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Original Article

Clinical and Biochemical Studies of the GenderSpecific Risk Factors for Css.ardiovascular Disease in Patients of Type 2 Diabetes with NAFLD

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ABSTRACT

Introduction*: Cardiovascular disease (CVD) remains a leading cause of morbidity and mortality in individuals with type 2 diabetes mellitus (T2DM). Additionally, the co-occurrence of non-alcoholic fatty liver disease (NAFLD) further exacerbates this risk. While numerous studies have explored the pathophysiological mechanisms underlying CVD in T2DM, there is a notable paucity of research focusing on the gender-specific nuances of this complex interplay.* ***Methods & Materials:*** *A hospital-based observational study was carried out in the Department of Medicine, Cumilla Medical Collesge and Hospital, Bangladesh, from 1st July to 31st December 2012. Fifty admitted patients of type2 diabetes with NAFLD were included in this study.* ***Results****: Out of 50 patients, 18(36%) were males and 32(64%) were females, with mean age 58.89 ± 8.38 and 54.6 ± 10.1 years respectively. The prevalence of hypertension (p <0.0001), obesity (measured by BMI) (p<0.0001), central obesity (measured by waist circumference and waist hip ratio) (p<0.0001), higher triglyceride levels (p<0.0001), higher LDL-C level (p<0.0001) and lower HDL-C levels (p<0.0001) were significantly higher among the study population. On statistical analysis, we*

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found increasing grades of NAFLD were significantly associated with hypertension (p=0.0083), obesity (p=0.0006), increasing levels of total cholesterol (p<0.0001), ALT (p<0.0001), AST (p<0.0001) and ALP (p<0.0001). ***Conclusion:*** *In our study we exposed that males exhibited a significantly higher propensity for smoking and possessed higher Body Mass Index (BMI) compared to females. On the other hand, females were more inclined to have higher Waist-to-Hip Ratios (WHRs) overall.*

Keywords: *Non-alcoholic fatty liver disease; cardiovascular disease; metabolic syndrome; cardiovascular risk factors*

INTRODUCTION

Type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) represent two significant public health challenges, with their prevalence steadily rising worldwide. Both conditions share common risk factors, including obesity, insulin resistance, and dyslipidemia, which contribute to their frequent co-occurrence. This synergistic relationship not only complicates management but also escalates the risk of cardiovascular disease (CVD), a leading cause of morbidity and mortality in patients with T2DM and NAFLD [1]. Recent studies have highlighted the intricate interplay between gender, T2DM, NAFLD, and CVD, shedding light on striking disparities in disease progression and outcomes. Males and females exhibit distinctive physiological responses to risk factors, hormonal influences, and lifestyle habits, all of which can significantly modulate the impact of these conditions on their cardiovascular health [2]. This study aims to delve into the nuanced differences in how cardiovascular disease manifests in male and female patients with T2DM and NAFLD, with a particular focus on key determinants such as smoking habits, Body Mass Index (BMI), waist-to-hip ratio, dyslipidemia, and elevated liver enzyme levels. By elucidating these gender-specific variations, we aspire to inform tailored clinical approaches that optimize care and outcomes for both male and female patients. The association between smoking and CVD risk is well-documented, with numerous studies emphasizing its differential impact on males and females. Emerging evidence suggests that smoking may exert distinct effects on insulin resistance, adipose tissue distribution, and liver metabolism in male and female patients with T2DM and NAFLD. Understanding these gender-specific nuances is pivotal in designing targeted smoking cessation strategies for optimal CVD prevention [3]. Obesity is a pivotal factor in the development and progression of T2DM, NAFLD, and CVD. However, the distribution of adipose tissue and its metabolic implications can vary substantially between genders. Exploring the differential effects of BMI on cardiometabolic risk factors in male and female patients is crucial for tailoring weight management interventions and refining risk stratification [4]. Waist-to-hip ratio is emerging as a valuable predictor of visceral adiposity and metabolic dysfunction. Recent studies have demonstrated gender-specific variations in the impact of this anthropometric measure on insulin sensitivity and lipid profiles in patients with T2DM and NAFLD [5]. Recognizing these disparities can enhance risk assessment and guide interventions to

mitigate CVD risk. Dyslipidemia is a hallmark feature of both T2DM and NAFLD, contributing significantly to the development of atherosclerosis. Gender disparities in lipid profiles, including triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol, may underlie differences in CVD risk [6]. Investigating these variations is essential for tailoring lipid-lowering strategies and optimizing cardiometabolic outcomes. Elevated liver enzyme levels are a common manifestation of NAFLD, reflecting hepatocellular injury and inflammation. Recent studies have indicated potential gender-specific differences in the relationship between liver enzymes, insulin resistance, and CVD risk [7]. Understanding these nuances is pivotal in refining risk assessment and implementing targeted interventions to mitigate CVD risk in male and female patients [8]. The pathogenesis of NAFLD is not fully understood, however, the finding that not all patients with steatosis develop hepatic inflammation and hepatocellular damage has led to the hypothesis that different pathogenic factors lead firstly to hepatic steatosis and secondly to hepatic damage ('the second hit') [13]. It is unknown what 'second hit' leads to the development of liver damage, although several factors have been implicated, including oxidative stress, mitochondrial abnormalities, and hormonal disturbances involving leptin and adiponectin. NAFLD exists as a histological spectrum of changes; simple steatosis refers to >5% hepatic steatosis in the absence of significant inflammation and hepatocellular damage, and sometimes fibrosis [14]. NAFLD may be progressive, resulting in cirrhosis that may be complicated by hepatocellular carcinoma and liver failure. Overall, about 5% of patients with NAFLD develop cirrhosis over an average of seven years, with 1.7% dying from complications of liver cirrhosis [15]. Most patients with nonalcoholic fatty liver disease have no symptoms or signs of liver disease at the time of diagnosis. The commonest symptoms are fatigue or malaise and a sensation of fullness or discomfort on the right side of the upper abdomen. Hepatomegaly is the only physical finding in most patients. Other features are pruritus, oedema, and stigmata of chronic liver disease, acanthosis nigricans, obesity, type2 diabetes, hypertension, and dyslipidemia [16]. The most frequent biochemical abnormality in NAFLD is persistent, fluctuating, and mild to moderately elevated transaminases (ALT and AST). Serum bilirubin and alkaline phosphatase are usually normal in patients with NASH. Gamma-glutamyl transferase is usually abnormal (>35U/L), and alkaline phosphatase may be upto twice normal, sometimes giving rise to a cholestatic variant [17]. Ultrasound is comparatively cheap and readily available but is less sensitive at detecting minimal (<30%) steatosis or obese patients (BMI of 35–40 kg/m2). Thus, a negative ultrasound does not necessarily exclude NAFLD. A good quality ultrasound can be highly sensitive and specific in diagnosing fatty liver. The classic finding is a hyperechoic (bright) liver. But this finding is non-specific (positive predictive value 62%) but sensitive (85-95%) [19]. A CT scan can detect fatty liver, even the degree of fat infiltration, but may be hampered by any liver iron deposition. Hepatic steatosis decreases the CT attenuation of the liver, while these features allow hepatic steatosis to be defined with a 76% positive predictive value.[20] MRI is overall the

best, most expensive imaging exam for fatty liver. The minimal advantages of MRI are balanced against the wider availability and lower cost of ultrasonogram [21].

METHODS AND MATERIALS

This study was designed as a hospital-based observational study at the Department of Medicine in Cumilla Medical College and Hospital, Bangladesh, for 6 months from 1st July 2012 to 31st December 2012. 50 (Fifty) adult patients, both male and female, with type2 diabetes and NAFLD admitted who fulfill the inclusion criteria were taken. Our sampling technique was purposive.

Inclusion criteria: All patients of type2 diabetes with Non–Alcoholic Fatty Liver Disease (NAFLD) were admitted to the department of medicine of Cumilla Medical College and Hospital at the time of July to December 2012 and had willing to give informed consent.

Exclusion criteria: The exclusion criteria were Alcoholic, known hepatic disease, HBsAg and Anti-HCV positive, history of ingestion of hepatotoxic drug and unwilling to give informed consent.

Key variables were age, gender, height, weight, waist-hip ratio (WHR), BMI (Body Mass Index), smoking, blood pressure (BP), Fasting lipid profile, Blood glucose-fasting and 2 hours after breakfast/75gm glucose load, Alanine aminotransferase (ALT) and Aspartate, aminotransferase (AST), HBsAg, Anti-HCV, ECG, Alkaline Phosphatase (ALP).

Operational definitions

Non-alcoholic fatty liver disease (NAFLD) was diagnosed by ultrasound examination of the liver and sonographic findings [21] graded as follows:

- Grade 0: normal echogenicity
- Grade 1: slight diffuse increase in fine echo's in liver parenchyma with normal visualization of the diaphragm and intra hepatic blood vessels borders.
- Grade 2: moderate diffuse increase in fine echo's in liver parenchyma with slightly impaired visualization of the diaphragm and intra hepatic blood vessels borders.
- Grade 3: marked diffuse increase in fine echo's in liver parenchyma with poor or non-visualization of the diaphragm, intra hepatic blood vessels borders and posterior lobe of the liver.

Dyslipidemia

Total cholesterol - >200mg/dl
HDL- cholesterol- <40 mg/dl (male), <50mg/dl (female)
Triglyceride- >150mg/dl
LDL- cholesterol- >130mg/dl

Hypertension: Hypertension was defined as the patients using of antihypertensive medication on admission or measuring blood pressure ≥140/90 mmHg.

Smoker: A smoker is defined as a person having smoked at least 100 cigarettes in a lifetime or currently smoking some days or every day.

Other causes of raised aminotransferases must be excluded (table-1) [18].

Table I: Causes of chronically elevated aminotransferase level

Hepatic causes	Non - hepatic causes
Alcohol abuse	Coeliac sprue
Medication	Inherited disorders of muscle metabolism
Chronic hepatitis B and C	Acquired muscle disease
Hereditary Haemochromatosis	Strenuous exercise
Wilson's disease (in patients <40 years old)	
Alpha 1 – antitrypsin deficiency	

Data collection tool and technique

Data were collected by prescribed data collection form by the investigator after the admission of the patient and by face-to-face interview. Information was collected by taking a medical history, clinical examination, and subsequent laboratory investigations. Proper permission was taken from the concerned department. All the participants were informed about the nature of the study. Their informed written consent was taken in a consent form before collecting data. A purposive non-probability sampling technique was used for data collection. A data collection form regarding the demographic data such as age, gender, hypertension, diabetes, smoking habit, family history, and previous history of CVD was recorded for each patient. A thorough physical examination was done on all patients. All the cases were investigated for blood glucose (fasting and 2 hours after breakfast/75gm glucose load), fasting lipid profile, ECG, S. Creatinine, and Liver enzymes. Ultrasonogram of the hepatobiliary system was carried out in all patients in Radiology Department using GE LOGIQ 200 pro series ultrasonography machine. All the information was recorded in the data collection form.

Statistical analyses

All data were recorded systemically, and quantitative data were expressed as mean and standard deviation and qualitative data as frequency distribution and percentage. Statistical analysis was done using SPSS (Statistical Program for Social Science) version 17.0. Chi-square test (with correction wherever values in the cells were less than five) was used for categorical data wherever comparisons were needed between the two groups or between two categories in the same group. Variations of $p<0.05$ were considered to be statistically significant.

Ethical considerations

Before the initiation of this study, the research protocol was approved by the Bangladesh College of Physicians and Surgeons (BCPS). The aims and objectives of the study were explained to the patients in an easily understandable local language, and then informed consent was taken from each participant. It was ensured that all were informed and records were kept confidential, and the procedure will be helpful for both the physician and the patients in making a rational approach to patient management.

RESULTS

This observational study was conducted in the Department of Medicine, Cumilla Medical College, and Hospital, Cumilla, from 1st July'12 to 31st December '12. 50 (Fifty) admitted patients of type 2 diabetes with NAFLD were included. There were 18 male patients and 32 female patients. The findings of the study are presented here.

Table II: Distribution of CVD risk factors parameter among patients with NAFLD (n=50)

Parameter	No of patients	%	P values*
Age ≥ 55 years	28	56	_
Family history of CVD	27	54	0.4237
Smoking	23	46	0.4237
Hypertension	35	70	<0.0001
BMI ≥ 23 kg/m^2 †	38	76	<0.0001
Total Cholesterol ≥200mg/dl	19	38	0.0897
HDL-C <40 mg/dl(male) <50mg/dl(female)	46	92	<0.0001
LDL-C >130g/dl	9	18	<0.0001
Triglyceride ≥150 mg/dl	41	82	<0.0001
WHR (≥ 0.9 –male; ≥0.8 -female)	46	92	<0.0001

**Chi-square test was done to measure the level of significance.*

† As per modified WHO criteria for Asian [22], BMI 18.5 – 22.9 kg/m^2 is normal, 23 – 24.9 kg/m^2 is overweight, 25 – 29.9 kg/m^2 is obese, and ≥ 30 kg/m^2 is morbidly obese. We used BMI ≥ 23 kg/m^2 as a cutoff point for obesity.

This table shows 28 (56%) patients were with age >55 and 27 (54%) patients had a positive family history of CVD risk factors. Hypertension, BMI, and dyslipidemia were significantly higher in the study population (p value<0.0001).

Table III: Comparisons of Clinical profile of NAFLD patients according to gender

Parameters	Male		Female		P values*
	Number	%	Number	%	
Total number (n=50)	18	36	32	64	-
Age (years)	58.89 ± 8.38		54.6 ± 10.1		-
Family history of CVD	14	77.78	13	40.63	0.6982
Smoking	18	100.00	5	15.63	<0.0001
Hypertension	15	83.33	20	62.5	0.1615
WHR (≥ 0.9 –male; ≥0.8 -female)	14	77.78	32	100	0.008
BMI ≥ 23 kg/m^2	15	83.33	23	71.86	0.0278

***Chi-square test was done to measure the level of significance.*

This table shows males were of a higher age than females. Prevalence of smoking and increased BMI were significantly higher in males (p-value < 0.0001 and 0.0278, respectively). At the same time, increased WHR prevalence is higher in females (p-value 0.008).

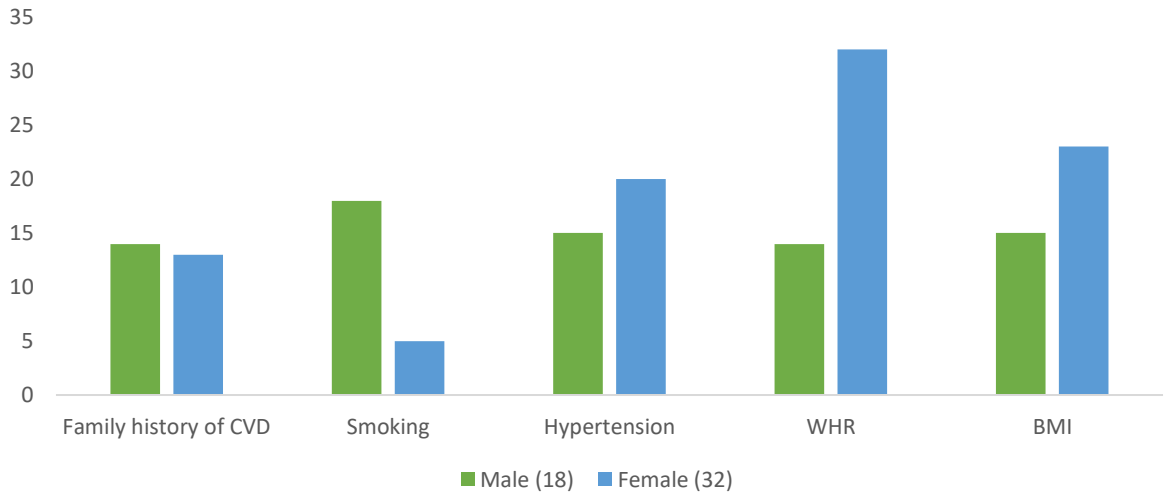

Figure 1: The Bar chart presentation of the clinical parameters.

It is clearly shown that female patients are at risk of CVD for hypertension and BMI, whereas male patients are at higher risk for genetic reasons and smoking.

Table IV: Comparisons of biochemical profile of NAFLD patients according to gender

Parameter	Male		Female		P values*
	Number	%	Number	%	
Total number (n=50)	18	36	32	64	_
Total Cholesterol ≥200mg/dl	7	38.89	12	37.5	0.0093
HDL-C <40 mg/dl(male) <50mg/dl(female)	16	88.89	30	93.75	0.0027
LDL-C >130mg/dl	2	11.11	7	21.88	0.0442
Triglyceride ≥150mg/dl	14	77.78	27	84.38	0.0014
ALT (10 – 50) U/L	7	38.89	13	40.63	0.0027
AST (10 – 40) U/L	7	38.89	12	37.5	0.0093
ALP (40 – 125) U/L	2	11.11	5	15.63	<0.0001

***Chi-square test was done to measure the level of significance.*

This table shows the prevalence of both dyslipidemia and liver enzyme levels were significantly higher in females.

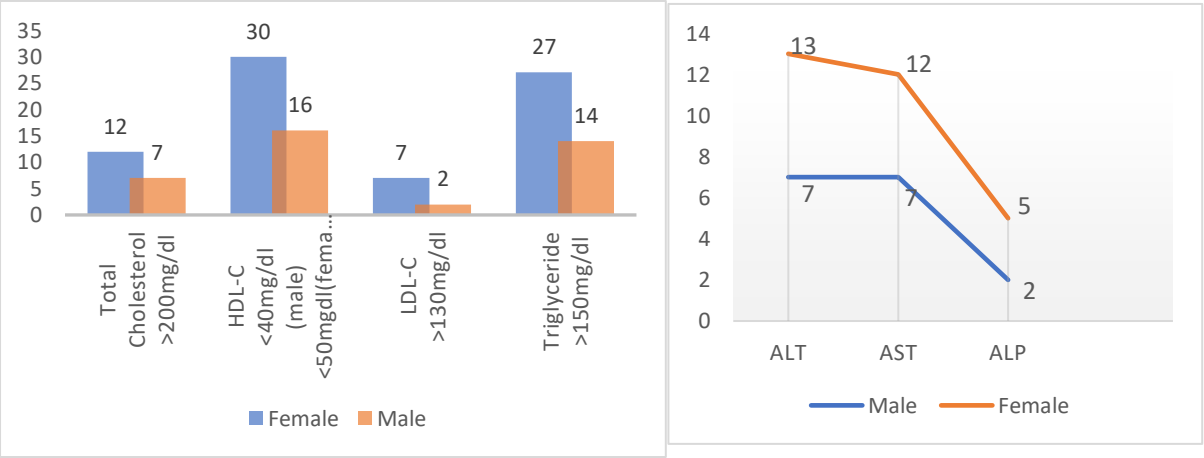

Figure 2: Graphical representation of the biochemical profile of the patients according to the gender

DISCUSSION

In this study, amongst the 50 patients, 18 (36%) were males, and 32 (64%) were females. Mean age ± SD among males and females were 58.89 ± 8.38 years and 54.6 ± 10.1 years, respectively. Overall, 28 (56%) patients were of age > 55 years. Family history for CVD, dyslipidemia, and hypertension were of clinical importance in NAFLD associated type2 diabetes patients [10]. In our study, 27 (54%) patients had positive family history of CVD risk factors. Gender comparison showed family history of CVD risk factors were more males than females (77.78 % vs 40.63 %). A similar finding was seen in

another study, where 57.7% of patients had a positive family history of CVD risk factors [11] . In the current study, 23(46%) patients were smokers. Of these, 18 (100%) patients were males, and 5 (15.63%) patients were females. These findings were higher than those reported by AK Agarwal et al., who found 18.3% smokers in their study population [9]. Hypertension was present in 35 (70%) patients in our study population, which is significantly higher ($p< 0.001$). Among males and females, 15 (83.33%) and 20 (62.5%) patients were hypertensive, respectively. A similar finding was seen in the study by AK Agarwal et al.[9] ;71.4% of patients were hypertensive. Using a cutoff point for abdominal/central obesity of WHR $\geq$ 0.9 for males and $\geq$ 0.8 for females, the prevalence is 92% among our study subjects which was very much significant statistically (p-value <0.0001). 14 (77.78%) male patients and 32 (100%) female patients were found to have abdominal obesity in the present study. This observation was similar to a study by Roli Agrawal et al. [23]. These also resembled the findings presented by Behl, who reported abdominal or central obesity in 92% of males and 100% of females, where WHR was 0.95 ± 0.47 in males and 0.95 ± 0.38 in females [24]. A significant proportion of our study population was obese, as almost 76% of patients had BMI $\geq$ 23 kg/m^2 (p-value <0.0001). A similar result was observed by AK Agarwal et al., where 82.2% of patients had BMI $\geq$ 23 kg/m^2 [9]. Using a cutoff point for obesity of BMI $\geq$ 23 kg/m^2, the prevalence is 83.33% among male and 71.86% among female patients. Accumulating evidence suggested that NAFLD with type2 diabetes could be linked to accelerated atherogenesis through the presence of abnormal lipoprotein metabolism. In NAFLD, hepatic apolipoprotein B–100 synthesis, a rate-determining step in the hepatic VLDL formation and in hepatocyte lipid export, was markedly reduced, and postprandial apolipoprotein B–100 responses were flat and strikingly dissociated from the concomitant increases of postprandial triglycerides [25, 26]. We also found that a significant proportion of our study population had dyslipidemia, as 41 (82%) and 46 (92%) patients had serum triglyceride >150mg/dl and serum HDL <40mg/dl (in male) and <50mg/dl (in the female) respectively. These findings were much higher than those reported by AK Agarwal et al. [9]. They found 54.9% and 50.7% of patients to have hypertriglyceridemia and low serum HDL levels, respectively. On the other hand, total cholesterol is present in 19 (38%) patients in our study. A similar observation was reported by Duseja et al., who reported hypercholesterolemia in 32% of patients [27]. In the current study, serum LDL cholesterol level was elevated in 18% of study subjects. Roli Agrawal et al. reported similar observation [23]. Among our study population, we also found that prevalence of dyslipidemia was higher in females than in males, as 84.38% and 93.75% of female patients had hypertriglyceridemia and low HDL level respectively, compared to 77.78% and 88.89% of male patients. Nonalcoholic fatty liver disease is the most common cause of elevated liver enzymes in patients of developed countries. Patients with NAFLD are often identified by asymptomatic elevation of liver enzymes, most frequently of serum alanine aminotransferase (ALT), and nonalcoholic hypertransaminasemia, in which viral or other causes of liver disease are excluded,

has been used as a surrogate marker for NAFLD [28,29]. In our study, serum ALT, AST and ALP levels are raised in 38.89%, 38.89% and 11.11% patients among males, respectively and 40.63%, 37.5%, and 15.63% patients among females, respectively.

CONCLUSION

Today's lifestyle represents a massive challenge because most meals are full of carbs and lipids and have high caloric contents, thus, helping to increase the prevalence of metabolic syndrome and its consequences, such as NAFLD and increased CVD-related mortality. NAFLD with type 2 diabetic is associated with an increased risk of developing cardiovascular disease, which augments as the hepatic damage progresses. In summary, our comprehensive analysis of the gathered data reveals notable disparities between males and females in various health parameters. Males exhibited a significantly higher prevalence of smoking and elevated BMI. Conversely, females demonstrated a greater prevalence of increased waist-to-hip ratio (WHR), suggesting potential areas of focus for health promotion efforts tailored to this group. Additionally, our findings shed light on gender-specific health concerns. Females exhibited significantly higher rates of dyslipidemia and elevated liver enzyme levels, warranting closer monitoring and intervention strategies. These insights underscore the need for gender-tailored approaches in healthcare provision and highlight areas where targeted interventions can yield the most significant impact on overall health and well-being.

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Approval: The study was approved by the Institutional Ethics Committee

LIMITATIONS OF THE STUDY

Most importantly, the study group was relatively small. It would be important to confirm these results in a larger cohort. However, study participants belonged to one ethnic group, which, in our opinion, strengthens the results. On the other hand, it is also important to stress that the diagnosis of NAFLD was based on ultrasonography and was not confirmed by liver biopsy. However, studies suggest that liver biopsy is seldom necessary to diagnose NAFLD [30, 31].

REFERENCES

1. *Seela Ramesh, Arun J. Sanyal. Evaluation and management of non-alcoholic steatohepatitis. Hepatology 2005; 42: Suppl S2-S12.*
2. *Clark JM, Brancati FL, Diehl AM. The prevalence and aetiology of elevated aminotransferase levels in the United States. Am J Gastroenterol 2003;98:960–7.*
3. *Bedogni G, Miglioli L, Masutti F, et al. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos Nutrition and Liver Study. Hepatology 2005;42:44–52.*
4. *Adams LA, Angulo P. Treatment of non-alcoholic fatty liver disease. Postgraduate Medical Journal 2006; 82; 315– 322.*
5. *Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346: 1221-31.*
6. *Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in nonalcoholic fatty liver disease: causal effect or epiphenomenon? Diabetologia 2008; 51: 1947 – 53.*
7. *Marchesini G, Moscatiello S, Di Domizio S, Forlani G. Obesity-associated liver disease. J Clin Endocrinol Metab 2008; 93: Suppl 1: S74 – S80.*

8. *20. de Alwis, Day CP. Non-alcoholic fatty liver disease: the mist gradually clears. J Hepatol 2008; 48: Suppl 1: S104 – S112.*
9. *Agarwal AK, Jain V, Singla S et al. Prevalence of non-alcoholic fatty liver disease and its correlation with coronary risk factors in patients with type 2 diabetes. JAPI, 2011; 59: 1 – 4.*
10. *Ghamar-Chehreh ME et al. Predictive value of having positive family history of cardiovascular disorders, diabetes mellitus, dyslipidemia, and hypertension in non-alcoholic fatty liver disease patients. Acta Medica Iranica, 2013; 51(5): 307 – 313.*
11. *Targher G, Mains F, Marchesini (3. lncreased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? Diabetologia 2008; 51(11): 1947-53.*
12. *Targher G. Bertolini L, Padovani R, Rodella S. Tessari R, Zenari L, Day C, Arcaro G. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care 2007; 30(5): 1212-18*
13. *Targher G, Bertolini L, Rodella S, Lippi G, Franchini M, Zoppini G, Muggeo M, Day CP. NASH predicts plasma inflammatory biomarkers independently of visceral fat in men. Obesity 2008;16(6): 1394-9.*
14. *Haque M, Sanyal AJ. The metabolic abnormalities associated with non-alcoholic fatty liver disease. Best pract Res Clin Gastroenterol 2002; 16: 709 – 31.*
15. *Neuschwander- Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summery of an NAFLD single topic conference. Hepatology 2003; 37: 1202 – 19.*
16. *Leon A. Adams, James F. Lymp, Jenny St. Sauver, Schuyler O. Sanderson, Keith D. Lindor, Ariel Feldstein and Paul Angulo. The natural history of Nonalcoholic Fatty Liver Disease: A Population-Based Cohort Study. Gastroenterology 2005; 129: 113-21.*
17. *Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346: 1221-31.*
18. *Sorbi D, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. Am J Gastroenterol 1999; 94(4): 1018-1022.*
19. *Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, et al. Insulin resistance-associated hepatic iron overload. Gastroenterology 1999; 117(5): 1155-63.*
20. *Ramesh S, Sanyl AJ. Evaluation and management of non-alcoholic steatohepatitis. Hepatology 2005;42: Suppl S2-12.*
21. *Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 2002; 123: 745 – 750.*
22. *WHO (2000): The Asia Pacific Perspective : Redefining Obesity Genes : World Health Organization*
23. *Agrawal R, Mishra S, Dixit VK, Rai S. Association of non – alcoholic fatty liver disorder with obesity. Indian J Prev Soc Med 2009; 40: 126 – 129*
24. *Behl Nitin, clinical pathological and radiogogical evaluation of non alcoholic fatty liver disease 2005.*
25. *Charlton M, Sreekumar R, Rasmussen D, Lindor K, Nair KS: Apolipoprotein synthesis in non-alcoholic steatohepatitis. Hepatology, 2002; 35: 898 – 904.*
26. *Musso G, Gambino R, De Michieli F, Cassader M, Durazz M, Faga E, Silli B, Pagano G: Dietary habits and their relations to insulin resistance and post-prandial lipemia in nonalcoholic steatohepatitis. Hepatology, 2003; 37: 909 – 916.*
27. *Duseja A, Das R, Nanda M, Das A et al. Non alcoholic steatohepatitis in Asian Indians in neither associated with iron overload with HF|E gene mutations. World J Gastroentero. 2005; 11(3): 393 -395.*
28. *Yu AS, Keeffe EB. Elevated AST or ALT to nonalcoholic fatty liver disease: accurate predictor of disease prevalence? Am J Gastroenterol, 2003; 98: 955 – 956.*
29. *Ruhl CE, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. Gastroenterology, 2003; 124: 71 – 79*

30. *Adams LA, Talwalkar JA. Diagnostic evaluation of nonalcoholic fatty liver disease. J Clin Gastroenterol 2006;40:34-8.*

31. *Caturelli E, Rapaccini GL, Sabelli C, et al. Ultrasonography and echo-guided fine needle biopsy in the diagnosis of focal fatty liver change. Hepatogastroenterology 1987;34:137.*