflammatory markers in diabetic non alcoholic fatty liver disease. The patient population for the study was selected from patients attending the Medicine OPD & IPD at Index Medical College & Hospital, Indore.

The study population included approximate 70% males and 30% females. The mean age of the patients was 50.55 years (SD 10.27), with an age range of 25 – 70 years. These study shows that NAFLD can affect people of any age; the highest prevalence of the disease seems at age of 45-54 years. Another study[7] also reported that the highest prevalence of NAFLD in the age group of 40 to 60 years. This was similar to our observations. In the present study majority of the patients were males (71%). Another two studies also have reported a higher prevalence of NAFLD amongst the males.[8] Duseja et al had attributed this high male prevalence to the existence of a referral bias.

Fasting blood sugar, the diagnosis of diabetes rests on the measurement of plasma glucose levels. The diagnostic criteria for diabetes were changed in 1997.[10] According to ADA FBS>126mg/dl is diagnostic of diabetes. In our study the mean FBS value of the NAFLD patients was 102.64±13.59 normal according to the ADA criteria and the PPBS which was within the limit (141.68±19.13) cut off value of 140 mg/dl, whereas control group had blood glucose value as 105.07±24.17 and 148.02±16.35 for FBS and PPBS respectively suggestive of normoglycemia. These values correlate well with clinical diagnosis

The association of oxidative stress in the pathogenesis of NAFLD stems from the observation that an increase in oxidative stress markers is a common feature in NAFLD. The present study was evaluated the oxidative stress profile regarding antioxidant enzymes (SOD and Catalase), and oxidative cell damage (LPO and MDA) in patients with NAFLD, AFLD, DM and control. The observation shows significantly increase in LPO and MDA in study groups. These results are verified by several clinical studies demonstrating an increase in oxidative stress markers in NAFLD patients[11] in other studies, changes of oxidative stress marker are followed by the decreasing of the hepatic content of reduced glutathione (GSH) and vitamin E along with the abnormal activity of antioxidant enzymes including GSH peroxidase, superoxide dismutase, and catalase. When changes of OS markers correlate with the clinical severity of the disease suggested that oxidative damages can involved to development of NAFLD.[12]

NAFLD has been considered a heterogeneous disease, which is also consistently linked with other diseases such as diabetes and cardiovascular and chronic kidney diseases[13]. Various researches have explained that NAFLD is a metabolic syndrome of hepatic manifestation, characterized by pathological alterations in carbohydrate and lipid metabolism.[14]

The increased lipid storage into the hepatocytes stimulate the over formation of reactive oxygen species (ROS), which leads to LPO[15]. LPO is a biological free radical chain reaction. The oxidation of unsaturated Fatty Acids or lipids produces peroxides of these compounds. An animal study showed increased LPO level.16 Extra-ordinary peroxidation happens in the liver cell and in circulating lipids, resulting to lipotoxicity with dysfunctions of metabolism[18].

Oxidative stress has been presumed as a main process which causes liver damage, stimulating the conversion from simple steatosis to NASH[19]. Dysfunction of mitochondrial in liver tissue during NAFLD was reported to disturb the liver lipid equilibrium, enhance production of ROS and stimulate LPO and cytokine release, resulting to cell death[20]. Polyunsaturated fatty acids (PUFA) with ROS are involved in formation of lipid peroxides. Lipid peroxides are very unstable rapidly decomposed into active 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA), resulting in cell damage[21]. Lipids are the most susceptible to ROS attack in fatty liver ischemia and reperfusion injury. However, FA accumulation and extensive lipid peroxidation are contained within NAFLD itself, making fatty liver ischemia and reperfusion injury more severe.[22]

In our study, we found elevated CRP levels were significant associated with NAFLD, and our findings are similar to Nigam et al[23]. Few studies have found elevated CRP levels are a useful diagnostic marker for NAFLD[24]. CRP level was associated with NAFLD in non-obese healthy men. The proposed mechanism between CRP and NAFLD is the rise in acute-phase cytokines. IL-6 is a strong booster and elevates CRP levels.

The disequilibrium between pro-inflammatory and anti-inflammatory cytokines aggravates the complications related to T2DM[25]. In the present study level, inflammatory markers (CRP, IL6, and TNF-α) were significantly higher in the study diabetic patients in comparison to the control group ($P<0.001$). Malik A et al[31] reported the levels of pro-inflammatory cytokines such as TNF-α, IL-6 and anti-inflammatory cytokine IL-10 were significantly higher in T2DM individuals compared to controls in their study. Similar results have been reported by Darko SN et al[26], in their study. Lemieux I et al[27] study reported on the correlation between fasting insulin levels and C-reactive protein levels in the plasma of diabetics showing that insulin resistance and inflammatory processes are linked. It has been observed that adipose tissue can synthesize main pro-inflammatory cytokines, tumor necrosis factor, and interleukin-1 and -6 and that inflammatory biomarkers are linked with body fat mass, suggesting that activated innate immunity and inflammation are important biological factors in the pathogenesis of diabetes mellitus and the complications of type 2 diabetes mellitus[28]. Furthermore, soluble and immobilized CRPs have been demonstrated to

mediate the uptake of native low-density lipoprotein (LDL) into macrophages[29]. CRP may also function as a substrate for membrane-associated neutrophil serine protease that cannot be up-regulated. Studies on inflammatory markers have noted that participants with a combined elevation of both IL-6 and IL-1β had about a three-fold increase in the risk of developing diabetes, whereas low levels of IL-1β alone demonstrated no substantial increase in risk[30]. Significant results were obtained with IL-6/IL-10 and IL-6/IL1β. Studies reviewing IL-6 and tumor necrosis factor–α (TNF-α) have established roles in the regulation of APRs with other investigations providing a possible link between these two biomarkers and cardiovascular events[29]. Additionally, an association with TNFα receptor 2, IL-6, and CRP has been demonstrated, with elevated levels of CRP noted as a strong independent predictor of T2DM. So our findings support the above pathology and noted the significantly higher inflammatory markers in diabetes patients with respect to normal healthy subjects.

CONCLUSION

In conclusion, at the current level of knowledge, the most important problem for oxidative stress in NAFLD remains the interpretation and correlation of some results obtained through experimental and clinical studies. A lot of these are difficult to translate into routine clinical practice, aimed at restoring a “healthy” reinforcing the antioxidant status and reduced the inflammatory and oxidative stress, because of a loss of strong evidences that support their application in the therapy of this disease. Certainly, we must admit that the evolution in the comprehension of the mechanisms that support NAFLD has been very fast in the last decade, and, at the same time, the analyzed fields represent some of the most promising topics of scientific research of the future.

REFERENCES

1. Nobili V, Svegliati-Baroni G, Alisi A, Miele L, Valenti L, Vajro P. A 360-degree overview of paediatric NAFLD: recent insights. J Hepatol. 2013; 58(6):1218-29.

2. NeuschwanderTetri BA, Chaldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology. 2003;37(5):1202-19.

3. Younossi ZM, Stepanova M, Afendy M, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. Clin Gastroenterol Hepatol 2011;9:524-530.

4. Amarapurkar DN, Hashimoto E, Lesmana LA, et al. How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? J Gastroenterol Hepatol 2007;22:788-93.

5. Stefan N, Häring HU. The metabolically benign and malignant fatty liver. Diabetes 2011;60:2011-7.

6. J Ludwig, T R Viggiano, D B McGill, B J Oh. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc.1980 Jul;55(7):434-8.

7. D , Kamani P, Patel N, Gupte P, Kumar P, Agal S, Baijal R, Lala S, Chaudhary D, Deshpande A. Prevalence of non-alcoholic fatty liver disease: population based study. Ann Hepatol. 2007 Jul-Sep;6(3):161-3.

8. Deepak Amarapurkar; Prafull Kamani; Nikhil Patel; Parijat Gupte; Pravin Kumar;Subhash Agal;Rajiv Baijal;Somesh Lala; Dinesh Chaudhary; Anjali Deshpande Prevalence of non-alcoholic fatty liver disease:population based study Annals of Hepatology 2007, 6(3): 161-3.

9. Sun X, Seidman JS, Zhao P, Troutman TD, Spann NJ, Que X, Zhou F, Liao Z, Pasillas M & Yang X et al. Neutralization of oxidized phospholipids ameliorates non-alcoholic steatohepatitis. *Cell Metabolism* 2020, Jan 7;31(1)189–206.

10. Zelber-Sagi S,Ivancovsky-Wajcman D, Fliss,Isakov N, Hahn M,Webb M, Shibolet O, Kariv R & Tirosh O Serum malondialdehyde is associated with non-alcoholic fatty liver and related liver damage differentially in men and women. Antioxidants 2020, Jul; 9(7): 578.

11. Notarnicola M., Osella A.R., Caruso M.G., Pesole P.L., Lippolis A., Tutino V., Bonfiglio C., De Nunzio V., Scavo M.P., Mirizzi A., et al. Nonalcoholic Fatty Liver Disease: Focus on New Biomarkers and Lifestyle Interventions. *Int. J. Mol. Sci.* 2021;22:3899.

12. Svegliati-Baroni G., Pierantonelli I., Torquato P., Marinelli R., Ferreri C., Chatgilialoglu C., Bartolini D., Galli F. Lipidomic Biomarkers and Mechanisms of Lipotoxicity in Non-Alcoholic Fatty Liver Disease. *Free Radic. Biol.Med.* 2019;144:293–309.

13. Yang S.Q., Lin H.Z., Lane M.D., Clemens M., Diehl A.M. Obesity Increases Sensitivity to Endotoxin Liver Injury: Implications for the Pathogenesis of Steatohepatitis. *Proc. Natl. Acad. Sci. USA.* 1997;94:2557–62.

14. Marra F., Svegliati-Baroni G. Lipotoxicity and the Gut-Liver Axis in NASH Pathogenesis. *J. Hepatol.* 2018;68:280–95.

15. Almeda-Valdes P., Altamirano-Barrera A., Méndez-Sánchez N. Insights in Non-Alcoholic Fatty Liver Disease Pathophysiology with Lipidomic Analyses. *Ann. Hepatol.* 2015;14:567–9.

16. Tang S.-P., Mao X.-L., Chen Y.-H., Yan L.-L., Ye L.-P., Li S.-W. Reactive Oxygen Species Induce Fatty Liver and Ischemia-Reperfusion Injury by Promoting Inflammation and Cell Death. *Front. Immunol.* 2022;13:870239.

17. Reiniers M.J., van Golen R.F., van Gulik T.M., Heger M. Reactive Oxygen and Nitrogen Species in Steatotic Hepatocytes: A Molecular Perspective on the Pathophysiology of Ischemia-Reperfusion Injury in the Fatty Liver. *Antioxid. Redox Signal.* 2014;21:1119–42.

18. Llacuna L., Fernández A., Montfort C.V., Matías N., Martínez L., Caballero F., Rimola A., Elena M., Morales A., Fernández-Checa J.C., et al. Targeting Cholesterol at Different Levels in the Mevalonate Pathway Protects Fatty Liver against Ischemia-Reperfusion Injury. *J. Hepatol.* 2011;54:1002–10.

19. Malaguarnera L., Madeddu R., Palio E., Arena N., Malaguarnera M. Heme Oxygenase-1 Levels and Oxidative Stress-Related Parameters in Non-Alcoholic Fatty Liver Disease Patients. *J. Hepatol.* 2005;42:585–91.

20. Puri P., Baillie R.A., Wiest M.M., Mirshahi F., Choudhury J., Cheung O., Sargeant C., Contos M.J., Sanyal A.J. A Lipidomic Analysis of Nonalcoholic Fatty Liver Disease. *Hepatology.* 2007;46:1081–90

21. Nigam P, Bhatt SP, Misra A, Vaidya M, Dasgupta J, Chadha DS. Non alcohol fatty liver disease is closely associated with sub-clinical inflammation: a case-control study on Asian Indians in North India. PLoS ONE 2013; 8(1):e49286.

22. Haukeland JW, Damås JK, Konopski Z, Loberg EM, Haaland T, Goverud I, et al. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. J Hepatol 2006; 44:1167–1174.

23. Oruc N, Ozutemiz O, Yuce G, Akarca US, Ersoz G, Gunsar F, et al. Serum procalcitonin and CRP levels in non-alcoholic fatty liver disease: a casecontrol study. BMC Gastroenterol 2009; 9:16.

24. Al-Dahhan NAA, Al-Dahhan HAA. Evaluation of ADA, IL-6 and TNF-alpha level in type 2 diabetes mellitus: with-and without hypoglycemic drugs. Evaluation. 2015;5:7

25. Aastha Malik, Rajesh Kumar Morya, Sarama Saha, Praveen Kumar Singh, Sanjay Kumar Bhadada, Satya Vati Rana. Oxidative stress and inflammatory markers in type 2 diabetic patients. Eur J Clin Invest. 2020;50:e13238.

26. Darko SN, Yar DD, Owusu-Dabo E, et al. Variations in levels of IL-6 and TNF-a in type 2 diabetes mellitus between rural and urban Ashanti Region of Ghana. BMC Endocr Disord. 2015;15:50.

27. Lemieux I, Pascot A, Prud'homme D, Alméras N, Bogaty P, Nadeau A, Bergeron J, Després JP. Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. Arterioscler Thromb Vasc Biol. 2001;21:961–7.

28. Pickup JC. Inflammation and activated immune system in the pathogenesis of type 2 diabetes. Diab Care. 2004;27:813–23.

29. Smitha CN, Soni S, Basu SK. Role of C-reactive protein and periodontal disease in systemic health: A review.JAdv Dent Res 2011;2(1):1-5.

30. Spranger J, Kroke A, Möhlig M, et al. . Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Diabetes. 2003;52(3):812–817.