# Factors Associated with Prognosis of Non-Alcoholic Fatty Liver Disease

**Abstract**:

**Background:**At the junction between obesity, metabolic syndrome and liver failure, lies Non-alcoholic fatty liver disease. Recent studies elaborated on role of metformin in patients with non-alcoholic fatty liver disease. This observation has not been studied at a global scale, neither it was investigated in different ethnical groups.

**Objectives:** We aim at determining the risk factors associated with prognosis of non-alcoholic fatty liver disease among a cohort of patients in Southern West Bank, Palestine.

**Methods:**A retrospective cohort study involving 300 NAFLD patients who visited the internal medicine department at Hebron Governmental Hospital fromOctober 2017 till September 2018. Two hundred and three patientsdiagnosed with non-alcoholic fatty liver disease, were included in this study. Lab test results within the past 6 months, comorbidity and medication history were collected from patients` profiles. Data was analyzed using SPSS V20. Liver Fibrosis score was determined by using non-alcoholic fatty liver diseasefibrosis score calculator.

**Results:**Two hundred and three non-alcoholic fatty liver disease patients (58.6% females), 54.78 (±12.27 ) years old were included in the study. Almost 65.5% of these patients have BMI>30 Kg/$m^2$. It was found that, 62.25% of the 58 diabetic patients in this study had liver fibrosis score >0.676 comparing to non-alcoholic fatty liver disease patients who are non-diabetic. There was a significant relationship between diabetes and fibrosis score, $\alpha$=0.000. There was also a significant relationship betweenhyperlipidemia and fibrosis score of non-alcoholic fatty liver disease patients, $\alpha$=0.023. We found a significant relationship between fibrosis score and hypertension, $\alpha$=0.000. In the same context, there was a significant relationship between NAFLD patients who were on statin therapy and those who were not using statin therapy, $\alpha$= 0.015. Metformin was not associated with significant relationship between users and non-users non-alcoholic fatty liver disease subjects.

**Conclusion**: Diabetes mellitus, hypertension, hyperlipidemia and statin use were associated with NAFLD prognosis.

**Key words**: Liver fibrosis score, hypertension, dyslipidemia, diabetes mellitus, Metformin, non-alcoholic fatty liver disease.

**Introduction:**

Non-alcoholic fatty liver disease (NAFLD) is considered as the first cause of end- stage liver disease in Western countries [1]. It occurs as a consequence of the accumulation of fat in hepatocytes without significant alcohol consumption. The American Association for the Study of Liver Diseases in 2018 defined NAFLD as the presence of 5% hepatic steatosis without evidence of hepatocellular injury in the form of hepatocyte ballooning [2].

The prevalence of NAFLD worldwide is thought to be on the rise over the next 20 years [3]. Global prevalence of NAFLD disease varying between 20-50% [4-7]. NAFLD disease was recorded the highest prevalence in the Middle East and South America (31.79% and 30.45%), respectively, while the lowest attribution was reported in Africa (13.48)[8]. Ultrasonography survey in the Mediterranean region indicated that the prevalence of NAFLD was 36.8% in men and 25.7% in women [9]. A study on general population in 2006 indicated that the prevalence of NAFLD in Israel was 30% [10].

The pathogenesis of NAFLD is associated with a variety of complex and multifactorial pathological conditions known as Metabolic Syndrome[11, 12]. Insulin resistance plays a central role in the development and progression of NAFLD [13, 11]. Insulin resistance cause hyperinsulinemia which results in the development of steatosis, hepatic denovolipogenesis, and increased adipose tissue lipolysis. This leads to rising in the level of free fatty acids and consequently increased fatty acids in the liver [11, 13, and 14]. Once in the liver, free fatty acids causes a chronic low-grade inflammation.This will provoke in turn an inflammatory response mediated by immune cells, chemical mediators and adipocytes leading to disease progression and liver damage[15].

Age, gender, obesity, body mass index (BMI), disease state and other concomitant diseases such as Diabetes Miletus, hypertension, dyslipidemia are risk factors contribute to the development and/ or progression of NAFLD.[16-31]. These factors affect to various degrees the prognosis of the disease.

Life style of patients such as smoking or stagnant life style, affect NAFLD prognosis too. Many studies considered moving time versus setting time as risk factors in developing and progression of the disease[35-42].

Controversial reports were found about role of various Anti-hyperglycemic medications (metformin, insulin, sulfonylureas) in NAFLD. In addition to that , antihypertensive and lipid lowering agents (statins) play major role in NAFLD[43-78].

Statins are the most widely used lipid lowering agents in dyslipedimia [75]. They reduce cholesterol levels by inhibiting HMG-CoA reductase enzyme. [76]. According to dose and type, they lower LDL cholesterol by 20 to 60%, triglycerides by 10 to 33% and increase HDL cholesterol by 5 to 10% by average [77]. It is approved to decrease liver fibrosis in NAFLD patients where it is effective and safe option [78]. Atorvastatin has been played a positive role in delaying lipid deposition in patients with NAFLD, but the overall effect is limited [79]. Current international guidelines are not recommending statins for the management of NAFLDs patients and suggest that more biopsy-proven benefits are mandatory from large randomized trials[80].

In this study we are going to evaluate for the first time in Palestine the factors associated with NAFLD prognosis and risk factors for developing terminal liver injury while looking for positive factors that might improve it.

**Methods:**

A retrospective cohort study involving all NAFLD patients who visited the internal medicine department at Hebron Governmental Hospital between October 2017 and September 2018 was done. We reviewed 3000 patients` electronic and/or paper-based profiles during that period. A face to face or telephone-based interview with the patient or his/her caregiver was done when necessary in order to get precise information or missing information from profile. Only 203 NAFLD patients were included in the study who have their laboratorytest results for ALT, AST, IGF, platelet count, and Albumindone within the past 6 months. SPSS version 20 was used to analyze the data. NAFLD fibrosis score was determined using NAFLD fibrosis score calculator by Angulo P. et. al, available on line.

**Results:**

As shown in table (1) below, 203 patients were included in the study, (58.6% females), age (54.78 ± 12.27 years old). Most of them, (50.2%), were in the age group of 40-59 years and 76.8 % of them were non-smokers. Majority of patients were living in villages, (59.6%)

In fact, 65.5% of subjects were obese, (BMI >30 Kg/m2). For daily activities, 39.9% of patients have a sitting time > 7 hours per day while 37.4% had moving time from 1-3 hours. For meals; 54.7 % of them had 2 meals per day while 34.5% had more than 3 meals per day.

In addition to NAFLD, we found that 119 subjects suffered from various comorbidities. Fibrosis score as main outcome of the study was calculated for all patients and they were categorized accordingly as shown in table (1) below.

As shown in table (2) below, there was a significant difference in fibrosis score between NAFLD patients who have diabetes and NAFLD patients without diabetic, $\alpha = 0.000$. There was no significant difference between the 2 groups according to years of diabetes, $\alpha = 0.167$.

Dyslipidemia was a major factor in prognosis of NAFLD. We found a significant difference between patients who have hyperlipidemia and who had not, $\alpha = 0.023$.

There wasalso a significant difference in fibrosis score of different patients` categories and hypertension, $\alpha = 0.000$

We also studied the effects of medications on NAFLD prognosis and fibrosis score. As shown in table (3) below, there was no significant difference between patients categories and the following independent factors; metformin, insulin or sulfonylurea use, $\alpha$ values were 0.975, 0.706 and 0.393 respectively.

Regarding anti-hypertensive agents; there was no significant difference between using antihypertensive agents; Beta-blocker, ACEI or CCB and fibrosis score, $\alpha$values were0.413, 0.182, and 0.304 respectively.

Concerning use of anti-hyperlipidemic agents, there was a significant difference between patients categories and anti-hyperlipidemic agents (statins), $\alpha = 0.015$

**Table (1): Socio-demographic characteristics of the NAFLDs patients (n=203)**

| Variables and its categories | | Frequency (n) | Percentage (%) |
|---|---|---|---|
| Residency | City | 80 | 39.4 |
| | Village | 121 | 59.6 |
| | Camp | 2 | 1 |
| Gender | Male | 84 | 41.4 |
| | Female | 119 | 58.6 |
| Age | 20-39 years | 25 | 12.3 |
| | 40-59 years | 102 | 50.2 |
| | ≥ 60 years | 76 | 37.4 |
| | Minimum | 20 | |
| | Maximum | 87 | |
| | Mean | 54.78 | |
| | Standard deviation | 12.27 | |
| BMI | 18.5-24.4 $Kg/m^2$ | 20 | 9.9 |
| | 24.5-30 $Kg/m^2$ | 50 | 24.6 |
| | More than 30 $Kg/m^2$ | 133 | 65.5 |
| Are you smoker | Yes | 42 | 20.7 |
| | No | 156 | 76.8 |
| | Missing | 5 | 2.5 |
| Sitting time | 1-3 hours | 84 | 27.6 |
| | 4-7 hours | 65 | 32.0 |
| | More than 7 hours | 81 | 39.9 |
| | Missing | 1 | 0.5 |
| Moving time | 1-3 hours | 76 | 37.4 |
| | 4-7 hours | 70 | 34.5 |
| | More than 7 hours | 56 | 27.6 |
| | Missing | 1 | 0.5 |
| Number of meals | 1 | 20 | 9.9 |
| | 2 | 111 | 54.7 |
| | ≥ 3 | 70 | 34.5 |
| | Missing | 2 | 1 |

Abbreviation: BMI, body mass index

*significance at $\alpha \leq 0.05$

| Variables and its categories | Fibrosis score | Total | P-value |
|---|---|---|---|

**Table (2): Diseases account for the development of NAFLD (n=119)**

| | | | more than 0.676 | -1.455_0.676 | less than -1.455 | | |
|---|---|---|---|---|---|---|---|
| Are you diabetic? | Yes | Count | 51 | 7 | 0 | 58 | 0.000* |
| | | % within fibrosis score | 62.2% | 21.2% | 0.0% | 48.7% | |
| | | % of Total | 42.9% | 5.9% | 0.0% | 48.7% | |
| | No | Count | 31 | 26 | 4 | 61 | |
| | | % within fibrosis score | 37.8% | 78.8% | 100.0% | 51.3% | |
| | | % of Total | 26.1% | 21.8% | 3.4% | 51.3% | |
| Number of years of diabetes | ≤ 2 years | Count | 13 | 5 | 0 | 18 | 0.167 |
| | | % within fibrosis score | 28.9% | 71.4% | 0.0% | 34.6% | |
| | | % of Total | 25.0% | 9.6% | 0.0% | 34.6% | |
| | 3-5 years | Count | 13 | 1 | 0 | 14 | |
| | | % within fibrosis score | 28.9% | 14.3% | 0.0% | 26.9% | |
| | | % of Total | 25.0% | 1.9% | 0.0% | 26.9% | |
| | 6-10 years | Count | 8 | 0 | 0 | 8 | |
| | | % within fibrosis score | 17.8% | 0.0% | 0.0% | 15.4% | |
| | | % of Total | 15.4% | 0.0% | 0.0% | 15.4% | |
| | 10> years | Count | 11 | 1 | 0 | 12 | |
| | | % within fibrosis score | 24.4% | 14.3% | 0.0% | 23.1% | |
| | | % of Total | 21.2% | 1.9% | 0.0% | 23.1% | |
| Do you have hyperlipidemia? | Yes | Count | 58 | 16 | 2 | 76 | 0.023 |
| | | % within fibrosis score | 71.6% | 48.5% | 50.0% | 64.4% | |
| | | % of Total | 49.2% | 13.6% | 1.7% | 64.4% | |
| | No | Count | 23 | 17 | 2 | 42 | |
| | | % within fibrosis score | 28.4% | 51.5% | 50.0% | 35.6% | |
| | | % of Total | 19.5% | 14.4% | 1.7% | 35.6% | |
| Number of years of dyslipidemia | ≤ 2 years | Count | 20 | 7 | 2 | 29 | 0.507 |
| | | % within fibrosis score | 40.0% | 46.7% | 100.0% | 43.3% | |
| | | % of Total | 29.9% | 10.4% | 3.0% | 43.3% | |
| | 3-5 years | Count | 15 | 5 | 0 | 20 | |
| | | % within fibrosis score | 30.0% | 33.3% | 0.0% | 29.9% | |
| | | % of Total | 22.4% | 7.5% | 0.0% | 29.9% | |
| | 6-10 years | Count | 13 | 2 | 0 | 15 | |
| | | % within fibrosis score | 26.0% | 13.3% | 0.0% | 22.4% | |

*Table 2 continued.*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | % of Total | 19.4% | 3.0% | 0.0% | 22.4% | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 10> years | Count | 2 | 1 | 0 | 3 | |
| | | % within fibrosis score | 4.0% | 6.7% | 0.0% | 4.5% | |
| | | % of Total | 3.0% | 1.5% | 0.0% | 4.5% | |
| **Variable and its categories** | | | **Fibrosis score** | | | | **P-value (sig)** |
| Are you hypertensive? | Yes | Count | 54 | 10 | 0 | 64 | 0.000* |
| | | % within fibrosis score | 65.9% | 30.3% | 0.0% | 35.8% | |
| | | % of Total | 45.4% | 8.4% | 0.0% | 35.8% | |
| | No | Count | 28 | 23 | 4 | 55 | |
| | | % within fibrosis score | 43.1% | 69.7% | 100.0% | 46.2% | |
| | | % of Total | 23.5% | 19.3% | 3.4% | 46.2% | |

*significance at $\alpha \leq 0.05$

**Table 3: Role of hypoglycemic , antihypertensive and lipid lowering agents in NAFLD management (n=119)**

| | | | more than 0.676 | -1.455_0.676 | Less than -1.455 | |
|---|---|---|---|---|---|---|
| **Diabetes mellitus medications:** | | | | | | |
| **Do you take Metformin.** | Yes | Count | 36 | 5 | 0 | .975 |
| | | % within fibrosis score | 72.0% | 71.4% | 0.0% | |
| | | % of Total | 63.2% | 8.8% | 0.0% | |
| | No | Count | 28.0% | 28.6% | 0 | |
| | | % within fibrosis score | 24.6% | 3.5% | 0.0% | |
| | | % of Total | 50 | 7 | 0.0% | |
| **Do you take Insulin.** | Yes | Count | 18 | 2 | 0 | .706 |
| | | % within fibrosis score | 36.0% | 28.6% | 0.0% | |
| | | % of Total | 31.6% | 3.5% | 0.0% | |
| | No | Count | 32 | 5 | 0 | |
| | | % within fibrosis score | 64.0% | 71.4% | 0.0% | |
| | | % of Total | 56.1% | 8.8% | 0.0% | |
| **Do you take Sulfonylurea** | Yes | Count | 23 | 2 | 0 | .393 |
| | | % within fibrosis score | 46.0% | 28.6% | 0.0% | |
| | | % of Total | 40.4% | 3.5% | 0.0% | |
| | No | Count | 27 | 5 | 0 | |
| | | % within fibrosis score | 54.0% | 71.4% | 0.0% | |
| | | % of Total | 47.4% | 8.8% | 0.0% | |
| **Hypertension medications:** | | | | | | |
| **Do you take Beta blockers** | Yes | Count | 34 | 4 | 1 | .413 |
| | | % within fibrosis score | 60.7% | 36.4% | 100.0% | |
| | | % of Total | 50.0% | 5.9% | 1.5% | |
| | No | Count | 22 | 7 | 0 | |
| | | % within fibrosis score | 39.3% | 63.6% | 0.0% | |
| | | % of Total | 32.4% | 10.3% | 0.0% | |
| **Do you take ACEIs** | Yes | Count | 25 | 3 | 0 | .182 |
| | | % within fibrosis score | 44.6% | 27.3% | 0.0% | |
| | | % of Total | 36.8% | 4.4% | 0.0% | |
| | No | Count | 31 | 8 | 1 | |
| | | % within fibrosis score | 55.4% | 72.7% | 100.0% | |
| | | % of Total | 45.6% | 11.8% | 1.5% | |
| | | ***Table 3 continued.*** | | | | |
| **Do you take** | Yes | Count | 22 | 3 | 0 | .304 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **CCBs** | | % within fibrosis score | 39.3% | 27.3% | 0.0% | |
| | | % of Total | 32.4% | 4.4% | 0.0% | |
| | No | Count | 34 | 8 | 1 | |
| | | % within fibrosis score | 60.7% | 72.7% | 100.0% | |
| | | % of Total | 50.0% | 11.8% | 1.5% | |
| **Dyslipidemia medications:** | | | | | | |
| **Do you take Atorvastatin** | Yes | Count | 51 | 10 | 1 | .015* |
| | | % within fibrosis score | 86.4% | 62.5% | 50.0% | |
| | No | Count | 8 | 6 | 1 | |
| | | % within fibrosis score | 13.6% | 37.5% | 50.0% | |

Abbreviations: ACEIs, angiotensin-converting-enzyme inhibitors; CCBs, Calcium channel blockers.
*significance at $\alpha \leq 0.05$

**Discussion:**

Insulin resistance and adipose tissue dysfunction which occur as a result of imbalance of adipokines (such as leptin and adiponectin) secretion [27], are the main contributing factors relate obesity to NAFLD rather than fat accumulation [28]. J. M. Clark et.al, (2002) reported that NAFLD occur in 30% of obese men and 40% of obese women [29].

Our results come in agreement with the previous report by J.M Clark where obesity was highly prevalent among our patients, 65.5% of our patients have BMI >30 Kg/m2.

Twenty patients were on insulin and 37 were not using insulin. There was no significant different between the 2 groups in termsof fibrosis score which implies insulin resistance in both categories (resistance to internally produced insulin or exogenously introduced insulin that lead to obesity which complicate NAFLD and increased fibrosis core in both).

Lifestyle modification consisting of diet, exercise, and weight loss has been advocated to treat patients with NAFLD in all guidelines[31].

Sedentary behavior can be defined as a state of prolong sitting, laying down, consuming very small amounts of energy in which the muscles are inactive (low-intensity exercises or movement) [35,36].

Sedentary behavior will increase in people who have a metabolic syndrome, excessive adiposity, cardiovascular disease and type 2 diabetes mellitus [37,38]. Sedentary time of NAFLD patient is nearly half an hour extra than healthy people[37]. We found that 39.9 % of subjects in our study have a sitting time more than 7 hours per day which was reflected on their high BMI and dyslipidemia.However this wasn`t shown to be significantly associated with NAFLD. This may be due to the fact that sitting time is not a direct risk factor for NAFLD. On the other hand, sedentary life style leads to obesity and dyslipidemia which in turn lead to prognoses of NAFLD.

The incidence of NAFLD is positively associated with the increase in sitting time independent of physical activity and exercises [39 and 40]. A study done in 2016 in China was reported that the prevalence of NAFLD depends on the sitting time and it will be higher in people with a sitting time

of7.1hours/day and longer [39]. Another study reported that the moderate or intense exercises have significant benefit for NAFLD patients [41]. The reduction in the physical activity have documented especially in NAFLD patient who also suffered from diabetes [42].
NAFLD has a close association with obesity, mainly visceral obesity. It is considered as the dominant risk factor for NAFLD occurrence [19, 25]. It is major leading cause of cardiovascular diseases[26].
In our study we have found that, 67 patients have dyslipidemia and 62 of them were on statins (atorvastatin). This was not associated with improvement of their overall NAFLD or dyslipidemia, rather they have worse condition comparing to other patients in this study whether they were using statins or not. This was clear as they have high fibrosis score. This might be explained in 2-ways; Patients are hesitant to use statins in our community due high cost, side effects or believes they are nt working for their condition.
On the other hand, we found that, patients in this studyuse statins at a late stage of their dyslipidemia or atherosclerosis. They were not using stains for reasons or doses related to NAFLD neither did they use them for sufficient time in order to be able to judge on its their efficacy.

Dyslipidemia is a critical comorbidity that is observed in NAFLD patients [66]. It is atherogenic in nature [67]. It is characterized by high triglyceride (TG) and LDL levels and low HDL levels [68]. This atherogenic abnormality increases risk of cardiovascular diseases [69]. The specific mechanism in which hyperlipidemia increases the risk of NAFLD is unclear. It may be associated with increased accumulation of lipids in liver cells[70] as a result of lipid metabolism abnormalities such as increased lipogenesis, ingestion of fatty foods and increase synthesis of very low density lipoprotein (VLDL) in addition to decreased oxidation of free fatty acid[71]
Our study was first study to predict the relationship between hypertension and NAFLD. One common factor for three conditions (dyslipidemia, hypertension and NAFLD) is obesity and or/ increased level of lipids in the body. Atherosclerosis is a major risk factor for hypertension among patients in our study. Hence come the indirect relationship between NAFLD and dyslipidemia that lead to atherosclerosis which lead to hypertension.
Our results came even more controversial where patient who took statins had worse score of NAFLD fibrosis comparing to those who didn't take it, as explained above.

**Conclusion:**Tight control of hypertension and dyslipidemia are mandatory among all NAFLD patients.Lifestyle modifications such as low fat and carb diets and exercise, are important measurement to fight obesity, hypertension and diabetes hence they will improve fibrosis in NAFLD patients. studies about use of stains, their dose, timing and adherence are mandatory to judge on statins benefit for NAFLD.

**Ethics approval:** this was a multistage process where we have to take permission from the IRC of our University to run the research. Then we took the permission of ministry of health in Ramallah and the district health office in Hebron to run the study in their hospital and to collect data from patients.
We prepared a consent form where we have to take consent from each patient or his guardian to participate in the research and to share information with us for the purpose of research only. We guarantee the confidentiality of his/her personal information. Participants and/or their care giver, or guardian had to sign the consent form on top of the interview-based questionnaire.

**Acknowledgement:** We would like to thank all staff members at Hebron Gov. Hospital for their cooperation with our team and providing all necessary logistic support.

**Funding:** We didn`t receive any fund for this study from anyone.

**Conflicts of Interest:** We declare no conflict of interest for this research.

**Abbreviations:** ACEIs: angiotensin-converting-enzyme inhibitors, CCBs: Calcium channel blockers, BMI: Body Mass Index.

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