

Different aspects of immunological profile in patients with Non-Alcoholic Fatty liver disease

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Abstract

Background: NAFLD is thought to affect approximately one-fourth of the world's population. Therefore, we evaluated the role of serum complement and immunoglobulins in the NAFLD pathogenesis.

Patients and methods: 200 participants were used in this study, divided into two groups; Group I: 100 NAFLD patients and Group II: 100 healthy volunteers. The diagnosis of NAFLD is based on non-invasive methods, following the EASL guideline 2022. IgG, IgM, IgA, C3, and C4 assays were performed on all participants.

Results: When the immunological profiles of patients with NAFLD and healthy controls were compared, it was found that the mean IgA in NAFLD patients was (4.20±5.07), whereas the mean IgA in healthy controls was (2.22±1.05) (P=0.000). Additionally, a significant increase in IgG was found in NAFLD patients (17.08±3.87) compared with healthy controls (11.59±3.34), with a P value of (p<0.001). complement C3 and complement C4 levels significantly increased in nonalcoholic fatty liver disease patients (1.28± 0.61 and 0.40 ± 0.19, respectively), compared to healthy controls (0.90 ±0.27 and 0.30 ±0.12, respectively), with a significant P value (p<0.001 for each).

Conclusions: Elevated IgA, IgG, C3 and C4 exist in patients with NAFLD and could be associated with fatty liver development and progression of hepatic fibrosis in patients with NAFLD. (*Acta gastroenterol. belg.*, 2024, 87, 274-281).

Keywords: IgG, IgM, IgA, C3, C4, NAFLD, fibrosis.

Introduction

With a nearly 30% prevalence worldwide, non-alcoholic fatty liver disease (NAFLD) is a major public health concern (1).

NAFLD has a spectrum of liver lesions that includes simple steatosis, steatohepatitis, fibrosis cirrhosis and eventually hepatocellular carcinoma (2).

Nonalcoholic steatohepatitis (NASH) is a combination of liver steatosis, inflammation and a different degree of fibrosis; it occurs in nearly 25% of patients with NAFLD and can lead end- stage liver disease (3).

The complex inflammatory process associated with NASH is largely mediated by the innate immune system, which also contributes to the development of the liver fibrosis that may ultimately necessitate liver transplantation (4-6).

Liver has long been regarded as a central immunological organ and vigorously participates in the maintenance of immune homeostasis (7).

When immune homeostasis is lost related to different liver diseases including NAFLD, both innate and adaptive immunity contribute to the onset and progression of liver diseases (8-9).

The pathogenesis of NAFLD remains unknown. Obesity is a suspected risk factor, related to adipose tissue inflammation which induces the production of proinflammatory cytokines, leading to obesity associated low grade chronic inflammation (10,11).

Moreover, adaptive immunity may have an impact on NASH-related hepatic inflammation (12,13).

As a component of adaptive immunity, immunoglobulins mirror the roles played by B cells (18). B cells induce insulin resistance and liberation of proinflammatory cytokines, which are responsible for the pathogenesis of NAFLD. So, serum immunoglobulins could be responsible for the pathogenesis of NAFLD (14,15).

In NAFLD patients, the complement system may be triggered by bacterial endotoxin, free fatty acids, cholesterol, and other body mediators (16).

Three distinct paths have been identified for the activation of the complement system, which is an essential part of innate immunity: the classical, lectin, and alternative pathways. Fraction C3 is produced because of all these processes. The conventional activation pathway involves the binding of antigen to C-reactive protein or immunoglobulin (IgM or IgG) molecules (17,18). Experimentally complement C4 activation involved in the development of hepatic fibrosis (19).

Few previous studies assessed the role of serum complement and immunoglobulins in patients with NAFLD. Therefore, we conducted a cross-sectional study to evaluate if serum complement and immunoglobulins changed in NAFLD patients compared to healthy patients.

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Materials and methods

This cross-sectional case-control study was conducted on 200 participants divided into two equal groups; Group I: consists of 100 adult patients with NAFLD who attend the outpatient's clinics of Tropical Medicine and Gastroenterology Department, Internal Medicine Department, Qena Faculty of Medicine, South Valley University and Internal Medicine Department, Sohag Faculty of Medicine, Sohag University between December 1, 2021, and September 30, 2022. Group II: 100 healthy participants.

Each participant underwent a thorough history taking and clinical examination with strict history taking on diabetes mellitus, insulin resistance, hypertension, hypothyroidism, dyslipidemia, polycystic ovary syndrome and obstructive sleep apnea.

Patients with viral hepatitis, autoimmune liver disease, hemochromatosis, Wilson disease, patients on steroid therapy or any medication that induce liver disease, alcohol intake ≥ 30 g/day for males or ≥ 20 g/day for females, and patients with any liver diseases other than NAFLD were excluded from the study.

It's known that liver biopsy is the most reliable method for diagnosis of liver steatosis and fibrosis in patients with NAFLD, but due to procedure-induced morbidity and mortality; liver biopsy is limited to some situations. So, the diagnosis of NAFLD was based on noninvasive methods following the European Association for the Study of the Liver guideline (EASL) (20).

Investigatory Workup

1. Fasting insulin ($\mu\text{IU/mL}$): measured using automated enzyme immunoassay system (TOSOH) (21).
2. Indices calculation: The following scores were calculated:

Fibrosis-4 score (FIB-4)

Age (years) $\times$ AST [U/L] / (platelets [$10^9/\text{L}$] $\times$ (ALT [U/L])^{1/2}) (22).

(Patients with score <1.45 are unlikely, whereas patients with score >3.25 are likely to have advanced fibrosis) (23).

AST to Platelet Ratio Index (APRI score)

$[(\text{AST}/\text{upper limit of the normal AST range}) \times 100] / \text{Platelet Count}$ (24).

1. Biochemical assays:

Blood sample obtained from all patients and controls to investigate complete blood count, blood urea, serum creatinine, ALT, AST, albumin, Alkaline phosphatase, bilirubin, prothrombin time, prothrombin concentration, INR, hepatitis B surface antigen and HCV antibody.

Immunoglobulin (IgG, IgM, IgA) and complement (C3&C4) assay: using Indiko Clinical Chemistry Analyzer.

2. Statistical analysis

Data entry and data analysis were done using SPSS version 19 (Statistical Package for Social Science). Data were presented as a number, percentage, the mean and standard deviation for parametric data. Chi-square test and Fisher exact test were used to compare qualitative variables. Independent T test used to compare between two quantitative variables for parametric data. We perform test for normality and the data was nonparametric. P-value was considered statistically significant when <0.05 .

3. Ethical approval:

The study was carried out in compliance with the Helsinki recommendations. This study, with the code SVU-MED-GIT023-4-22-9-448, was authorized by the Institutional Review Board of the Qena Faculty of Medicine in Egypt. All subjects provided informed consent to participate.

Results

Demographic data of studied participants

Total of 200 subjects were included in this case control study, they were divided into 100 patients with fatty liver (group I) and 100 healthy controls (group II). The mean age of patients with NAFLD was 46.38 ± 14.36 year. 82 (82%) women and 18 (18%) men.

No statistically significant difference between NAFLD and healthy volunteer's groups regarding age and sex.

12 (12%) NAFLD patients had hypertension, 10 (10%) had type II DM, and 3 (3%) were smokers, while none of the healthy controls had any of these conditions (Table 1).

Laboratory data of studied groups

Among the patients with NAFLD compared with healthy controls, different abnormalities in serum biochemical parameters were observed including, liver function tests abnormalities in the form of significant raised ALT, AST, total bilirubin, and GGT. Also, complete blood count abnormalities in the form of significant decreased Hemoglobin level and significant increase total white blood cells, Neutrophils count and percentage with significant decrease in lymphocytic count and percentage. At the same time, a significant increase in serum ferritin observed in patients' group compared with controls ($p < 0.001$).

Patients with NAFLD showed significant increase in fasting serum insulin and random blood sugar 20.84 ± 27.06 and 134.86 ± 50.43 respectively, compared

Table 1. — Demographic data of studied group

Variables	NAFLD group (N= 100)	Healthy controls (N=100)	P. value
Age (years)	46.38±14.36	45.51±14.35	0.67
Sex (N%) Male Female	18 (18%) 82 (82%)	19 (19%) 81 (81%)	0.86
Weight (Mean ± SD)	72.405±19.979	60.504±10.904	< 0.001
BMI (Mean ± SD)	29.03 ±6.74	23.17±3.26	< 0.001
Hypertension (N%)	12 (12%)	----	-----
Diabetes Mellitus (N%)	10 (10%)	-----	-----
Smoking (N%)	3 (3%)	-----	-----
Drinking behavior	None	None	-----

with healthy controls where fasting serum insulin and random blood sugar were 6.94 ± 5.39 and 113.11 ± 27.79 respectively, ($p < 0.001$ each).

Regarding lipids profile in studied group, the mean level of triglyceride was 159.25 ± 87.88 in patients with NAFLD compared with 136.22 ± 60.13 in controls with significant P. value (0.032), and the mean level of VLDL was 33.07 ± 19.87 in patients group compared with 27.25 ± 12.02 in healthy volunteers with significant P. value (0.013). Data are summarized in Table 2.

Different indices used for fibrosis assessment.

For assessment of hepatic fibrosis for our included subjects, different fibroses scores have been used; in patients with NAFLD a significant elevation of APRI (0.26 ± 0.20), FIB-4 (1.06 ± 0.82) indices have been detected in comparison to healthy controls where APRI and FIB-4, 0.19 ± 0.08 and 0.67 ± 0.38 respectively ($p < 0.001$ each) Table 3.

Immunoglobulin and Complement profile among studied participants

Comparing between immunological profile in patients with NAFLD and healthy controls, the mean IgA in patients with nonalcoholic fatty liver disease was 4.20 ± 5.07 , while its mean in healthy controls was 2.22 ± 1.05 ($P < 0.001$), also, significant increase of IgG was detected in patients with nonalcoholic fatty liver disease (17.08 ± 3.87) compared with healthy controls (11.59 ± 3.34) $p < 0.001$.

Both complement C3 and complement C4 showed marked increase in patients with nonalcoholic fatty liver disease (1.28 ± 0.61 and 0.40 ± 0.19 , respectively) comparing with healthy controls (0.90 ± 0.27 and 0.30 ± 0.12 , respectively) with significant P. value ($p < 0.001$, each). Detailed data in Table 4.

Discussion

The development of non-alcoholic fatty liver disease, a chronic liver disease that affects both adults and children, is a complicated multifactorial process that are worldwide linked to overweight and obesity (25).

The progression of this condition starts with steatosis and progresses to nonalcoholic steatohepatitis, cirrhosis, and liver failure. it is the second factor leading to liver transplantation in the United States (26).

We attempt to assess immunoglobulins and complements levels in patients with varying degrees of hepatic fibrosis and steatosis because, as far as we are aware, this study is the first one in Egypt and only a few studies have been conducted globally to assess immunoglobulins and complements profiles in patients with NAFLD.

In our study, a positive relationship was detected between high body mass index and the development and severity of steatosis and fibrosis.

According to Razmpour et al. 2023 research, body mass index is one of the key factors that influence the development of fatty liver disease (27).

In this study, the serum Hb concentration increased in patients with NAFLD compared with healthy controls.

It is confirmed previously by Eva Juárez-Hernández et al 2018 and Goh Eun et al. 2017 in their studies. It is suggested that, increased hemoglobin had a protective role in the presence of steatosis (28-29).

A high WBC count is a sign of low-grade inflammation. NAFLD has been linked to elevated WBC counts, even when they are within the normal range (30).

The most prevalent WBC subtype in the human circulatory system is the neutrophil and among the immune cells; neutrophils is the first cells that respond to inflammation. Recent research has shown that neutrophils are crucial in the development and maintenance of chronic inflammation in nonalcoholic fatty liver disease patients. They do this by assisting macrophages in their engagement with antigen-presenting cells (31).

Table 2. — Laboratory data of studied group

Variables	NAFLD group (N= 100)	Healthy controls (N=100)	P. value
Hgb (g/dl) (Mean ± SD)	13.07±1.81	12.15±2.14	<0.001
Platelets (10 ³ /mm ³) (Mean ± SD)	287.1±97.01	286.35±70.27	0.975
WBC (x10 ³ /mm ³) (Mean ± SD)	7.88±2.94	7.04±2.79	0.04
Neutrophils (x10 ³ /mm ³) (Mean ± SD)	4.81±2.74	3.66±2.23	<0.001
Neutrophils (%) (Mean ± SD)	59.20±15.72	49.53±13.26	<0.001
Lymphocytes (x10 ³ /mm ³) (Mean ± SD)	2.45±1.02	2.11 ±1.06	0.02
Lymphocytes (%) (Mean ± SD)	38.14±12.28	29.10±13.25	<0.001
Monocytes (x10 ³ /mm ³) (Mean ± SD)	0.70±0.94	0.57± 0.27	0.172
Monocytes (%) (Mean ± SD)	8.17±3.24	8.56±3.10	0.368
Basophil (x10 ³ /mm ³) (Mean ± SD)	0.05±0.10	0.08±0.12	0.03
Basophil (Mean ± SD)	0.67±1.35	1.41±2.33	0.007
Eosinophil (x10 ³ /mm ³) (Mean ± SD)	0.22±0.26	0.16±0.16	0.06
Eosinophil (%) (Mean ± SD)	2.43±2.49	2.20±1.86	0.641
Blood Urea (%) (Mean ± SD)	22.15±15.22	19.85±13.25	0.225
S. creatinine (mg/dl) (Mean ± SD)	1.03±0.27	0.95±0.17	0.013
ALT (IU/L) (Mean ± SD)	21.59±13.54	18.61±5.31	0.042
AST (IU/L) (Mean ± SD)	24.74±13.09	20.37±6.93	0.004
Total bilirubin (mg/dl) (Mean ± SD)	0.84±1.98	0.43±0.29	0.042
Direct bilirubin (mg/dl) (Mean ± SD)	0.33±0.82	0.23±0.24	0.234
Albumin (g/dl) (Mean ± SD)	3.81±0.81	4.09±0.45	0.003
ALP (U/L) (Mean ± SD)	74.74±34.52	72.31±28.76	0.589
GGT (U/L) (Mean ± SD)	120.18±54.93	61.56±64.79	<0.001
Serum Ferritin (Mean ± SD)	111.57±121.09	51.79±60.32	<0.001
Serum C-RP (mg/dl) (Mean ± SD)	7.46±6.82	7.31±5.52	0.863
Fasting serum insulin (mg/dL) (Mean ± SD)	20.84±27.06	6.94±5.39	<0.001
RBG (mg/dL) (Mean±SD)	134.86±50.43	113.11±27.79	<0.001
HBA1C (Mean ± SD)	5.55±1.56	5.50±0.88	0.750
Serum ionized Calcium Ca ⁺ (mmol/l) (Mean ± SD)	1.16±0.098	1.25±0.09	<0.001
Prothrombin time (seconds) (Mean ± SD)	12.28±1.12	11.97±0.66	0.018
Prothrombin Concentration (Mean ± SD)	94.33±10.67	95.35±7.57	0.438
INR (Mean ± SD)	1.04±0.09	1.07±0.09	0.09
Triglycerides (mg/dL) (Mean ± SD)	159.25±87.88	136.22±60.13	0.032
Total cholesterol (mg/dL) (Mean ± SD)	179.92±44.45	178.67±64.16	0.873
HDL (mg/dL) (Mean ± SD)	57.22±45.77	52.28±28.55	0.360
LDL (mg/dL) (Mean ± SD)	86.48±40.69	92.46±49.27	0.349
VLDL (mg/dL) (Mean ± SD)	33.07±19.87	27.25±12.02	0.013

In our study, even though the changes in WBCs were within the normal range, patients with nonalcoholic fatty liver disease had a significantly higher total white blood cell count, neutrophil count, and neutrophil percentage

compared with healthy controls, put in consideration that, the participants varied in terms of age, gender, and smoking status.

Table 3. — Comparison between different fibrosis indices in patients with NAFLD and healthy controls

Variables	NAFLD group (n=100)	Healthy controls (N=100)	P value
APRI (Mean ± SD)	0.26±0.20	0.19±0.08	<0.001
Participants with APRI score less than cut off value (Mean ± SD) (N%)	0.16±.05 68 (68%)	0.13±.02 52 (52%)	<0.001
participants with APRI score more than cut off value (Mean ± SD) (N%)	0.5±.2 32 (32%)	0.3±.07 48 (48%)	<0.001
FIB-4 (Mean ± SD)	1.06±0.82	0.67±0.38	<0.001
participants with FIB-4 score less than cut off value (Mean ± SD) (N%)	0.6±.1 51(51%)	0.4±.1 51(51%)	<0.001
participants with FIB-4 score more than cut off value (Mean ± SD) (N%)	1.6±.4 49 (49%)	0.9±.1 49 (49%)	<0.001

Table 4. — Immunological parameters of studied group

Variables	NAFLD group (N= 100)	Healthy controls (N=100)	P value
IgA (g/l) (Mean ± SD)	4.20±5.07	2.22±1.05	< 0.001
IgM (g/l) (Mean ± SD)	1.82±0.639	1.72±1.002	0.373
IgG (g/l) (Mean ± SD)	17.08±3.87	11.59±3.34	< 0.001
Complement C3 (g/l) (Mean ± SD)	1.28±0.61	0.90± 0.27	< 0.001
Complement C4 (g/l) (Mean ± SD)	0.40±0.19	0.30±0.12	< 0.001

This comes with Zhang et al., 2019 study, where his included NAFLD patients showed raised total white blood cells count and neutrophils compared with non-NAFLD group (32).

Its known that, the liver normally contains different types of lymphocytes. Earlier research demonstrated a link between the malfunction of the lymphocytes and the abnormal serum lipid profile (33).

In addition, massive lymphocyte infiltration exacerbates hepatic inflammation and liver cell damage. Also, lymphocytes are widely distributed in obese adipose tissues and control macrophages' production of inflammatory mediators (34).

Our included NAFLD patients showed significant increase in lymphocyte count and percentage compared with healthy subjects.

In agreement with our results, Zhou et al., 2022 who found a significant increase in lymphocyte count of his included NAFLD patients (35).

On the contrary, Alkhouri et al., 2011 found decrease lymphocytic count in patients with steatohepatitis compared with patients with simple steatosis and considered that advanced hepatic fibrosis was closely related to lymphopenia (36).

This can be explained by the fact that most of our included NAFLD patients had early stages of hepatic fibrosis as evaluated by both APRI and FIB-4 indices.

Peripheral eosinophilia might be seen in conjunction with eosinophilic liver infiltration, which is frequently connected to inflammation with or without necrosis (37).

In this study, patients with NAFLD had a significantly higher peripheral eosinophil count than non-NAFLD participants.

Starling, 2017 found that, eosinophils were present in NASH livers, in addition to, overexpression of eosinophil function-related genes (38).

Recent studies have connected serum creatinine to a number of metabolic disorders, including diabetes, obesity, and dyslipidemia, and they have shown that greater serum creatinine concentrations are linked to higher risks of metabolic syndrome even when serum creatinine level are within the normal range. So, serum creatinine may be capable of anticipating the risk of NAFLD is raised by the interactions between serum creatinine levels and metabolic syndrome (39).

Niu et al., 2022 found in his study that, NAFLD patients had higher serum creatinine than those without NAFLD. Furthermore, Moreover, the levels of serum creatinine were linked with ALT, AST, GGT, and insulin resistance (40) parison to imaging or histology-based research, liver enzyme has low accuracy in the detection of NAFLD and normal range of liver transaminases can be found in people with NAFLD (41).

Although changes in serum levels of ALT, AST, total bilirubin, and albumin were within normal reference ranges, the current study found that patients with NAFLD had significantly altered liver function test parameters when compared to non-NAFLD participants. These changes included elevated ALT, AST, total bilirubin, GGT, INR and decreased serum albumin levels.

This comes in agreement with Sanyal et al., 2015 in his study on large number of patients with NAFLD (42).

Despite the fact that ferritin is an acute phase reactant, its rise in the serum of fatty liver disease patients may be an indication of disease activity. It may also be linked to the amount of hepatic iron in people with NAFLD, indicating that iron metabolism in these people may be abnormal (43).

In this study we found significant increase in the serum ferritin level in patient with NAFLD compared with Non-NAFLD subjects.

Serum ferritin levels were significantly higher in NAFLD patients than in non-NAFLD individuals, according to Modares et al., 2018 study. However, this higher level may not be a valid predictor of the severity of the disease histologically (44).

In this study, we had remarkable increase in random blood sugar, fasting serum insulin, serum triglyceride and VLDL with low HDL in patients with NAFLD compared with Non-NAFLD participants.

Pérez-Mayorga et al. 2022 found raised triglyceride, random blood sugar, HOMA-IR with decreases HDL in patients with NAFLD compared with non-NAFLD group in his study and considered that, triglyceride index and the triglyceride/HDL ratio were accurate clinical tools to identify patients with NAFLD. Also, considered triglyceride index and the triglyceride/HDL ratio as a practical screening tool compared with imaging studies (45).

It takes a complex, heterogeneous mechanism to cause inflammation in NAFLD. The role of the innate and adaptive immune systems in the development of liver cirrhosis, liver failure, and mortality caused by liver disease is poorly understood (46).

Our study showed increase hepatic fibrosis indices (APRI and FIB-4) in patients with fatty liver compared with Non-NAFLD subjects.

Our finding comes with other previous studies with increase hepatic fibrosis indices values with liver disease progression (47-48).

In this study, our patients showed significant elevation of immunoglobulin A, immunoglobulin G, complement C3 and complement C4.

Some previous studies showed decrease IgG level in patients with NAFLD, while others showed not only increase of IgG level but also considered that elevated IgG level was associated with high risk of hepatic decompensation and death in patients with nonalcoholic fatty liver disease (49-50).

Indeed, the bacterial products produced in the stomach, notably endotoxin, do affect the pathogenesis

of NAFLD. Therefore, it is conceivably accepted that the plasma IgG levels in NAFLD patients were shown to be slightly elevated.

In a study that is similar to ours, Maleki et al. 2014 found that the level of IgA rose as hepatic fibrosis progressed in NAFLD patients compared to healthy controls, and that NAFLD cases can be followed up by repeated IgA level monitoring (51).

Complement system is a main part of both innate and adaptive immune system. The main elements of the complement system are the complement proteins C3 and C4. The likelihood of metabolic syndrome, one of the risk factors for NAFLD, was linked to C3 and C4 (52).

Our patients with NAFLD showed marked elevation of C3 and C4 compared with Non-NAFLD participants.

In concordance with our study, Li et al., 2021 found that, both C3 and C4 levels of patients with NAFLD were higher than those without NAFLD (53).

Conclusions

Elevated IgA, IgG, complement component C3 and C4 exist in patients with NAFLD and could be associated with fatty liver disease development and hepatic fibrosis and steatosis progression in patients with non-alcoholic fatty liver disease. Future studies on complement inhibition might offer a way to prevent the onset of fatty liver disease or even slow the condition's progression in NAFLD patients.

Authors' contributions

Heba Ahmed Osman, Mohammed Tag-Adeen, Usama Mohamed Abdelaal, E.E. KAN, Asmaa Nafady: study concept and design, study supervision, analysis and interpretation of data, drafting of the manuscript; Heba Ahmed Osman, Mohammed Tag-Adeen, Usama Mohamed Abdelaal, E.E. KAN, Asmaa Nafady: acquisition of data, statistical analysis, critical revision of the manuscript; Heba Ahmed Osman, Mohammed Tag-Adeen, Usama Mohamed Abdelaal, E.E. KAN, Asmaa Nafady: administration.

Data availability

Data from the study is available by request to the corresponding author

Conflicts of interest

None.

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