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Research Paper

Histopathological Association of Non-alcoholic Fatty Liver Disease With Coronary Artery Atherosclerosis Grade

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ABSTRACT

Background: The association between the severity of coronary atherosclerosis and histopathologic findings in patients with non-alcoholic fatty liver disease (NAFLD) is not entirely understood. Considering the gold standard method, this study evaluates the histopathologic association between the severity of NAFLD and the grades of coronary atherosclerosis.

Methods: In this descriptive-analytical study, data from 205 cadavers who were referred to an Iranian (Tabriz) forensic medicine organization between 2015 and 2017 and underwent simultaneous liver and coronary artery biopsies were examined. Finally, 168 cases were entered based on the inclusion criteria. First, pathological slides of these cadavers were extracted from the forensic medicine archive and re-examined. Then, the selected cases' blocks were extracted from the tissue block bank, and again, after preparing a new slide, they were stained with trichrome for accurate estimation of liver fibrosis.

Results: The assessment of NAFLD histological status in the studied cases revealed that 75.6% of the cases were classified as severity I, 18.4% as severity II, and 6% as severity III. Most cases with coronary atherosclerosis were classified as American Heart Association staging (AHA), type V (19.6%), and normal (19.6%). There was no statistically significant relationship between the severity of simple steatosis, steatohepatitis, and NAFLD, with coronary atherosclerosis. In subjects with higher severity of coronary atherosclerosis, the liver fibrosis rate is also higher, but no statistically significant difference was observed.

Conclusion: The present study revealed no significant histopathological association between NAFLD and coronary artery atherosclerosis grade.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common liver disease that causes chronic liver disease worldwide [1]. The pathogenesis of NAFLD is associated with numerous complex genetic and environmental factors. Hepatic fat accumulation results from an imbalance between fat influx and fat efflux [2]. Recent research based on noninvasive and nonspecific methods has shown that NAFLD is correlated with the development of atherosclerosis and subclinical cardiovascular disease (CVD) [1-3]. Atherosclerosis is identified as a chronic inflammatory CVD of large/medium-sized arteries, and it is one of the most frequent causes of mortality in the elderly [4]. It is a progressive disease that is known by the accumulation of lipids and fibrous elements in the large arteries [5].

It has been shown that NAFLD is the potential determinant of greater intima–media thickness, independent of the other potent effectors like age, sex, visceral fat mass, and insulin resistance/insulin secretion, in individuals with the problem in fasting glucose and or impaired glucose tolerance [6]. The risks of carotid intima-media thickening, which is impaired with endothelial function, calcification of coronary artery, and greater arterial stiffness are higher in patients with NAFLD [6, 7]. CVD is the main cause of mortality in individuals who suffer from NAFLD [8, 9]. The common method established for NAFLD diagnosis is ultrasound or computed tomography (CT), which can only diagnose simple steatosis, but not advanced stages of NAFLD and steatosis [2, 7]. The gold standard for NAFLD diagnosis is liver biopsy. It has been demonstrated that the mortality rate in patients with liver biopsy-proven NAFLD is higher in total than in the general population [10, 11]. The concentration of hepatic fat, evaluated by magnetic resonance imaging (MRI), helps diagnose NAFLD [12]. It has been observed that the liver fat quantity is a predictor of CVD risk and metabolic syndrome [13]. In this study, for the first time, we evaluate the association between the severity of NAFLD and the grade of coronary atherosclerosis through a specific examination (histopathological evaluation) in deceased cases.

Materials and Methods

The study cases were those who died from 2015 to 2017 and underwent autopsy and simultaneous liver and coronary artery biopsy. The total number of cases was 205, the 37 subjects were excluded from the study due to exclusion criteria including a history of alcohol consumption, viral hepatitis, autoimmune hepatitis, hemochromatosis, alpha-1 antitrypsinase deficiency, Wilson disease, history of CVDs, diabetes, using cytotoxic drugs, and chemotherapy [2, 3, 6, 7, 10, 13, 14], and finally, 168 subjects were analyzed. The pathological slides of these cases were obtained from the East Azerbaijan General Department of Forensic Medicine archive and re-examined based on the grading and staging mentioned in this study. Two pathologists analyzed the first ten slides separately, and the kappa agreement coefficient was calculated. With obtaining a kappa coefficient greater than 70%, one pathologist continued examining the slides based on Mendler et al's method [15]. The simple steatosis grading of the cases was determined in 4 grades based on fatty changes: Grade 1) Fatty change <5% liver cell involved, 2) Fatty change: 5-33% liver cell involved, 3) Fatty change: 34-66% liver cell involved, and 4) Fatty change 66% liver cell involved [15]. The group of cases with steatohepatitis was characterized by collecting scores for lobular inflammation and necrosis (LIN: 0–3), Mallory bodies (MB: 0–3), and hepatocyte ballooning (HB: 0–3) based on the method by Mendler et al. Each LIN, MB, and HB was individually scored as follows: 0) Absent, 1) Focal involvement of some lobules, 2) Focal involvement of most lobules, 3) Focal involvement of most or all lobules, with diffuse involvement of some or most of the lobules. The steatohepatitis grade was calculated by adding these individual scores, resulting in a total score ranging from 0 to 9 [15]. Then, these selected cases' blocks were extracted from the tissue block bank, and again, after preparing a new slide, they were stained with trichrome for accurate estimation of liver portal fibrosis in 6 scores (portal fibrosis or NAFLD staging) based on Mendler et al's method [15]. An activity score (AS: 0–12) was calculated by the sum of scores of lobular inflammation and necrosis (LIN: 0–3), Mallory bodies (MB: 0–3), hepatocyte ballooning (HB: 0–3), and perisinusoidal fibrosis (PSF: 0–3). Finally, an order for the severity of NAFLD or NAFLD grade was provided based on previous literature: Grade 1 (PF: 0–2 and AS: 0–4), grade 2 (PF: 3 or AS: 5–7), and grade 3 (PF: 4–6 or AS: 8–12) [1, 15].

Microscopic grading of coronary atherosclerosis was evaluated by the American Heart Association (AHA) atherosclerosis stage classification [16] criteria in 8 classes. Intima and media thickness and lumen area were analyzed by microscopic examination with x25 magnification. The clinical and demographic data of subjects were collected. All data were recorded in a researcher-made checklist, and samples with fatty liver and 11 cases without fatty liver were analyzed to compare the severity of coronary atherosclerosis based on sex, weight, height,

Table 1. Frequency of various grades of steatohepatitis in the histopathologic evaluation of liver samples in the studied subjects

Steatohepatitis Grade	No. (%)
0	97(57.8)
1	2(1.2)
2	10(5.9)
3	11(6.5)
4	16(9.5)
5	19(11.3)
6	8(4.8)
7	2(1.2)
8	2(1.2)
9	1(0.6)

Note: Descriptive statistics (frequencies) were used.

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body mass index (BMI), and the reason for referral to the General Department of Forensic Medicine. Then, the association between histopathological changes in NAFLD severity and coronary atherosclerosis grade was statistically analyzed.

Statistical analysis: Descriptive statistics were used to analyze the characteristics of the study population, and the results were reported using tables and text. Data were analyzed by t-test, chi-square test, Pearson correlation coefficient, and regression. Data were analyzed by SPSS software, version 26. $P<0.05$ was considered statistically significant.

Results

Baseline characteristics of studied subjects

A total of 168 cadavers met the inclusion criteria of this study. The mean age of the study population was 49.42±15.52 years, and 126(75%) cases were male. The average BMI of the study group was 27.16. The reasons for referring the cadavers to the General Department of Forensic Medicine of East Azerbaijan, Tabriz, Iran, are sudden death, trauma, poisoning, and anaphylaxis. The studied cases did not have any documented underlying diseases.

Histopathologic analysis of studied samples

Histopathologic analysis showed that 6.5% (11 cases) of studied samples had liver with normal features and 93.5% had different grades of simple steatosis, respectively: 51 cases (30.4%) steatosis severity I, 65 cases (38.7%) steatosis severity II, 29 cases (17.3%) steatosis severity III, and 12 cases (7.1%) steatosis severity IV. The highest prevalence of simple steatosis was related to the severity II. Also, histopathologic analysis of samples showed various grades of steatohepatitis (Table 1). Trichrome staining of liver specimens categorized studied samples into six stages of portal liver fibrosis. 57.8% of the individuals were in stage 0, and 30.9% of the samples were in stage 1 of fibrosis (Table 2). The evaluation of NAFLD grades among the 168 study subjects showed that 75.6% had severity I, 18.4% had severity II, and 6% had severity III of NAFLD.

The frequency of different types of atherosclerosis in the studied samples classified based on AHA classification [16] of atherosclerotic lesions are mentioned in Table 3. The majority of samples fall into grade V (19.6%) and grade I (19%).

Data analysis and association of NAFLD grades and coronary atherosclerosis types

The results of BMI association with NAFLD grades analyzed by the Pearson test showed that there is no statistical significance between BMI (P=0.29), height (P=0.54),

Table 2. Frequency of microscopic portal fibrosis staging and non-alcoholic fatty liver disease staging, based on trichrome staining of liver samples in the studied subjects [15]

Fibrosis Trichrome or Portal Fibrosis Stages			No. (%)
NAFLD staging stages	S0	No fibrosis	97(57.8)
	S1	Fibrous expansion of some portal areas (with or without short fibrous septa)	52(30.9)
	S2	Fibrous expansion of most portal areas (With or without short fibrous septa)	16(9.5)
	S3	Fibrous expansion of most portal areas with occasional portal-to-portal (P-P) and/or portal to central (P-C) bridging	2(1.2)
	S4	Fibrous expansion of portal areas with marked bridging (P-P as well as P-C)	1(0.6)
	S5	Marked bridging (P-P and/or P-C) with occasional nodules (Incomplete cirrhosis)	0
	S6	Cirrhosis (probable or definite)	0
Total			168(100)

NAFLD: Non-alcoholic fatty liver disease.

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Note: Descriptive statistics (frequencies) were used.

weight (P=0.25), and age (P=0.47) with NAFLD grades. Also, the chi-square test analysis showed no significant association between gender and NAFLD grade (P=0.39).

Pearson test revealed a significant association between atherosclerosis type and age in the studied groups (P<0.001). Accordingly, the atherosclerosis of the cases enhances with age increasing. Also, no significant association between gender and atherosclerosis types was observed in studied samples (P=0.8). Statistical analysis demonstrated no significant association between atherosclerosis types and BMI (P=0.34). Moreover, no significant association was observed between atherosclerosis type and steatosis grades (P=0.54), atherosclerosis type and steatohepatitis (P=0.89), and atherosclerosis type and fibrosis grades (P=0.34). It is worth mentioning that in subjects with higher severity of fibrosis, the grade of coronary atherosclerosis is also higher than in other groups, but statistical analysis showed no significant correlation between fibrosis and coronary atherosclerosis. In 11 subjects without fatty liver disease, moderate to high degrees of atherosclerosis were seen.

The association between NAFLD grades and coronary atherosclerosis type was evaluated. The Fisher test results showed no significant association between the severity of NAFLD and coronary atherosclerosis in subjects with fatty liver (P=0.79).

Discussion

There is some uncertainty regarding the relationship between NAFLD and the risk of atherosclerotic disease [17-19]. Thus, this study evaluated the histopathological association among deceased individuals. We were unable to identify any significant association between fatty liver and coronary atherosclerosis. The sole noteworthy finding was an association between the degree of liver fibrosis and the severity of coronary atherosclerosis.

It has been observed in various studies, often conducted using less specific and less sensitive methods [20], that NAFLD, which is considered a hepatic manifestation of the metabolic syndrome, might elevate the risk of atherosclerosis through various mechanisms [21]. Nevertheless, various studies have yielded different results [22-24].

NAFLD is a wide spectrum of liver abnormalities, including steatosis, non-alcoholic steatohepatitis, and fibrosis [25]. Additionally, NAFLD has a strong relationship with extrahepatic diseases, including obesity, insulin resistance, and dyslipidemia [3, 10, 13, 17, 18, 23]. Besides, the risk of developing NAFLD is elevated in patients with hypertension, enhancing the risk of CVD. Overall, it has been shown that the severity of NAFLD can lead to increased morbidity and mortality rates due to CVD [8, 25, 26]. Nowadays, the most common cause of death in patients with NAFLD is CVD in

Table 3. Frequency of microscopic grading of coronary atherosclerosis in heart samples from the studied subjects [16]

Microscopic Grading of Atherosclerosis Based on AHA Criteria			No. (%)
Coronary atherosclerosis types	0	Absent	33(19.6)
	1	Initial lesion with foam cells	32(19.0)
	2	Fatty streak with multiple foam cell layers	6(3.6)
	3	Pre-atheroma with extracellular lipid pools	12(7.1)
	4	Atheroma with a confluent extracellular lipid core	24(14.3)
	5	Fibro-atheroma	33(19.6)
	6	Complex plaque with possible surface defect or hemorrhage or thrombus or some combination	6(3.6)
	7	Calcified plaque	21(12.5)
	8	Fibrotic plaque without a lipid core	1(0.6)
		Total	168(100)

AHA: American Heart Association.

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Note: Descriptive statistics (frequencies) were used.

the world [27]. The data shows that NAFLD has a very close correlation with atherosclerotic vascular diseases and is also a risk factor for CVD. So far, several studies have been conducted regarding the relationship between NAFLD in liver sonography and ultrasound findings, such as carotid intima-media thickness, coronary artery calcification, and carotid and femoral artery pulse wave velocity findings [7, 12, 28]. The distinguishing advantage of this study, setting it apart from previous research, is the sampling of the right lobe of the liver, measuring 3×3 cm. This approach enhances the accuracy of detection, a task that is not feasible in living individuals.

The histological evaluation of NAFLD in the study group showed that 75.6% of the samples were severity I, 18.4% severity II, and 6% severity III. On the other hand, 80.4% of the samples had coronary atherosclerosis, and most of the samples were in type 5 (19.6%) and type 1 (19%) according to the AHA classification. According to the results of this study, among the 11 cases without NAFLD, some cases had average or high grades of atherosclerosis, and other 33 cases with different grades of NAFLD did not have any atherosclerosis type. Thus, it seems that NAFLD is not the essential condition for atherosclerosis incidence. Alkhouri et al.'s study demonstrated the relationship between liver inflammation and atherosclerosis, and a direct correlation between NAFLD severity and histological features (steatosis, inflammation, and ballooning) with increased CVD risk and atherogenic lipid profile [29]. Our results showed no significant correlation between steatosis, steatohepatitis, and liver fibrosis in the specimen and the severity of coronary atherosclerosis. Also, there is not a significant relationship between the severity of NAFLD and the severity of coronary atherosclerosis. Furthermore, it seems that subjects with high grades of liver fibrosis experience a higher severity of coronary atherosclerosis; however, a statistically significant difference has not been observed between these groups. Kim et al.'s study showed a direct relationship between the severity of coronary artery calcification and NAFLD severity in women in postmenopausal periods of life that could be inconsistent with our results [30]. Chen et al.'s study showed a direct relationship between the prevalence of NAFLD and the increased severity of the coronary artery calcification score [31] in nonspecific and less sensitive diagnostic methods [6, 20]. The studies in animal models have observed that liver inflammation and insulin resistance played a crucial role in dyslipidemia and enhanced susceptibility to atherosclerosis [32-34]. Also, liver fibrosis was high in liver biopsy in patients with higher severity of coronary atherosclerosis, which is similar to our results [32-34]. Dai et al.'s study demonstrated no significant association between non-obese NAFLD and incidents of CAD [35]. Another study indicated no direct association between the presence of CAD and hepatic steatosis, which is consistent with our results [36]. In general, it can be said that according to the specific method used in our study on the one hand and the limited number of samples on the other hand, additional studies with a larger sample

size can be helpful, and currently, we may not establish a direct relationship between the severity of fatty liver and the severity of the atherosclerosis disease.

The novelty of the present study is the histological examination of the coronary arteries and its comparison with the histology of the liver in NAFLD subjects. Because biopsy is the gold standard for NAFLD diagnosis and the ability to differentiate steatohepatitis from simple steatosis in this method, and due to the inability of ultrasound to detect fatty liver with a fat content of 30% and lower than that, the sensitivity of ultrasound is 84%, and the specificity is 93% [6, 20, 37]. Therefore, these results are possibly more reliable in clinical decisions. On the other hand, in addition to the small number of cases, the main limitation of the present study is the inadequacy of sampling NAFLD cases based on the grade of the disease, so that 75.6% of the studied subjects had severity I disease, and also 11 samples had normal liver, which seems that this bias has been created and no significant relationship has been found. However, the sampling of the present study was conducted based on the blind sampling of cadavers, and systematic sampling was impossible for us. For this reason, it is suggested to collect the same number of samples from each grade and the same amount of normal liver samples and compare them in future studies.

Conclusion

The results of the present study show no direct relationship between the histopathologic correlation of NAFLD and coronary atherosclerosis in the studied subjects. While it appears that in subjects with NAFLD who exhibit greater severity of fibrosis, there is a trend of increased grade of coronary atherosclerosis compared to other groups, this trend does not reach statistical significance. These findings may be related to the limited population of the studied group. Therefore, more similar studies should be conducted to confirm these results.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Tabriz University of Medical Sciences (Code: IR.TBZMED.REC.1398.877). Also, the dead individuals' information remained confidential in this study.

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Authors' contributions

Conceptualization and supervision: Ali Banagozar Mohammadi, Maryam Zaare Nahandi, Manouchehr Khoshbaten, Behnaz Gholami, Ali Ostadi, Artin Kamali Sabeti and Hamidreza Eftekhari; Scientific investigation: Ali Banagozar Mohammadi, Maryam Zaare Nahandi, Manouchehr Khoshbaten, Behnaz Gholami and Hamidreza Eftekhari; Sample collection: Behnaz Gholami and Hamidreza Eftekhari; Data collection: Behnaz Gholami, Hamidreza Eftekhari, Ali Ostadi, Artin Kamali Sabeti, Bahram Samadi Rad and Alireza Najafi; Sample analysis: Hamidreza Eftekhari, Amir Vahedi and Behnaz Gholami; Data analysis: Morteza Ghojazadeh, Behnaz Gholami and Saiedeh Razi Soofiyani; Writing the original draft: Behnaz Gholami, Saiedeh Razi Soofiyani and Ali Banagozar Mohammadi; Review and editing: Maryam Zaare Nahandi, Ahad Banagozar Mohammadi and Manouchehr Khoshbaten; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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