International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

The role of Inflammatory and Oxidative stress markers as predictive marker for Non-alcoholic fatty liver disease in Type 2 diabetic patients

Preeti Vijaysing Padvi[1], Kavita More[2*], Sandeep Rai[3]

[1]Ph.D. Scholar, Department of Biochemistry, MGM Medical College, MGM Institute of Health Sciences, Navi Mumbai, India
[2]Professor, Department of Biochemistry, MGM Medical College, MGM Institute of Health Sciences, Navi Mumbai, India*
[3]Professor, Department of General Medicine, MGM Medical College, MGM Institute of Health Sciences, Navi Mumbai, India

Abstract

Diabetes mellitus is a prevailing, potentially devastating, multifactorial metabolic disease characterized by hyperglycemia. Persistent hyperglycemia induced oxidative stress can lead to increased levels of pro-inflammatory proteins which can predispose an individual to an increased risk of developing non-alcoholic fatty liver disease or further deteriorate the condition. Thus, we aimed to determine the levels and association of OS markers (MDA, SOD, and Uric acid), Inflammatory markers (Hs-CRP and IL-6) with HbA1c, as well as their predictive role for NAFLD in diabetic patients. A total of 150 participants (51% being female) (50 in each group: G1 – Control, G2 – T2DM with NAFLD, G3 – T2DM without NAFLD) were enrolled from diabetes specialty clinic at MGM Medical college, Navi Mumbai. Demographic and Anthropometric with detailed patients' history were noted. Biochemical parameters were analyzed after blood collection. The mean levels of MDA, SOD, Hs-CRP, IL-6 and Uric acid were significantly higher as compared to the controls ($p<0.001$). Correlation analysis and multiple linear regression analysis revealed a positive significant correlation and predictive association of HbA1c ($p<0.05$) with OS and Inflammatory markers. In ROC analysis to determine the predictive utility of MDA, SOD, Uric acid, Hs-CRP and IL-6 with AUC (0.99, 0.97, 0.92, 0.84, 1.0) for G2 and (0.95, 0.91, 0.77, 0.82, 0.99) for G3 respectively ($p<0.001$) was obtained. Increased levels of OS markers, inflammatory markers and uric acid in response to HbA1c enlightens the fact of hyperglycemia induced oxidative stress leading to increased secretion of proinflammatory proteins. These changes can aggravate hepatic inflammation and worsen NAFLD to NASH. Thus, this multi-marker approach can greatly improve early detection of patients with high risk of NAFLD in T2DM and its further progression.

Keywords: Hyperglycemia, Oxidative stress, Inflammation, diabetes mellitus, Fatty liver

Full length article **Corresponding Author*, e-mail: drkavitajadhav2020@gmail.com

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent hepatic manifestation of metabolic disorder that is closely associated with insulin resistance (IR). It is highly common among individuals with obesity or type 2 diabetes mellitus (T2DM) [1]. The average incidence of NAFLD in India has been reported to be 9-32%, and it is known to rise with additional associated risk factors such as diabetes and dyslipidemia [2]. More than 60-70% of T2DM patients are thought to have NAFLD [1]. Fatty liver is referred to as having a hepatic fat concentration that exceeds 5% of the total liver weight. The NAFLD spectrum advances from benign fatty liver, non-alcoholic steatohepatitis (NASH), cirrhosis to the life-threatening condition of hepatocellular carcinoma [2,3]. Fatty infiltration of hepatocytes is common to all of these stages of NAFLD. T2DM promotes lipolysis and limits the uptake of glucose, resulting in increased TG production by adipose tissues. Several adipokines have been found to contribute to peripheral insulin intake [4]. The etiology of NAFLD seems to entail multiple hits. The first hit is steatosis, which is thought to be caused by IR, and the second hit involves cytokine modifications and oxidative stress through lipid peroxidation, culminating in disease development. Pro-inflammatory cytokines and adipokines have been

associated to the pathophysiology of NAFLD [3]. Impaired glucose tolerance and β-cell dysfunction cause persistent hyperglycemia, abnormal carbohydrate, protein, and lipid metabolism, leading to oxidative stress, chronic inflammation, and elevated hepatotoxic cytokines [5]. Hyperglycemia promotes oxidative stress in type 2 diabetes by multiple pathways, including glucose autoxidation, non-enzymatic protein replication, polyol pathway activation, glycolysis pathway, and pentose phosphate pathway [6]. Lipid peroxidation constitutes an endogenous chain reaction in which free radical species, such as reactive oxygen species (ROS), cause oxidative destruction of phospholipids, resulting in the formation of a diverse range of oxidation products. Malondialdehyde (MDA), a significant aldehydic metabolite, is the most common lipid peroxidation byproduct and has been identified as a strong indicator of lipid peroxidation. In contrast, superoxide dismutase (SOD) is an established intracellular antioxidant that catalyzes the dismutation of superoxide radical into molecular oxygen and H_2O_2 [7]. Increased uric acid concentrations (hyperuricemia) has been added to the list of metabolic abnormalities linked to IR and/or hyperinsulinemia in metabolic syndrome. Uric acid, a natural end product of purine catabolism, can behave as a pro-oxidant and hence be employed as a marker of OS [8]. This OS has the ability to change the structure of the cell membrane, damage cells, and produce cytokines that promote inflammation [6]. Interlukin-6 (IL-6) is a cytokine produced by mononuclear phagocytes, endothelial cells, fibroblasts, epithelial cells, and activated cells. IL-6 has been associated with increased visceral fat and IR [9]. Furthermore, high-sensitivity C-reactive protein (Hs-CRP) is one of the primary acute phase proteins, and as inflammation is critical in NAFLD, Hs-CRP has been employed as an inflammatory marker throughout different investigations [10]. All these factors are implicated in the pathophysiology of NAFLD in diabetes mellitus and further its progression. Thus, keeping all these factors and diagnostic uses of OS and inflammatory markers into consideration the present study was aimed to elucidate the association of these biochemical parameters with glycemic control and to determine the specificity and sensitivity of these markers and assess their utility as predictive biomarker for the diagnosis and progression of NAFLD in T2DM.

2. Materials and Methods

The present observational study was conducted in the Department of Biochemistry and Diabetes specialty clinic in the Department of General Medicine at MGM Medical College and hospital Navi Mumbai. The study was undertaken for ethical consideration and approved by the Institutional ethical committee (ECR/457/Inst/MH2013/RR-20).

2.1. Inclusion criteria

Type 2 diabetes mellitus patients as per ADA guidelines for diagnosis and classification of diabetes mellitus with their HbA1c levels >6.5% [3] without NAFLD of age group between 35-75 years were included in the study group 3. Diagnosed T2DM patients and NAFLD (diagnosed by USG) with similar age group were included in group 2. Apparently, healthy individuals with similar age group were enrolled in control group. All the participants who voluntarily participate in the study were enrolled.

2.2. Exclusion Criteria

Type 1 diabetes mellitus patients or any other type of diabetes were excluded from group 3. For group 2 type 1 diabetes mellitus patients or any other type of diabetes and patients having history of any other liver disease were excluded. Detailed history of alcohol consumption (if more than 40 units/week), smokers, pregnant women were excluded from the study groups.

2.3. Sample size

Sample size was calculated using the formula

$$N = Z^2\, p*q \div L^2$$

Where p=prevalence and q=100-p, L= Margin of error=5. Therefore, N obtained is 147.

2.4. Procedure

A total of 150 participants were enrolled in the study i.e. 50 in each group (G1 – Control, G2 – T2DM with NAFLD, G3 – T2DM without NAFLD). All the participants enrolled in the study were informed about the study and written consent was obtained. A detailed clinical history of the participants was noted down including family history of diabetes, demographic as well as anthropometric details were noted using standard procedures and calculations. Aseptic blood collection for biochemical analysis which included HbA1c by HPLC technique on D10 analyzer, AU480 autoanalyzer was used for estimation of FBS and PPBS by GOD-POD method, liver enzymes (SGOT and SGPT IFCC without pyridoxal method, Alk PO_4 by IFCC (PNPP kinetic method), Total cholesterol by CHOD-PAP method, TG by GPO-TOPS method, HDL cholesterol by selective inhibition method, LDL by calculation, Uric acid by uricase POD method. Oxidative stress markers (MDA, SOD) were measured calorimetrically and inflammatory markers (Hs-CRP by turbidimetric method, IL-6 by ECLIA method).

2.5. Statistical analysis

The data was recorded and analyzed using SPSS software version 25. Quantitative data was represented in the form of Mean ±standard deviation (SD) and frequencies, differences in the means between 2 groups was analyzed using unpaired t-test. Pearsons's correlation analysis was also performed to determine the association between the variables. Multiple linear regression analysis was employed to assess the predictive value of OS and inflammatory markers analyzed in T2DM and NAFLD. Specificity and sensitivity of the parameters was determined using ROC curve. A p-value of <0.05 was considered statistically significant.

3. Results and discussion

Insulin resistance and compensatory hyperinsulinemia can lead to impaired lipid metabolism and hepatic TG buildup in NAFLD, or β-cell dysfunction in T2DM. Compared to non-diabetic individuals' patients with T2DM appear to have a greater risk of acquiring advanced liver diseases [11] similar reported in the recent study done by Hariharan et al., in the year 2021 describing the high prevalence of NAFLD in T2DM participants [12]. The final phases, fibrosis and cirrhosis, occur as a result of collagen deposition and subsequent vascular remodeling. Finally, within the scope of the disease, hepatocellular carcinoma is included as a consequence following these series of pathophysiological events [13]. Currently, NAFLD is the most common chronic liver disease with an estimated prevalence of 25% worldwide, 9-32% in the general Indian population [14] and 12.5-87.5% in Indian type 2 diabetic population [15]. High risk population include those with hypertension, obesity and dyslipidemia. [13]. A total of 150 participants were enrolled in the study which were further grouped into 3 groups based on the radiological findings of USG abdomen (50 in each group: G1 – Control, G2 – T2DM with NAFLD, G3 – T2DM without NAFLD). The demographic and anthropometric data with significantly increased BMI and dyslipidemia in G2 and G3 as compared to the controls was observed which is demonstrated in Table 1. There are various factors that influence fibrosis progression in NAFLD and NASH, but IR and T2DM are by far the most significant predictors. Inter-group comparison on the biochemical parameters was done which depicted poor glycemic control through FBS, PPBS and HbA1c with increased levels in G2 as compared to the G3 and control which are similar to the study findings of Hariharan et al., with similar study groups [12], and other consonant studies by Akbar et al., and Das K et al., [16,17]. Significantly increased levels of liver enzymes (SGOT, SGPT, Alk PO4), total cholesterol, TG, OS markers (MDA, SOD, Uric acid) and Inflammatory markers (Hs-CRP, IL-6) whereas significantly decreased levels of HDL cholesterol in G2 and G3 as compared to the controls has also been observed and is represented in Table 2. According to Melania G et al., other indicators of NAFLD progression include BMI, higher SGPT and SGOT levels, and the degree of hepatic fat accumulation [18]. In the Indian population, a high prevalence of all the components of metabolic syndrome in cases of NAFLD has been reported by Gaharwar R et al., [19], and Sanal MG et al., [20] similar to the present study reports. The IR disrupts the haemostasias of glucose and lipid metabolism, allowing more FFAs to reach the bloodstream for processing by the liver. The presence of dyslipidemia (hypercholesterolemia, hypertriglyceridemia or both) has been reported in 20-80% of cases associated with NAFLD [21] which is similar to the findings of the present study. Hyperglycemia is known to generate ROS, which causes damage to the cells in many ways, leading to further complications in DM [22]. Correlation analysis of HbA1c with OS markers (MDA, SOD and Uric acid) analyzed in the study demonstrated a positive significant correlation in G2 and G3 complementary to the results of previous studies done by Klisic et al. which provided with increased MDA concentrations in Fatty liver Index group (FLI>60) indicating MDA as a strong predictor of OS [1], Kumar et al., found significantly elevated levels of pro-inflammatory and OS markers in NAFLD participants [3] similarly, Samy et al., suggested obesity, dyslipidemia causing OS and impaired glucose tolerance prevalent in NAFLD [23]. Higher MDA levels could be linked to increase ROS. Several routes are thought to be contributing to the upsurge in OS in hyperglycemic states. Hyperglycemia causes increased oxidative stress through the first pathway because it enhances non-enzymatic glycation. The Amadori molecule, an intermediate metabolite, then generates ROS, which leads to the creation of metabolites known as advanced glycosylation end products (AGE) as a result of a glycation reaction. Another mechanism involves the mitochondrial electron transfer system, resulting in OS. Furthermore, the hexosamine pathway additionally serves as a source of OS [24]. Similarly, positive significant correlation of serum uric acid in relation to HbA1c as found in the present study groups G2 and G3 can be the result of underexcretion of urate. Reaven et al., attributed the presence of hyperuricemia in MS to a secondary response to hyperinsulinemia [25]. Corresponding results were reported in a study done by Azhar Hussain et al., suggesting a strong relation between T2DM and hyperuricemia due to direct effect on the oxidation of the purine nucleotides [26]. Hyperinsulinemia may stimulate the hexose phosphate shunt, promoting purine biosynthesis and transformation and consequently increasing the rate of uricogenesis [27]. At the same time, insulin may stimulate uric acid reabsorption from the kidneys by activating the urate anion transporter on the border membrane of the proximal tubular brush, resulting in an increase in serum uric acid concentration. Uric acid can serve as a prooxidant, especially in high concentrations, and hence may be an indicator of OS. Some investigations have hypothesized that uric acid may directly influence the accumulation of fat and hepatic steatosis by preventing insulin signaling, resulting in IR, including mitochondrial OS, or creating ER stress [28]. Positive significant correlation of SOD with HbA1c in G2 and G3 was similar to the findings of the studies done by Mizobuchi et al., Turk et al., Kimura et al., Soliman and Bandeira et al., all demonstrated an increase in Extracellular-SOD enzyme activity in diabetic group as compared to healthy controls [29-33]. This could be due to increased expression of the enzyme responsible for eliminating oxidative attack and peroxidation of polyunsaturated fatty acids from diabetic patient's cell membrane, thereby compensating for free radicals. [34]. Positive correlation of Hs-CRP and IL-6 with HbA1c in G2 and G3 confirms the hypothesis that low-grade inflammation is involved in the pathogenesis of T2DM according to a study done by Pitsavos et al., [35]. Similar findings with elevated OS, inflammatory markers were observed by Pallavi M et al., [5], Wong et al., [36], Klesic et al., suggesting increased OS and pro-inflammatory markers in group with FLI>60 [1] and Kumar et al., with elevated levels of pro-inflammatory cytokines and OS with significant association of Il-6 with IR in NAFLD participants was recorded [3]. OS is most likely caused by more than just the saturation of the antioxidant machinery as an outcome of increased pro-oxidant species production in NASH.

Table 1. Represents the demographic data of the enrolled participants

Parameters	Mean ± SD					
	G1 (n=50) Control		G2 (n=50) T2DM with NAFLD		G3 (n=50) T2DM without NAFLD	
Age (years)	44.86±10.38		47.56±11.12		54.7±9.57	
Gender (%)	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
	26(52%)	24(48%)	23(46%)	27(54%)	25(50%)	25(50%)
Family H/o DM (%)	11 (22%)		47 (94%)		50 (100%)	
Duration of diabetes (years)	-		8.05±2.05		6±1.05	
Height (cm)	164.78±10.38		165.82±8.84		165.03±10.03	
Weight (Kg)	57.51±9.69		78.38±11.62		65.51±8.48	
Waist: Hip ratio	0.80±0.02		0.96±0.06		0.92±0.03	
BMI (kg/m²)	21.14±2.72		28.57±4.13		24.11±3.28	

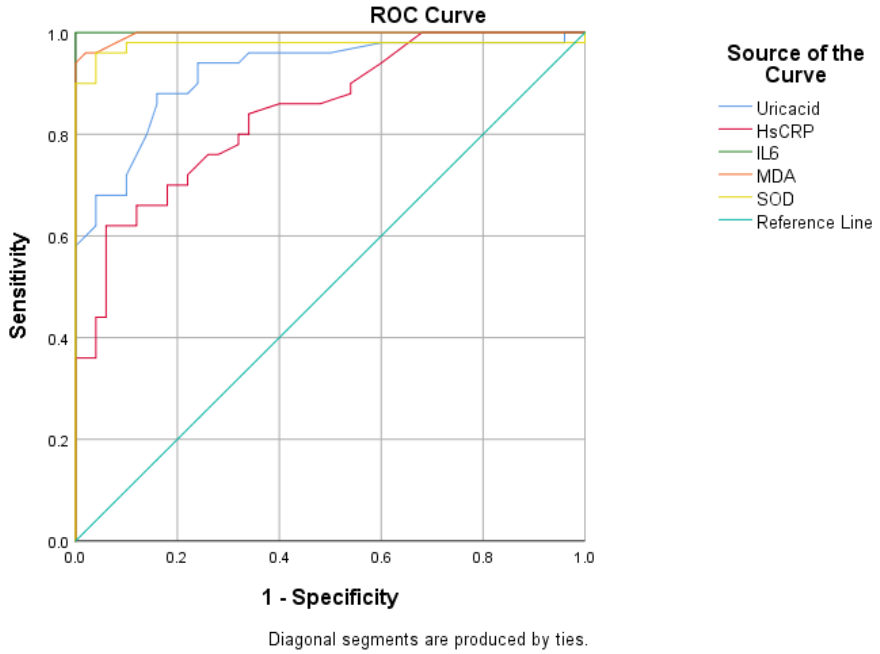

Figure 1. Receivers Operating Characteristics curve for MDA, SOD, Uric Acid, Hs-CRP, IL-6 for group 2

Table 2. Represents the intercomparison of different groups of the enrolled population

Parameters	Mean ± SD		
	G1 (n=50) Control	**G2 (n=50) T2DM with NAFLD**	**G3 (n=50) T2DM without NAFLD**
HbA1c (%)	4.67±0.48	9.59±1.59***	$8.51\pm1.48^{\#\#\#\alpha\alpha\alpha}$
FBG (mg/dl)	87.77±7.79	181.89±46.5***	$162.15\pm44.79^{\#\#\#\alpha\alpha}$
PPBG (mg/dl)	122.77±14.05	243.85±64.54***	$234.68\pm65.98^{\#\#\#\alpha\alpha}$
SGOT (U/l)	26.16±6.38	32.82±14.24***	$32.37\pm16.23^{\#\#\#\alpha}$
SGPT (U/L)	22.93±10.13	35.07±20.19***	$30.96\pm17.60^{\#\#\#\alpha}$
Alk PO4 (U/L)	74.66±16.05	99.10±22.29***	$90.2\pm22.23^{\#\#\#\alpha\alpha\alpha}$
Total Chol (mg/dl)	162.55±39.30	190.14±39.44***	$172.49\pm44.55^{\#\alpha\alpha\alpha}$
TG (mg/dl)	114.90±52.55	228.94±167.22***	$179.40\pm92.99^{\#\#\#\alpha\alpha}$
HDL (mg/dl)	48.11±7.38	43.93±6.93***	$45.26\pm5.85^{\#\#\#\alpha}$
LDL (mg/dl)	92.57 ± 34.21	101.54 ± 37.96*	$90.99 \pm 35.72^{\#\alpha\alpha}$
MDA (nmol/ml)	1.06±0.67	6.52±1.58***	$3.37\pm1.13^{\#\#\#\alpha\alpha\alpha}$
SOD (U/ml)	163.86±17.96	281.84±54.32***	$244.46\pm36.51^{\#\#\#\alpha\alpha\alpha}$
Hs-CRP (mg/L)	2.13±1.65	9.45±10.57***	$4.02\pm5.02^{\#\#\alpha\alpha\alpha}$
IL-6 (pg/ml)	1.38±1.16	21.22±9.77***	$12.13\pm6.73^{\#\#\#\alpha\alpha\alpha}$
Uric Acid (mg/dl)	4.51±1.04	7.13±1.55***	$6.14\pm1.79^{\#\#\#\alpha\alpha}$

Group 1 vs 2 - **$p \leq 0.05$ significant, ***$p \leq 0.001$ highly significant, *$p \geq 0.05$ non-significant.
Group 1 vs 3 - $^{\#\#}p \leq 0.05$ significant, $^{\#\#\#}p \leq 0.001$ highly significant, $^{\#}p \geq 0.05$ non-significant.
Group 2 vs 3 - $^{\alpha\alpha}p \leq 0.05$ significant, $^{\alpha\alpha\alpha}p \leq 0.001$ highly significant, $^{\alpha}p \geq 0.05$ non-significant.

Table 3. Represents the multiple linear regression analysis between dependent variable (HbA1c) and independent variables (OS and inflammatory markers) in group 2 and 3

Parameters	β- coefficient	R^2 value	F value	p-value
G2 (T2DM with NAFLD)				
MDA (nmol/ml)	.361	.131	7.21	0.010*
SOD (U/ml)	.434	.188	11.11	0.002*
Hs-CRP (mg/L)	.521	.271	17.84	0.000**
IL-6 (pg/ml)	.436	.190	11.26	0.002*
Uric Acid (mg/dl)	.399	.159	9.08	0.004*
G3 (T2DM without NAFLD)				
MDA (nmol/ml)	.479	.230	14.31	0.000**
SOD (U/ml)	.401	.161	9.22	0.004*
Hs-CRP (mg/L)	.487	.238	14.95	0.000**
IL-6 (pg/ml)	.559	.313	21.83	0.000**
Uric Acid (mg/dl)	.579	.335	24.18	0.000**
*$p \leq 0.05$ significant, **$p \leq 0.001$ highly significant, $^{\#}p \geq 0.05$ non-significant.				

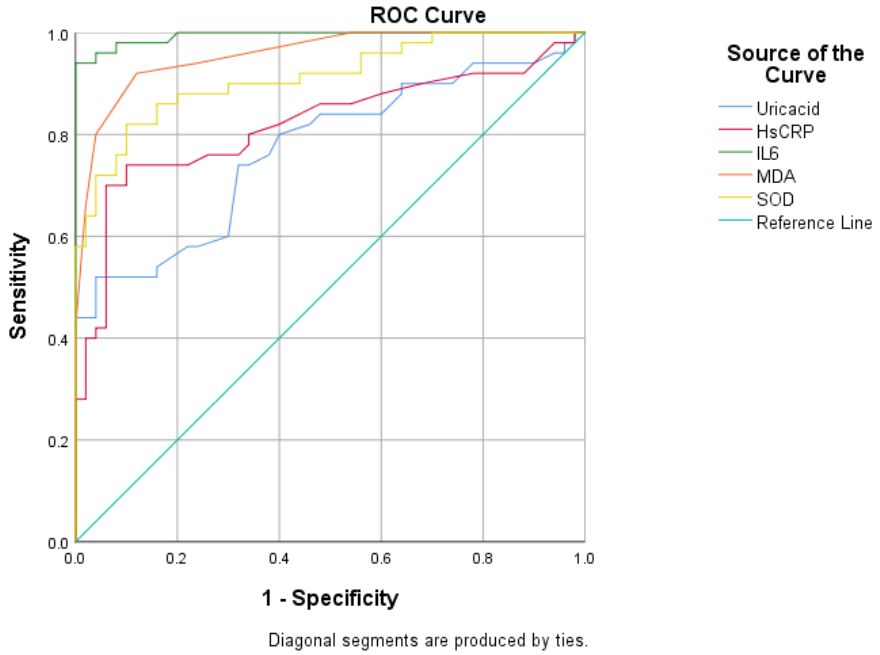

Figure 2. Receivers Operating Characteristics curve for MDA, SOD, Uric Acid, Hs-CRP, IL-6 for group 3.

Table 4. Represents ROC analysis of OS and inflammatory parameters included in the study group 2 and 3

Parameters	AUC (95%CI)	Std Error	Sensitivity (%)	Specificity (%)	p Value
G2 (T2DM with NAFLD)					
MDA (nmol/ml)	.997	.003	92.5	100	0.000**
SOD (U/ml)	.976	.020	89.2	100	
Hs-CRP (mg/L)	.844	.038	64.3	88.8	
IL-6 (pg/ml)	1.000	.000	94.3	100	
Uric Acid (mg/dl)	.920	.028	77.5	88.09	
G3 (T2DM without NAFLD)					
MDA (nmol/ml)	.957	.018	53.7	100	0.000**
SOD (U/ml)	.912	.029	64.9	100	
Hs-CRP (mg/L)	.820	.047	70.1	90.9	
IL-6 (pg/ml)	.994	.005	83.3	100	
Uric Acid (mg/dl)	.771	.047	65.2	83.87	
*$p \leq 0.05$ significant, **$p \leq 0.001$ highly significant, #$p \geq 0.05$ non-significant.					

In the liver, these circumstances cause lipid peroxidation and the generation of ROS. OS promotes inflammation by increasing macrophage infiltration, activating stress-activated kinases, and secreting a wide range of pro-inflammatory adipokines and cytokines, resulting in impaired insulin action and dyslipidemia. Inflammation in adipose tissue may therefore precede to hepatic inflammation [1]. Further, to strengthen this correlational analysis multiple linear regression analysis represented in Table 3 was performed to predict the value of the OS markers, Inflammatory markers and uric acid. The R^2 value obtained are (0.13, 0.18, 0.15, 0.27, 0.19) for G2 and (0.23, 0.16, 0.33, 0.23, 0.31) for G3 of MDA, SOD, Uric acid, Hs-CRP and IL-6 respectively. Which explains that 13%, 23% of variation is due to MDA, 18%, 16% due to SOD, 15%, 33% due to uric acid, 27%, 23% due to Hs-CRP and 19%, 31% variation is obtained due to IL-6 in G2 and G3 respectively. Thus, all these parameters can be considered as predictive markers to assess OS and Inflammation, which can lead to further progression of the disease condition. To assess the predictive utility of OS, Inflammatory markers including Uric acid to predict NAFLD in T2DM participants, Receiver Operating characteristic curve was plot represented in Table 4 for the same which provided with AUC of (0.99, 0.97, 0.92, 0.84, 1.0) for G2 and (0.95, 0.91, 0.77, 0.82, 0.99) for G3 respectively ($p<0.001$) with high sensitivity and specificity depicted in Fig 1 and 2 suggesting the multi-marker approach including OS markers, uric acid and Inflammatory markers beneficial to determine the risk of the NAFLD in T2DM and its further progression to NASH.

4. Conclusions

Type 2 DM is the predominant form of DM worldwide and NAFLD the commonest manifestation in T2DM. Thus, early diagnosis of the disease and to assess further progression of NAFLD is important for better management of the condition in T2DM participants. In addition to the traditionally used liver enzymes and dyslipidemia parameters to rule out NAFLD a multi-marker approach including oxidative stress markers, uric acid, and inflammatory markers, which represents a remarkable specificity and sensitivity in predicting the severity of the disease, is suggested. All this together could greatly put an impact on the improvement in early identification of those

individuals who are at a higher risk of developing NAFLD and its progression.

Acknowledgement

We would like to thank all the physicians, students and lab mates who were of constant help throughout the research process and all the participants who volunteered in our study.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Klisic, A. Isakovic, G. Kocic, N. Kavaric, M. Jovanovic, E. Zvrko, A. Ninic. (2018). Relationship between oxidative stress, inflammation and dyslipidemia with fatty liver index in patients with type 2 diabetes mellitus. Experimental and Clinical Endocrinology & Diabetes. 126 (06) 371-378.

[2] R. Kumar, Y.C. Porwal, N. Dev, P. Kumar, S. Chakravarthy, A. Kumawat. (2020). Association of high-sensitivity C-reactive protein (hs-CRP) with non-alcoholic fatty liver disease (NAFLD) in Asian Indians: A cross-sectional study. Journal of Family Medicine and Primary Care. 9 (1) 390-394.

[3] R. Kumar, S. Prakash, S. Chhabra, V. Singla, K. Madan, S.D. Gupta, S.K. Acharya. (2012). Association of pro-inflammatory cytokines, adipokines & oxidative stress with insulin resistance & non-alcoholic fatty liver disease. Indian Journal of Medical Research. 136 (2) 229-236.

[4] M.E. Shams, M.M. Al-Gayyar, E.A. Barakat. (2011). Type 2 diabetes mellitus-induced hyperglycemia in patients with NAFLD and normal LFTs: relationship to lipid profile, oxidative stress and pro-inflammatory cytokines. Scientia pharmaceutica. 79 (3) 623-634.

[5] M.S. Pallavi, A.A.L. Sachan, S. Rao. (2019). Role of adipokines, oxidative stress, and endotoxins in the pathogenesis of non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus. Int J Res Med Sci. 7 (5) 1644.

[6] R. Sunita, S. Sahidan, R. Hidayat. (2020). Evaluation of Malondialdehyde in Type 2 Diabetes Mellitus Patients as Oxidative Stress Markers in Bengkulu Population. Bioscientia Medicina: Journal of Biomedicine and Translational Research. 4 (3) 45-54.

[7] S.C. Shabalala, R. Johnson, A.K. Basson, K. Ziqubu, N. Hlengwa, S.X. Mthembu, P.V. Dludla. (2022). Detrimental effects of lipid peroxidation in type 2 diabetes: Exploring the neutralizing influence of antioxidants. Antioxidants. 11 (10) 2071.

[8] M. Nadeem. (2022). Comparison Of Oxidative Stress Level In Diabetes Mellitus As Compare To Normal Individual. Journal of Cardiovascular Disease Research; 2022.

[9] S. Muratoğlu Severcan, A. Bilgihan, G. Erkan, U. Erçin, Ç. Severcan. (2019). Oxidative stress markers are increased in non-alcoholic fatty liver disease patients with high serum alanine aminotransferase levels. Medical Studies.

[10] T. Jamialahmadi, S. Bo, M. Abbasifard, T. Sathyapalan, A. Jangjoo, S.A. Moallem, A. Sahebkar. (2023). Association of C-reactive protein with histological, elastographic, and sonographic indices of non-alcoholic fatty liver disease in individuals with severe obesity. Journal of Health, Population and Nutrition. 42 (1) 30.

[11] W. Dai, L. Ye, A. Liu, S.W. Wen, J. Deng, X. Wu, Z. Lai. (2017). Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a meta-analysis. Medicine. 96 (39) e8179.

[12] L.R. HARIHARAN, J.V.B. MANIYAN. (2021). Prevalence and Clinical Characteristics of Nonalcoholic Liver Disease in Type 2 Diabetes Mellitus Patients: A Cross-sectional Study. Journal of Clinical & Diagnostic Research. 15 (4).

[13] A. Campos-Murguía, A. Ruiz-Margáin, J.A. González-Regueiro, R.U. Macías-Rodríguez. (2020). Clinical assessment and management of liver fibrosis in non-alcoholic fatty liver disease. World journal of gastroenterology. 26 (39) 5919.

[14] L.J. Heyens, D. Busschots, G.H. Koek, G. Robaeys, S. Francque. (2021). Liver fibrosis in non-alcoholic fatty liver disease: from liver biopsy to non-invasive biomarkers in diagnosis and treatment. Frontiers in medicine. 8 615978.

[15] M. Prashanth, H.K. Ganesh, M.V. Vima, M. John, T. Bandgar, S.R. Joshi, N.S. Shah. (2009). Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. J Assoc Physicians India. 57 (3) 205-210.

[16] D.H. Akbar, A.H. Kawther. (2003). Nonalcoholic fatty liver disease in Saudi type 2 diabetic subjects attending a medical outpatient clinic: prevalence and general characteristics. Diabetes care. 26 (12) 3351-3353.

[17] K. Das, P. Kar. (2005). Non-alcoholic steatohepatitis. The Journal of the Association of Physicians of India. 53 195-199.

[18] M. Gaggini, M. Morelli, E. Buzzigoli, R.A. DeFronzo, E. Bugianesi, A. Gastaldelli. (2013). Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients. 5 (5) 1544-1560.

[19] R. Gaharwar, S. Trikha, S.L. Margekar, O.P. Jatav, P.D. Ganga. (2015). Study of clinical profile of patients of non alcoholic fatty liver disease and its association with metabolic syndrome. The Journal of the Association of Physicians of India. 63 (1) 12-6.

[20] M.G. Sanal, S.K. Sarin. (2011). Association of nonalcoholic fatty liver disease with metabolic syndrome in Indian population. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 5 (2) 76-80.

[21] E.M. Brunt, V.W.S. Wong, V. Nobili, C.P. Day, S. Sookoian, J.J. Maher, M.E. Rinella. (2015). Nonalcoholic fatty liver disease. Nature reviews Disease primers. 1 (1) 1-22.

[22] M. Jaganjac, O. Tirosh, G. Cohen, S. Sasson, N. Zarkovic. (2013). Reactive aldehydes–second messengers of free radicals in diabetes mellitus. Free Radical Research. 47 (sup1) 39-48.

[23] W. Samy, M.A. Hassanian. (2011). Paraoxonase-1 activity, malondialdehyde and glutathione peroxidase in non-alcoholic fatty liver disease and the effect of atorvastatin. Arab journal of gastroenterology. 12 (2) 80-85.

[24] S. Kawahito, H. Kitahata, S. Oshita. (2009). Problems associated with glucose toxicity: role of hyperglycemia-induced oxidative stress. World journal of gastroenterology: WJG. 15 (33) 4137.

[25] G.M. Reaven. (1997). The kidney: an unwilling accomplice in syndrome X. American journal of kidney diseases. 30 (6) 928-931.

[26] A. Hussain, O.B. Latiwesh, F. Ali, M.Y. Younis, J.A. Alammari. (2018). Effects of body mass index, glycemic control, and hypoglycemic drugs on serum uric acid levels in type 2 diabetic patients. Cureus. 10 (8).

[27] I.H. Fox. (1981). Metabolic basis for disorders of purine nucleotide degradation. Metabolism. 30 (6) 616-634.

[28] L. Xu, T. Li, J. Yin, G. Lin, Y. Xu, Y. Ren, L. Chen. (2019). Association between serum uric acid and nonalcoholic fatty liver disease in community patients with type 2 diabetes mellitus. PeerJ. 7 e7563.

[29] N. Mizobuchi, H. Nakata, T. Horimi, I. Takahashi. (1993). Serum superoxide dismutase (SOD) activity in diabetes mellitus. Rinsho byori. The Japanese Journal of Clinical Pathology. 41 (6) 673-678.

[30] H.M. Turk, A. Sevinc, C. Camci, A. Cigli, S. Buyukberber, H. Savli, N. Bayraktar. (2002). Plasma lipid peroxidation products and antioxidant enzyme activities in patients with type 2 diabetes mellitus. Acta diabetologica. 39 117-122.

[31] F. Kimura, G. Hasegawa, H. Obayashi, T. Adachi, H. Hara, M. Ohta, T. Yoshikawa. (2003). Serum extracellular superoxide dismutase in patients with type 2 diabetes: relationship to the development of micro-and macrovascular complications. Diabetes care. 26 (4) 1246-1250.

[32] G.Z.A. Soliman. (2008). Blood lipid peroxidation (superoxide dismutase, malondialdehyde, glutathione) levels in Egyptian type 2 diabetic patients. Singapore medical journal. 49 (2) 129.

[33] S.D.M. Bandeira, G.D.S. Guedes, L.J.S.D. Fonseca, A.S. Pires, D.P. Gelain, J.C.F. Moreira, M.O.F. Goulart. (2012). Characterization of blood oxidative stress in type 2 diabetes mellitus patients: increase in lipid peroxidation and SOD activity. Oxidative medicine and cellular longevity, 2012.

[34] D. Bonnefont-Rousselot, J.P. Bastard, M.C. Jaudon, J. Delattre. (2000). Consequences of the diabetic status on the oxidant/antioxidant balance. Diabetes and metabolism. 26 (3) 163-177.

[35] S.A. Kavouras, D.B. Panagiotakos, C. Pitsavos, C. Chrysohoou, C.A. Anastasiou, Y. Lentzas, C. Stefanadis. (2007). Physical activity, obesity status, and glycemic control: the ATTICA study. Medicine and science in sports and exercise. 39 (4) 606.

[36] V.W.S. Wong, G.L.H. Wong, P.C.L. Choi, A.W.H. Chan, M.K.P. Li, H.Y. Chan, H.L.Y. Chan. (2010). Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. Gut. 59 (7) 969-974.