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Obesity and related parameters of non-alcoholic steatohepatitis

Nonalkolik steatohepatit gelişiminde obesite ve ilişkili parametreler

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Background/aims: *The aim of this prospective study was to evaluate the clinical, biochemical and histopathological parameters of nonalcoholic steatohepatitis and the conditions associated with this disease.* ***Methods:*** *Twenty-four patients were included in the study, each having been diagnosed with nonalcoholic steatohepatitis on the basis of liver biopsy and elimination of other possible causes of elevated aminotransferase levels. Measurements of degree of obesity, liver enzymes and serum lipids were recorded before liver biopsy and reevaluated after one or two months of a standard exercise and diet program. Serum insulin levels were also measured. Each liver biopsy was histologically examined for steatosis, inflammation, fibrosis, necrosis and iron storage, and semiquantitave assessment of these was recorded for three separate hepatic zones.* ***Results:*** *The prevalence of obesity in the group was 79.2%, while the figure for overt and latent diabetes mellitus combined was 33.3%, and for hyperlipidemia was 83.3%. Compared to the rest of the group, the patients with severe steatosis had significantly higher serum lipid (particularly high triglyceride) and insulin levels ($p<0.05$ for both). There was a correlation between steatosis and obesity ($p=0.06$). More severe obesity, higher insulin and elevated aspartate aminotransferase were positively correlated with inflammation, whereas elevated serum triglyceride was negatively correlated with inflammation. There was a tendency towards normalization of liver enzyme levels after weight loss and dietary restrictions.* ***Conclusions:*** *Obesity and hyperlipidemia were associated with nonalcoholic steatohepatitis in the group studied. Obesity is not a factor in every case, but the study showed that restricted diet and exercise are significant forms of therapy for nonalcoholic steatohepatitis.*

Key words: Nonalcoholic steatohepatitis, steatosis, triglyceride, cholesterol, insulin, diabetes mellitus, obesity.

Amaç:*Nonalkolik steatohepatitli hastalarda klinik, biokimyasal, histopatolojik parametreler ve eşlik eden diğer durumları değerlendirmeyi amaçladık.* ***Yöntem:*** *Karaciğer biyopsisi ve diğer aminotransferaz yüksekliği yapabilecek sebepler ekarte edilerek nonalkolik steatohepatit tanısı konulan 24 hasta çalışmaya alındı. Obesite derecesi, karaciğer enzimleri ve serum lipidleri karaciğer biyopsisi öncesi ve standart egzersiz ve diet programından 1-2 ay sonra değerlendirildi. Karaciğer biyopsileri histolojik olarak steatoz, inflamasyon, fibrozis, nekroz ve demir birikimi açısından değerlendirildi ve 3 ayrı hepatik zon için semikantitatif skorlama uygulandı.* ***Bulgular:*** *Obesite %79.2, aşikar ve gizli diabet toplam olarak %33.3 , hiperlipidemi %83.3 sıklığında idi. Şiddetli steatozu olanlarda serum lipidleri (özellikle trigliserid) ve insülin düzeyleri daha yüksekti (her ikisi için $p<0.05$). Steatoz ve obesite arasında istatistik öneme yakın korelasyon gözlendi ($p=0.06$). Daha şiddetli obesite, yüksek insülin ve aspartat aminotransferaz düzeyleri inflamasyon ile pozitif korelasyon gösterirken, yüksek serum trigliserid düzeyi inflamasyonla ters ilişki gösterdi. Zayıflama sonrasında karaciğer enzim düzeylerinde belirgin düzelme gözlendi.* ***Sonuç:*** *Obesite ve hiperlipidemi nonalkolik steatohepatit ile anlamlı birliktelik gösterdi. Obesite her vakada olmamasına rağmen çalışma sonuçları diet sınırlaması ve egzersizin nonalkolik steatohepatit'de faydalı bir tedavi şekli olduğunu ortaya koymaktadır.*

Anahtar kelimeler: Nonalkolik steatohepatit, steatoz, trigliserid, kolesterol, insülin, diabetes mellitus, obesite.

INTRODUCTION

Any etiology of steatosis can lead to necroinflammation and fibrosis, so-called "steatohepatitis" and even cirrhosis (1). The various types of steatohepatitis that result from diverse causes share common histological features (2), but it is not known exactly how this unique pathological entity develops, why some patients with steatosis never progress to nonalcoholic steatohepatitis (NASH), whether or not the severity of steatosis is important in terms of progression to fibrosis or inflammation, or which factors actually determine the development of NASH. We collected histological data from patients with NASH and determined whether these findings correlated with degree of obesity, serum levels of lipids, insulin and transaminases, certain hematological parameters,

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and some more specific indicators of liver function. Additionally, we aimed to investigate the effect of weight loss on the improvement of some biochemical abnormalities due to NASH.

MATERIALS AND METHODS

Consecutive patients with elevated transaminase levels were investigated for NASH. The diagnosis of NASH was established based on retrospective and prospective data, as follows: historical information (consumption of alcohol and drugs known to cause steatohepatitis, jaundice, family members with liver disease, blood transfusions, surgery, dental procedures), laboratory findings and serologic testing (using viral markers for hepatitis B and C viruses and immunologic markers for antinuclear, antimitochondrial and smooth muscle antibodies), serum measurements of ceruloplasmin, alpha-1-antitrypsin, blood urea nitrogen and creatinine and abdominal ultrasonography (US). All of the patients included in the study denied alcohol consumption. If the initial US imaging demonstrated a "bright liver appearance," the patient was given the option of percutaneous liver biopsy as further work-up. If the first liver US did not reveal this specific type of image, we waited two months and then reevaluated the patient's transaminase levels. In this way, we avoided misdiagnosing viral or toxic hepatitis as steatohepatitis. The patients whose pathologic examination showed steatosis and portal and/or lobular inflammation were accepted to have NASH and included in the study group. In this way, we accumulated a total of 24 patients (17 men and 7 women; mean ages 39.8 ± 7.5 and 45.9 ± 7.3 years, respectively).

Once the group of 24 patients was established, many data were collected from each individual, as follows: economic status, smoking status, hypertensive status, history of familial hyperlipidemia, presence of diabetes mellitus (DM), presence of atherosclerotic heart disease, occurrence of significant weight loss, use of intravenous glucose or parenteral nutrition in recent months, symptoms attributable to liver disease, measurements of body weight and height, fasting blood glucose level (glucose tolerance testing was done in all patients with normal fasting levels), levels of serum cholesterol, triglyceride, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gammaglutamyl transpeptidase (GGT), alkaline phosphatase, bilirubin, albumin, insulin, blood iron parameters, hemoglobin and apolipoprotein A and B, as well as leukocyte count, platelet count, prothrombin time and protein electrophoresis results. Patients with fasting serum glucose levels >120 mg/dL in a minimum of two separate samples were identified as having DM, and a finding of 140-200 mg/dL at two hours after standard oral glucose loading was considered an abnormal glucose tolerance test.

The measurements pertinent to body mass were recorded close to the day of liver biopsy and were reevaluated after one or two months. We determined the degree of obesity by calculating the body mass index (BMI), and used the same formula to group patients as normal (BMI<25), mildly obese (BMI: 25-30), manifest or moderately obese (BMI: 31-39) and morbidly obese (BMI>40) (3). To estimate fat tissue mass, we used the waist/hip calculation. The waist/hip calculation is a simple method that reveals the distribution of subcutaneous and intraabdominal adipose tissue, and distinguishes android from gynoid obesity. The upper limit values are 1.0 for men and 0.8 for women (4).

Liver biopsies were performed using a Tru-cut biopsy needle, and samples were collected before each patient started their trial period on a specific diet regime (ideal weight . 25 Cal . kg-1) and a standard exercise program (walking for 30 min once a day). Basic histologic evaluation was done in sections stained with hematoxylin and eosin. Tissue iron storage was assessed using Perls' Prussian blue method, and hepatitis B surface and core antigen and hepatitis C virus (HCV) status were assessed using immunohistochemical staining. We recorded the histological findings semiquantitively, as shown in Table 1. According to Perls' staging, a score of 0-1 is normal and a score of 2-4 indicates increased iron storage (5). We statistically analyzed the results and tested correlations between parameters using the chi-square and Mann-Whitney U tests. Probability values less than 0.05 were considered significant.

RESULTS

Table 2 lists some of the clinical and demographic characteristics of the patients. There were no symptoms apart from a few cases of right upper quadrant abdominal pain and fatigue. Some of the patients with abdominal pain were observed to be pain-free during follow-up after having followed recommendations for managing irritable bowel syndrome. Thus, we were unable to document any

Table 1. Examined parameters and semiquantitative scoring system used for their assessment.

Parameters	*Appearance**	*Scoring*
Fat	Macrovesicular	1. Hepatocytes containing fat < 33%
		2. 33% – 66
		3. >66%
Steatohepatitis	İnflammation	Grade 0-3
	Ballooning of Hepatocytes	Grade 0-3
	Mallory body	Grade 0-3
	Necrosis of Hepatocytes	Grade 0-3
	Vacuolization	Grade 0-3
Fibrosis		Grade 0-3
Hemosiderosis		Grade 0-4(Perls)

*Individual scoring for every parameter was performed in the periportal , perivenular and acinar regions.

statistical result for symptoms attributable to NASH.

The histologic scoring of steatosis and inflammation (0-3) was modified to reflect mild as 0-1 and severe as 2-3 to facilitate the statistical calculations, since the total number of patients was low. When this was done, we found steatosis to be significantly associated with insulin level (p=0.01) and less strongly associated with obesity (p=0.06). The mean insulin levels in patients with mild and severe steatosis were 18.0 ± 7.7 IU/ml, and 29.6 ± 8.0 IU/ml, respectively. The mean BMI values in the same groups were 44.1 ± 27.5 and 31.1 ± 3.6, respectively. Although the statistical correlation between serum lipids and steatosis was not significant, patients with severe steatosis tended to have higher serum lipid values (triglyceride in particular) than patients with mild steatosis (triglyceride 283.5 ± 111.5 mg/dL vs. 226.5 ± 89.9 mg/dL, and cholesterol 219.3 ± 28.6 mg/dL vs. 195.4 ± 49.0 mg/dL, respectively).

Table 2. The demographic and clinical characteristics of patients

Parameters	
Age (mean)	39.8 ± 7.5
Sex	
Male	17
Female	7
Degree of obesity	
Normal (obesity absent)	4 (16.7%)
Mild	8 (33.3%)
Moderate	10 (41.7%)
Severe	2 (8.3%)
Diabetes mellitus *	2 (8.3%)
Glucose intolerance *	6 (25%)
Hyperlipidemia	9 (37.5%)
High cholesterol	20 (83.3%)
High Triglyceride	4 (16.7%)
Hyperlipidemia absent	
Hypertension	8 (33.3%)
Smoking	9 (37.5%)
Family history	
Liver disease	7 (29.1%)
Known hyperlipidemia	4 (16.7%)
DM	8 (33.3%)
Atherosclerotic heart disease	3 (12.5%)
Economic status	
Poor	1 (4.2%)
Moderate	12 (50%)
High	4 (16.7%)

*The incidence of diabetics in our group was comparable with the incidence of DM in our region by an unpublished study and glucose intolerance was more frequent than regional one.

The presence of obesity had a significant effect on the severity of inflammation. BMI values for patients with mild and severe inflammation were 28.2 ± 3.7 and 34.1 ± 8.1, respectively (p<0.05). There was significant association between insulin and inflammation (insulin; 27.2 ± 24.0 IU/ml for mild and 41.9 ± 25.8 IU/ml for severe inflammation patients, p=0.05). Serum triglyceride concentration was significantly higher in patients with mild inflammation than in individuals with severe inflammation (296.4 ± 99.1 mg/dL vs. 190.1 ± 84.6 mg/dL, p<0.05). The relationship between cholesterol and inflammation showed a similar trend, but was not significant (p=0.2).

Regarding fibrosis, we found that serum cholesterol was the only parameter that was significantly higher in patients with higher-grade fibrosis (232 ± 31.0 mg/dL vs. 194.5 ± 38.7 mg/dL, p<0.05).

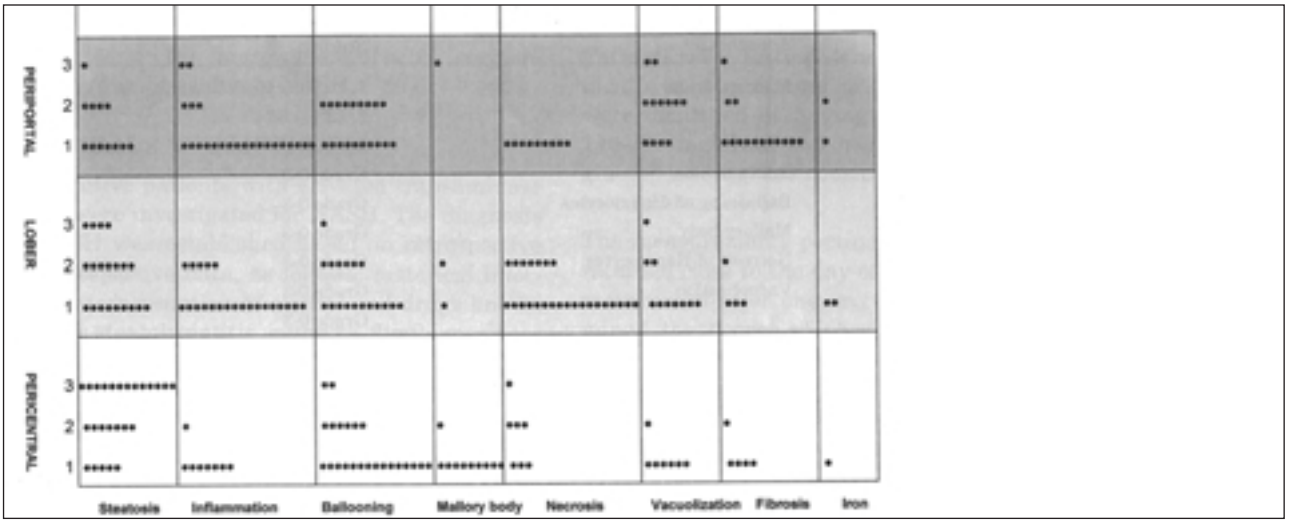

Figure 1. Distribution of histologic findings with respect to severity and location the three hepatic zones

In testing the correlations between the parameters and the different liver enzyme levels, we found that patients with severe inflammation had higher AST and ALT values, although the association was not significant in the case of ALT (68.1 ± 60.7 U/L vs. 50.3 ± 10.8 U/L, $p<0.05$; 91.8 ± 27.1 U/L vs. 76.8 ± 36.4 U/L, respectively). Regarding steatosis, there was no significant association between AST or ALT and steatosis, but patients with severe steatosis tended to have higher ALT values (93.1 ± 35.1 U/L) vs. 74.2 ± 30.9 U/L, $p=0.2$). The situation was similar with respect to insulin, where patients with higher insulin values tended to have higher AST and ALT levels. The AST levels in patients with higher and lower insulin levels were 70.4 ± 57.3 U/L vs. 52.5 ± 15.0 U/L, respectively, and the corresponding ALT levels were 86.8 ± 27.2 U/L vs. 67.1 ± 28.6 U/L.

After they had completed the diet and exercise therapy programs, the patients were asked to attend monthly rechecks. At each appointment we recorded BMI, liver enzyme levels and serum lipid levels in order to assess the changes that might have occurred with weight loss. Figure 1 shows the distribution of histologic findings (steatosis, inflammation, ballooning degeneration, Mallory's bodies, necrosis, vacuolization, fibrosis and iron accumulation) with respect to severity and localization to the three hepatic zones.

DISCUSSION

Although it has not yet been proven, there may be a genetic basis to the pathogenesis of NASH (6,7). We were able to collect information about family members with liver disease from 18 of our 24 patients, and seven individuals had a positive family history. The liver problems in family members included probable NASH in one patient's brother, three cases of cirrhosis of unknown etiology in the parents of three patients, hepatitis B virus carrier status in the mother and sister of one patient and one case of fatty liver and history of jaundice probably due to viral hepatitis in the mother of one individual. Since our information about liver functions in patients family members was limited we were unable to make any conclusions about familial tendency to develop NASH. Based on the findings in our study, we can also rule out hypolipoproteinemia as a familial cause of NASH because none of our patients exhibited hypotriglyceridemia or hypoapolipoptroteinemia.

Even though we did not specifically assess dietary content, the moderate or high economic backgrounds of most of our patients (67%) suggest sufficient nutrition on a quantitative basis. It would be valuable to learn whether the common nutritional practices in different populations play any role in the development of NASH. Some animal studies have demonstrated that the type and amount of dietary fat can alter the degree of fibro-

sis and steatosis in the liver (8,9). In countries such as Turkey, it is likely that the components of daily nutrition influence the development of NASH.

Similar to the findings of previous studies, our NASH patients had elevated ALT and AST values, with ALT levels showing a more prominent peak. We noted a mild increase in GGT, but alkaline phosphatase levels were normal in all cases. Our findings for these two enzymes combined indicate that the damage in NASH is hepatocellular in nature.

George et al examined the role of heterozygocity for hemochromatosis in the development of mild iron load, a condition sometimes seen in patients with NASH.. Using Perls' staging, a score of 1 indicates a pathologic condition (6). We detected stainable iron in five of the 21 (23.8%) patients we tested for tissue iron. Four of the five scored 1 using Perls' method and the other scored 2. The latter patient had elevated serum ferritin concentration but normal transferrin saturation. Three of the four patients who had elevated ferritin in their blood showed stainable iron on histologic liver tissue assessment. It is possible that iron induces hepatic injury through initiating the lipid peroxidation that is known to occur in NASH. On the other hand, iron loading in the liver may be the result of phagocytosis of injured hepatocytes in patients with NASH. In cases of NASH and/or iron loading inflammation, necrosis and iron accumulation must all occur in close proximity to each other. We did note that inflammation, necrosis, steatosis and iron accumulation occur together in any one zone. This may suggest that the stainable iron in our patients represents a normal histologic finding that would be seen in normal healthy liver.

Regarding fat distribution, our observations in the three hepatic zones were in agreement with those of previous reports. In our patients, zone 3 contained the largest proportion of fat, and zone 1 contained the least. Although it is not clear what factors influence zonal fat distribution, part of the reason for the differences may be the functional heterogeneity of hepatocytes in the three regions (10).

We found that necrosis, inflammation and fibrosis were not distributed in parallel with steatosis. In contrast, these types of changes were observed predominantly in zones 1 and 2, a finding somewhat different from which has been described previously. Other reports have observed inflammation mostly in the centrilobular zone, and to a lesser extent in the portal and periportal regions (11,12). It should also be noted that the inflammation in NASH is not strictly limited to perivenular regions. Histologically, zonal distribution of hepatic inflammation is assessed on the basis of types of inflammatory stimulus (13). Lymphocytes can penetrate the various liver zones via different paths, but it is not clear what governs this process. In normal liver tissue, T lymphocytes are distributed in marked quantities in the portal tracts and in a scattered pattern in the parenchyma. Elevated numbers of lymphocytes in the portal and lobular regions of our NASH patients' livers can be accepted as an indication of increased levels of tissue necrosis in these areas. However, we cannot speculate as to what actually caused these tissue changes. The severity and distribution of fibrosis would be expected to follow the same pattern as necrosis, and we did observe this in our patients' specimens.

Currently, there is no satisfactory explanation for why steatosis does not progress to steatohepatitis in some individuals. Obesity may be important, but does not provide the entire answer. Although some authors have reported a positive correlation between obesity and NASH, obese individuals who are diagnosed with steatosis based on US examination do not always have increased transaminase levels. In our study, there was a significant association between the presence of obesity and severity of steatosis or inflammation, but presence of obesity was not correlated with transaminase levels. Furthermore, the fact that not all studies have found a significant association between severity of steatosis and inflammation may suggest that other factors are significant in the progression to steatohepatitis.

Although most of our patients had above-normal triglyceride concentrations, there was no correlation between this finding and severity of steatosis. It is noteworthy, however, that patients with severe steatosis tended to have higher mean cholesterol and triglyceride levels than those with less severe steatosis. We also found no significant association between serum lipids and transaminase values. The fact that NASH is sometimes diagnosed in patients with normal triglyceride levels, the evidence that antilipidemic drugs do not resolve NASH, and the findings of this study

and previous reports (14) all point to the conclusion that hypertriglyceridemia is probably not an absolute etiologic factor in NASH.

We found endogenous insulin levels to be correlated with severity of steatosis and inflammation, and it does appear that this hormone plays some part in the pathogenesis of NASH. There was no significant difference between the transaminase levels in patients with elevated and normal insulin levels; however, enzyme levels tended to be higher in patients with above-normal insulin. Obesity is accompanied by various degrees of hyperinsulinemia, and this is attributed to beta cell hypersecretion, reduced hepatic insulin extraction or both (15,16). Increased steatosis of the liver was shown to be associated with reduced insulin clearance (17). Analysis of the changes in various parameters on our individual patients' graphs suggests that weight loss (associated with decreased transaminase values) had a beneficial effect on biochemical improvement in NASH, probably through reduced insulin resistance (IR). Insulin resistance does not always accompany obesity. In a study conducted in Asian Indians, some of the men who were categorized as nonobese on the basis of BMI calculations showed IR. In these individuals, IR was directly related to serum triglyceride levels and proportions of visceral, not subcutaneous, adipose tissue (18). Seven of our patients were normal or only mildly obese according to the obesity scoring scheme, and three of these seven had elevated serum insulin levels. As mentioned, we found the prevalence of DM in our patients to be similar to the general population, but the prevalence of glucose tolerance was significantly higher in the study group. Above-normal waist/hip results (not shown in the results) of eight patients can be accepted as a reflection of abnormal fat accumulation or fat distribution in the body. Six of these eight had hyperinsulinemia, as did two of the five patients with glucose intolerance. Although these findings were not statistically significant, they may be a reflection of hyperinsulinemia or IR in patients with NASH (19).

NASH is a debilitating disorder that can usually be treated with simple therapy. Our findings in this study indicate that some of the significant factors are obesity, hypertriglyceridemia and hyperinsulinemia; however, more in-depth investigation involving larger numbers of patients is required to clarify the mechanisms that are at work in this disease.

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