インスリン抵抗性改善薬

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abstract

インスリン抵抗性が非アルコール性脂肪肝炎（non-alcoholic steatohepatitis：NASH）/非アルコール性脂肪性肝疾患（non-alcoholic fatty liver disease：NAFLD）の発症機序に深く関与していることは，以前より多くの研究者によって指摘されており，インスリン抵抗性改善薬はNASH治療薬の有力な候補として注目されてきた．インスリン抵抗性改善薬として代表的なものとしてはチアゾリジン誘導体とビグアナイド系の薬剤が挙げられる．ピオグリタゾンは同系統のチアゾリジン誘導体であるロシグリタゾンと異なり肝障害の副作用が少ないことから，これまでに複数のNAFLDの臨床研究で検討され，有効性を認める報告がなされている．今後は，より基礎・臨床の両面で研究を進めてNAFLDの病態に対する理解を深め，その病態ごとに有効な治療法の検討を行うことが重要な課題である．

I　はじめに

インスリンは膵臓のβ細胞で産生され，末梢組織（主に骨格筋）では糖の取り込み促進，肝臓では肝細胞における糖新生の抑制作用を有し，食後の急峻な血糖上昇を抑制する働きをしている．糖尿病の初期段階では，インスリンの効果が低下するために末梢組織による糖の取り込み遅延と肝細胞の糖新生の亢進が起こり，膵β細胞ではむしろインスリンの過剰分泌をきたす．この病態をインスリン抵抗性と称し[1]，インスリンの効果が低下する機序としては，内臓脂肪の増加に伴う脂肪組織由来のアディポサイトカインの分泌異常，血中遊離脂肪酸の増加，腸管細菌叢の変化などが関与していると考えられている．インスリン抵抗性は糖尿病のみならず，高血圧，脂質異常症の発症にも深く関与しており，メタボリックシンドロームの病態形成における中心的な因子として広く認められている．

糖尿病患者に脂肪肝が多いことは以前より指摘されていたが，近年，非アルコール性脂肪肝炎（non-alcoholic steatohepatitis：NASH）の存在が広く認知されるにあたり，NASHの病態とインスリン抵抗性の関連が注目され，多数の研究が行われるようになった[2),3)]．その結果，インスリン抵抗性は肝脂肪化のみならず肝の炎症，線維化，癌化，肝再生不全など，多岐にわたってNASHの発症進展に関与していることが報告された[4),5)]．こういった背景から，インスリン抵抗性改善薬がNASHの治療法を検討する際に注目されたのはいわば必然的なことであった．今回述べるチアゾリジン誘導体，そしてビグアナイド剤はそのインスリン抵抗性改善薬の代表的なもので，いずれも糖尿病の治療薬として保険認可を受けて以前から臨床で使用されているものである（図1）．

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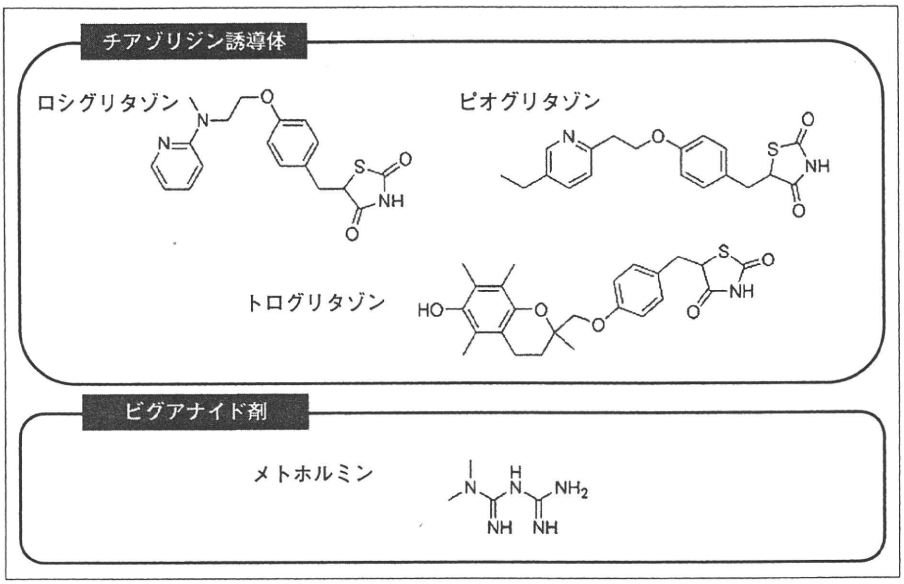

図1
チアゾリジン誘導体とビグアナイド剤

II チアゾリジン誘導体

チアゾリジン誘導体は脂質異常症の薬剤開発中に発見され，血糖降下作用を有することが知られていたが[6]，核内受容体スーパーファミリーのひとつであるペルオキソゾーム活性化受容体（peroxisome proliferator activated receptor：PPAR）γのリガンドとして作用することが明らかになったことから，より注目されるようになった．PPARは遺伝子解析によってリガンドが不明なまま発見された，いわゆるオーファン受容体であった．PPARはα，β/δ，γの3つのサブタイプがあり，組織によって発現が異なり，PPARγは脂肪組織，免疫組織に特に強く発現し，筋肉，肝臓にも発現している．チアゾリジン誘導体はPPARγのリガンドとして作用し，脂肪細胞の分化誘導，マクロファージの分化および活性化作用を有することが示されたが，そのほかにも肝の糖新生抑制，骨格筋の糖の取り込み促進，脂肪細胞のアディポネクチンの分泌促進，脂肪組織由来のTNF-αの分泌抑制という多岐にわたる効果が認められ，複数のプロセスで総合的にインスリン抵抗性を改善させることが明らかにされた．臨床面においては，本邦ではトログリタゾンが最初に商品化され，1997年にチアゾリジン誘導体として初めて保険認可された．しかし，その後一部のトログリタゾンを投与した患者で肝障害が起こり，劇症肝炎からの死亡例も出たため，2000年には発売中止になってしまった．もうひとつのチアゾリジン誘導体であるピオグリタゾンは遅れて1999年に保険認可されたが，肝障害をきたしにくいチアゾリジン誘導体として現在も臨床で使用されている．もうひとつのチアゾリジン誘導体としてはロシグリタゾンがあり，米国では認可されているが，本邦ではまだ使用できない．近年米国で報告された多施設共同のコホート研究で，ロシグリタゾン投与の患者は，ピオグリタゾンによって治療されている患者と比較して有意に心筋梗塞や心不全などの発症率が高いと報告され[7]，虚血性心疾患に関してはピオグリタゾンに軍配が上がる結果となった．

NASHに対するチアゾリジン誘導体の効果に関する基礎的研究はこれまでに多数報告されている．Kawaguchiらは，ラットにコリン欠乏アミノ酸添加食を与え，ピオグリタゾンを同時に添加すると肝の脂肪化および線維化が抑制されたと報告した[8]．また，Otaらは肥満ラットのOLETFにコリンメチオニン欠乏食＋高脂肪食を添加して線維化を伴う脂肪性肝炎を発症させ，同時にピオグリタゾンを投与すると，血中アディポネクチンの増加，肝脂肪化の減少，血清ALTの低下，活性化星細胞の減少を認め，肝組織中のtransforming growth factor（TGF）-β，sterol regulatory element-binding protein（SREBP）

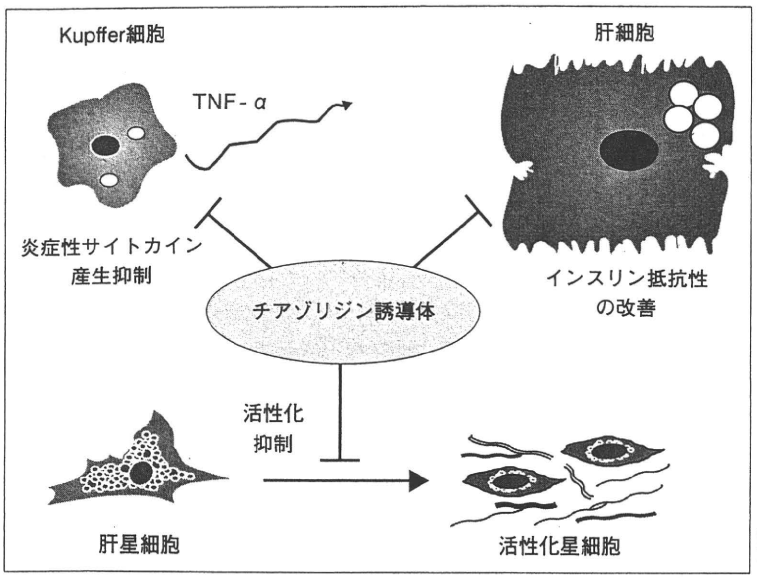

図2
チアゾリジン誘導体のNASHに対する効果

-1c，脂肪酸合成酵素（fatty acid synthase：FAS）の発現がそれぞれ低下していることを示した[9]．われわれの研究室でも以前より肝病態に対するピオグリタゾンの効果を注目しており，まずピオグリタゾンが単離したラット肝星細胞の活性化に対して抑制効果を示すことを見いだし，2002年に報告した[10]．最近では，インスリン抵抗性を示す肥満マウスのKK-Aʸマウスを用いて実験を行い，KK-Aʸマウスがコントロールマウスと比較して肝再生能が低下しており，ピオグリタゾンを投与するとインスリン抵抗性が改善し，肝再生能が上昇することを示した[5]．これらの報告は，ピオグリタゾンがNASHの病態に対して肝脂肪化，炎症，線維化といった病態の主体となる各因子に対して改善効果を有することを分子レベルから明らかにしただけではなく，肝再生の改善など，病期進展にかかわる因子に対しても有効である可能性を示している（図2）．

ヒトのNASHを対象とした臨床試験は，2003年以降，主に糖尿病合併のNASH患者を対象とした投与例が散見されるようになった．その報告の多くは少数例で，かつ肝逸脱酵素の数値に対する効果を評価するのにとどまるものが多かったが，近年ようやく肝組織を評価に加えた大規模研究の結果が報告されるようになった．Aithalらは，非糖尿病のNASH患者74名に対して無作為化プラセボ比較試験を行い，ピオグリタゾンがプラセボと比べて有意に体重を増加させたが，ALTおよびγ-GTPレベルを低下させ，肝組織上の炎症および線維化を改善させたと報告した[11]．GastaldelliらはヒトのNASH患者47名に対してピオグリタゾンの投与を行い，脂肪組織のインスリン抵抗性の改善が肝脂肪化・肝炎症の減少と相関したと報告した[12]．また，MussoらはNASHに対するチアゾリジン誘導体の効果に関するメタ解析を行い，チアゾリジン誘導体は体重減少には有意な効果がなく，肝の脂肪化および炎症を軽減させる効果があると報告した[13]．このメタ解析の対象となった文献の多くは解析対象の治療期間が1年未満であり，小規模の群を対象とした解析が多かった．SanyalらはNASH患者を対象とした初の大規模臨床試験として，プラセボおよびビタミンEを対照としたPioglitazone versus vitamin E versus placebo for the treatment of non-diabetic patients with non-alcoholic steatohepatitis（PIVENS）試験を米国で行った．ここでは非糖尿病NASH患者247名を対象としてピオグリタゾン30mg/日およびプラセボ，ビタミンE投与群に無作為に割り付け，96週間の追跡調査を行った．結果として，ピオグリタゾン投与はビタミンE単独投与と比較して有効な結果は得られず，プラセボと比較するとピオグリタゾンの投与によってAST，ALTの改善効果は得られたものの，この追跡期間中には肝組織スコアの改善は認められなかったと報告された[14]．

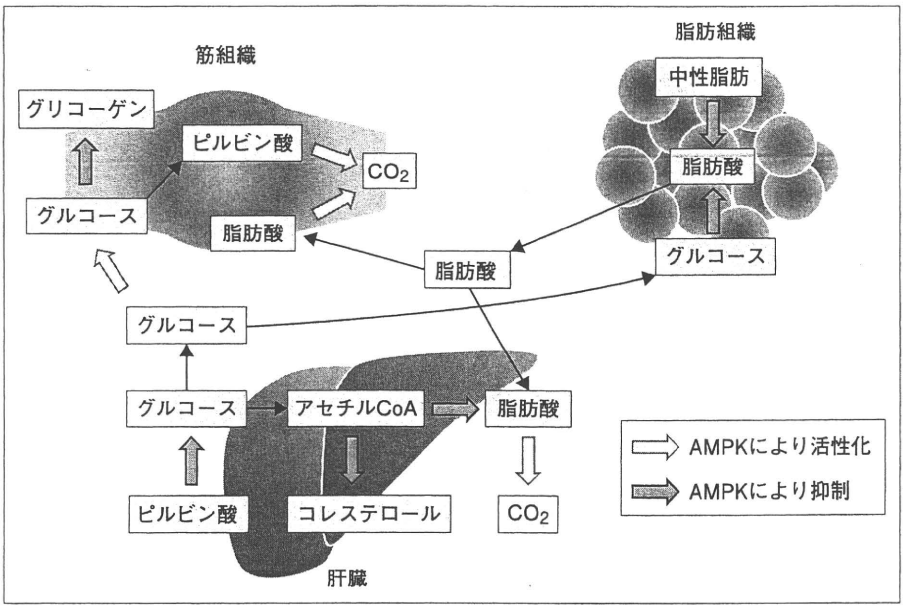

図3
AMPKと糖脂質代謝
〔参考文献15）より引用改変〕

これらを総合して考えると，ピオグリタゾンはNASH患者の肝逸脱酵素値を減少させる効果が確認された数少ない薬剤ではあるが，NASHの病期に対する治療効果に関してはいまだ十分な証明がなされておらず，より長期間のフォローを交えた検討が必要である．また，NASHの病態に対する治療効果を検討するうえで大きな問題となるのが，NASHの診断基準が主に除外診断であることからもNASHは必ずしも単一の疾患とはいえず，表現系が酷似した疾患群ともいえる点である．前述の大規模研究に関しては非糖尿病のNASH患者を対象としたものであり，インスリン抵抗性が明確に病態に関与している症例を対象にした場合は結論が変わってくる可能性もある．今後は，NASHの病態に対する基礎・臨床両面の研究を進め，どのような病態のNASHに対してピオグリタゾンが有効であるのかを検討していく必要がある．

III ビグアナイド剤

ビグアナイド剤の糖尿病治療薬としての歴史は，前述したチアゾリジン誘導体よりもはるかに古い．1918年にエール大学病理化学のC. K. Watanabeによりグアニジンが血糖降下作用を有することが報告されたのが始まりであり，以後数種のグアニジン誘導体が開発されたが，その多くは肝毒性や胃腸症状などの副作用が強いために使用されなくなった．メトホルミンもグアニジン誘導体のひとつで，本邦での保険認可は1961年であった．しかし，同系統のフェンホルミンが乳酸アシドーシスの副作用のために発売中止となり，メトホルミンも同じ副作用に対する懸念から，実際に治療に使われることは少なくなってしまった．1990年代より欧米を中心としてメトホルミンの再評価が行われ，低血糖や体重増加を起こしにくい面が注目された．本邦でも見直しが行われ，臨床で用いられるケースが増えている．

メトホルミンの作用機序にはAMP-activated protein kinase（AMPK）の活性化作用が重要であると考えられている．AMPKは本来，AMP/ATP比の低下によって活性化し，エネルギー代謝を促進させる役割をもつ[15]（図3）．メトホルミンはAMPKの活性化により，筋肉組織の糖取り込みの促進，肝細胞における糖新生の抑制，肝でのVLDL分泌抑制，脂肪酸合成抑制といった多彩な働きをする．これらのメトホルミンの作用機序を考慮すると，NASHの病態に対する改善作用も期待がもたれる．しかしながら，NASHの病態に対するメトホルミンの効果に関しては，まだ少数例を対象とした報告が散見されるのみである．その先駆けとして，Nairらは15名のNAFLD患者に対してpilot studyを行い，メトホル

ミンを20mg/kg/日で48週間投与し肝組織所見も含めた治療効果の評価を行った．その結果，メトホルミン投与によるAST，ALT値の減少は限定的で，かつ治療後の肝生検が可能であった10名のうち肝脂肪量の減少を認めたのが3名，炎症の改善は2名，線維化の改善を認めたのは1名に留まったと報告した[16]．Garinisらは，非糖尿病のNAFLD患者50名に対して食事療法単独群を対照としたランダム化比較試験（RCT）を行い，25名の患者に対してメトホルミンの少量投与＋食事療法を半年間行った．その結果，両群で体重減少，肝脂肪量の減少が認められたが，メトホルミン投与群ではインスリン抵抗性の改善，アディポネクチンの減少を有意に認めたと報告した[17]．また，HaukelandらはNAFLDの患者48名に対してプラセボを対照としたRCTを行い，24名に6カ月間のメトホルミン投与を行った．この試験ではメトホルミンに肝脂肪沈着に対する効果は認められず，LDLコレステロールおよび空腹時血糖，HbA_{1c}の減少が得られた[18]．これらの報告をみると，NAFLDに対するメトホルミンの効果はインスリン抵抗性の改善については認められるものの，肝障害に対する効果としてはほぼ肝脂肪化に対する効果に限定されており，肝の炎症，線維化に対する効果を示した研究はほとんどない．ただし，これらの検討はすべて少数例に留まっており，今後の検討でNAFLDの病態によっては効果が得られる可能性もある．なお，メトホルミンの副作用として乳酸アシドーシスが知られているが，肝機能が低下すると乳酸が増加しやすく，また，腎不全ではメトホルミンの蓄積が起こるため，メトホルミンによる乳酸アシドーシスが起こりやすいとされている．このため，肝機能が低下している患者に対しては乳酸アシドーシスのリスク増加から使用すべきではなく，NASHに対する治療としても投与は肝予備能が低下していない症例に限られるべきである．

Ⅳ おわりに

NASHの治療におけるインスリン抵抗性改善薬の現状について述べた．NASHの発症・進展にかかわるメカニズムを考えるうえでインスリン抵抗性は最も重要な因子であり，インスリン抵抗性改善薬はNASHの治療法として最も有望視されているもののひとつである．なかでもピオグリタゾンはNASH症例における肝酵素の減少効果が確認された数少ない薬剤であるが，長期予後に対する効果に関しては今後の検討を要する．また，ビグアナイド剤についてはまだNASHに対する治療効果を十分に示した報告はなく，今後の研究の進展が望まれるところである．

病態の多様性を考慮すると，NASHの治療を画一的に行うことはおそらく困難である．特に，インスリン抵抗性改善薬の治療効果に関しては，各症例ごとにインスリン抵抗性の存在と病態に対する関与を解析し，病態形成のメカニズムに踏み込んだ形で臨床試験を行っていかなければ，治療効果の評価を誤る危険性がある．今後は基礎・臨床両面からより深くNASHの病態メカニズムを解明し，病態に合わせた治療法の選定を可能にすることが重要になってくると思われる．

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INTERNATIONAL FORUM

Prevalence, gender, ethnic variations, and prognosis of NASH

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Abstract We provide an update review on the prevalence, gender, ethnic variations, and prognosis of nonalcoholic steatohepatitis (NASH). According to annual health checks, 9–30% of Japanese adults have nonalcoholic fatty liver disease (NAFLD) by ultrasonography (US) and prevalence of NASH is estimated to be 1–3%. These conditions are strongly associated with the presence of obesity and life-style-related diseases. NAFLD and NASH exhibit age and gender differences in both prevalence and severity. Among younger patients, these conditions are more common in men (2–3 times); however, after 60 years of age, the prevalence of NASH is higher in women. According to a systemic analysis of histological findings for NASH, 37.6% of patients had progressive fibrosis, 20.8% improved, and 41.6% remained stable over a mean duration of follow-up of 5.3 years. Age and presence of inflammation on initial biopsy were independent predictors of progression to advanced fibrosis. The frequencies of development of cirrhosis in NASH are 5–25% during around 7-year follow-up periods. Survival in NASH is lower than the expected survival of the matched general population due to the higher prevalence of cardiovascular and liver-related death. In patients with cirrhotic NASH, hepatocellular carcinoma (HCC) and liver failure are the main causes of morbidity and mortality (5-year cumulative HCC development rate 11.3%, 5-year survival rate 75.2%, respectively). The cumulative rate of recurrence of HCC at 5 years was 72.5%. Regular screening for complications of liver cirrhosis and HCC is extremely important for cirrhotic NASH patients.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is now recognized as the most common cause of chronic liver disease in Asian and Western countries. NAFLD consists of a wide spectrum of conditions, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) which can progress to cirrhosis and hepatocellular carcinoma (HCC). Since there are no accurate noninvasive diagnostic methods for NASH, such as biochemical markers or imaging techniques, liver biopsy is needed to make a definite diagnosis. Unfortunately, liver biopsy is associated with pain, risks, hospitalization, high cost, and sampling errors. Moreover, the pathologic diagnosis of it is subjective and exhibits pathologist-dependent bias and no clear consensus exists regarding the definition of NASH. Therefore, in general practice, NAFLD is a term convenient for the diagnosis and management of these patients.

The diagnosis of NAFLD is based on the detection of steatosis by liver histology or imaging modalities, and the exclusion of other liver diseases, such as alcoholic liver disease and viral hepatitis [1]. However, imaging modalities have several limitations in this respect. The most important limitation of ultrasonography (US) and computed tomography (CT) is that they can detect only moderate to severe steatosis, which affects more than one-third of hepatocytes, and cannot detect mild steatosis [2].

As a result of the difficulties in diagnosis of NAFLD and NASH and referral bias, it is difficult to determine the true prevalence and prognosis of NAFLD and NASH. This review provides an update on the prevalence,

gender, ethnic variations, and prognosis of NAFLD and NASH.

Epidemiology

Nonalcoholic fatty liver disease patients are usually asymptomatic and transaminases are not helpful in making the diagnosis of NAFLD, since 50–80% of patients with it have normal transaminase levels. In addition to the difficulties in diagnosis of steatosis, this has made study on the epidemiology of NAFLD difficult in the general population.

Prevalence in general population

According to Japanese annual health check reports, 9–30% of Japanese adults demonstrate evidence of NAFLD by US [3–5]. Since it is known that almost 10–20% of individuals with NAFLD have NASH, the prevalence of NASH is estimated to be 1–3% of the adult Japanese population, an extremely large number of potential patients. These prevalences are similar in Asian and Western countries. Tominaga et al. [6] examined the prevalence of fatty liver on US examination in 810 Japanese children, and found a prevalence of NAFLD of 2.6% in the general pediatric population, with NAFLD identified in children as young as 6 years of age. Tsuruta et al. [7] conducted a population-based cross-sectional study in Nagano prefecture among 249 and 288 junior high school students in 2004 and 2007, respectively. The prevalence of NAFLD was 4.4 and 4.5% in 2004 and 2007. The presence of obesity and an alanine transaminase (ALT) level of 30 U/L or more were independent predictors of NAFLD (odds ratio 16.9, $P = 0.001$ and odds ratio 16.6, $P = 0.001$, respectively).

Recently, a nationwide survey of the etiology of liver cirrhosis was performed in Japan [8]. Hepatitis C-related cirrhosis accounted for 60.9% of cases, followed by hepatitis B at 13.9% and cirrhosis caused by NASH at 2.1%. The incidence of cirrhotic NASH will increase soon given the rising incidence of obesity. In the USA, among newly diagnosed chronic liver diseases, NAFLD accounted for 9% of the cases [9].

NAFLD and NASH in high-risk populations

It is well known that NAFLD and NASH are strongly associated with the presence of obesity and lifestyle-related diseases, especially type 2 diabetes mellitus.

According to annual health check findings in Japan, the prevalence of NAFLD by US increased with body mass index (BMI); it was 10–20% in non-obese individuals, around 50% in those with a BMI more than 25 kg/m^2 but less than 30 kg/m^2, and around 80% in those with a BMI over 30 kg/m^2 [3, 4, 10]. Jimba et al. [10] reported that the crude prevalence of NAFLD increased with deterioration of glucose homeostasis, from 27% in those with normal fasting glucose, 43% in those with impaired fasting glucose, and 62% in those with newly diagnosed and thus untreated diabetes.

Wanless et al. [11] reported that the prevalence of NAFLD was 70% in obese and 35% in lean patients and prevalence of NASH was 18.5% in obese and 2.7% in lean patients in a consecutive autopsy study. Dixon et al. [12] assessed the relationship between presence of NASH and complications of type 2 diabetes and hypertension among obese patients undergoing bariatric surgery. If an obese patient (BMI $>$ 35 kg/m^2) had neither condition, the prevalence of NASH was 7%; with diabetes, it was 62%, while with both of these conditions the prevalence was 75%. A synergistic effect of obesity and lifestyle-related diseases is evident in relation to NASH incidence.

It is conceivable that the duration and severity of obesity and lifestyle-related disease play a role in the development of NAFLD. Further study is needed to fully understand the risk factors for development of NAFLD.

Gender and ethnic variations

Concerning NAFLD and NASH, it is well known that these diseases exhibit age and gender differences in both prevalence and severity. These age and gender differences are caused by differences in prevalence of obesity and lifestyle-related diseases [13]. According to annual health check findings in Japan, prevalence of NAFLD in men was around 27% for all ages above 30 years [4] (Fig. 1). In contrast, in women it gradually increased from 7% in the 30s to 23% above 60 years of age. Among 492 biopsy-proven NASH patients diagnosed at our university hospital, male patents were significantly younger in age; however, the number of NASH cases in women is higher than that in men over 50 years of age (probably after menopause) (Fig. 2). Concerning cirrhotic NASH patients, the prevalence in women was higher than that in men (57% in women and 43% in men) [14]. In contrast, the prevalence of HCC was higher in men (38% in women vs. 62% in men) [15] (Fig. 3). This gender difference may be attributable to differences in exposure to risk factors for HCC, such as cigarette smoking. However, it was recently reported that estrogen-mediated inhibition of IL-6 production in mouse models explains the gender disparity in development of HCC [16]. Estrogen may play a role in the pathogenesis of HCC. Further studies are needed to clear the pathogenesis of gender differences.

Ethnic differences with respect to the prevalence and severity of obesity and metabolic syndrome are well

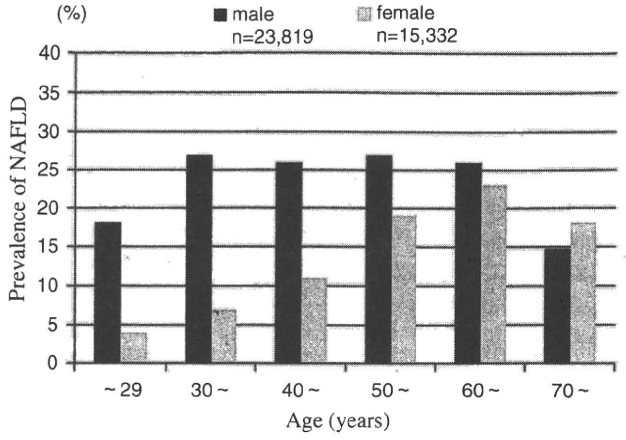

Fig. 1 The prevalence of NAFLD by age and sex. Among men, the prevalence of NAFLD is around 27% in each age group. In women the prevalence gradually increased with age. Updated data from Kojima et al. [4]

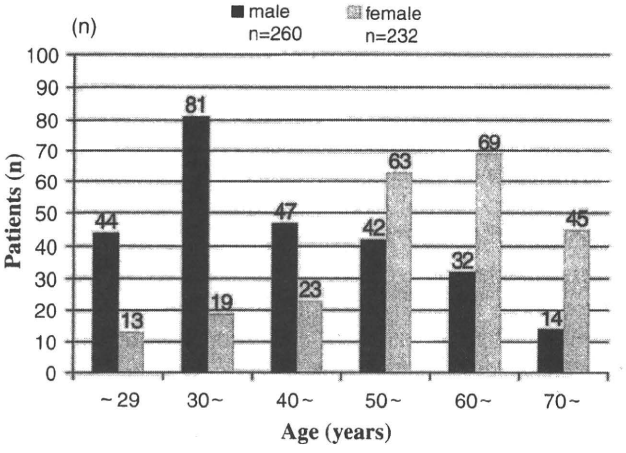

Fig. 2 Age and sex distribution of the patients with biopsy-proven NASH. Female patients were significantly more common above 50 years of age

documented. The definition of obesity varies among ethnic groups; for example, since Asians have been shown to have more visceral fat than Caucasians, the definition of obesity for Japanese is a BMI greater than 25, instead of greater than 30 as it is for Caucasians. Concerning NAFLD, among NAFLD patients who have undergone laparoscopic Roux-en-Y gastric bypass surgery for severe obesity, it was reported that the liver dysfunction in Japanese with severe obesity tended to be more severe than that in non-Japanese patients [17]. In the USA, it was reported that the frequency of hepatic steatosis varied significantly with ethnicity (Hispanics > whites > blacks) [18]. The differences in prevalence of hepatic steatosis were associated with similar differences in visceral adiposity and metabolic response. This accumulating evidence reveals that the effects of age, gender, and ethnic differences need to be considered when studying NAFLD and NASH.

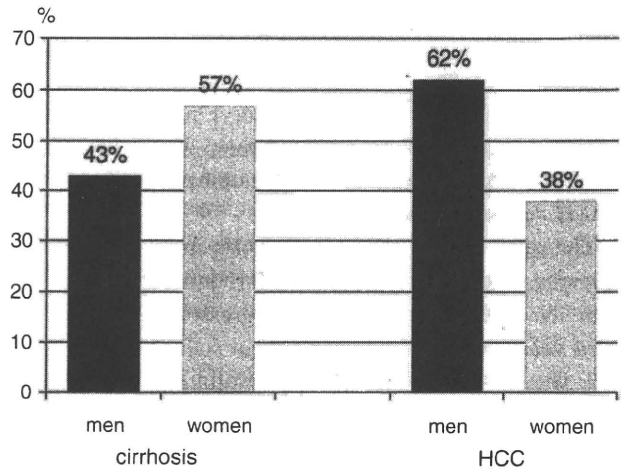

Fig. 3 The gender differences in prevalence of cirrhotic NASH and NASH with HCC. Among cirrhotic NASH, the prevalence in women was higher than that in men. In contrast the prevalence of HCC was higher in men

Several case reports of familial clustering of NAFLD and NASH have been reported [19], further suggesting that genetic factors may play roles in the pathogenesis of NAFLD. Recently, Romeo et al. [20] reported that genetic variation in PNPLA3 confers susceptibility to NAFLD. We [21, 22] reported that tumor necrosis factor gene single-nucleotide polymorphisms (SNPs) were associated with the progression to NASH from simple steatosis, and that adiponectin SNPs were associated with progression to liver fibrosis and insulin resistance, especially in females. These findings suggested that genomic analyses as well as lifestyle surveys are needed to clarify the pathogenesis and progression of NAFLD and enable the development of appropriate treatments for it.

Prognosis

There are several studies on paired biopsy analyses of simple steatosis, NAFLD and NASH, and the natural history of these diseases. In addition to the diagnostic shortcomings associated with NAFLD and NASH, there are several limitations in evaluation of the natural history of these slowly progressive liver diseases, including retrospective studies, referral bias (originating from tertiary referral due to histological diagnosis), limited generality of findings, short-term follow-up, and use of small samples. Furthermore, in histological longitudinal studies, second biopsies at study end are usually optional for ethical reasons, resulting in selection biases.

Histological course of NAFLD and NASH

It has been reported in several papers that the clinical course of simple steatosis is benign [23, 24]. However,

some recent studies have shown that, among patients with non-NASH (simple steatosis and steatosis with inflammation), around 40% progressed to fibrosis and over half developed NASH after 4–13 years [25, 26]. Although mortality significantly differs between NASH and non-NASH, histologically a substantial number of cases of non-NASH develop NASH.

The rates of progression of fibrosis in NAFLD among previous reports are very similar; around 40% of patients with NAFLD will develop progressive fibrosis over 3–14 years, around 40% will remain stable, and less than 30% will regress (Table 1). Age, insulin resistance, severe obesity, type 2 diabetes, high levels of aspartate transaminase (AST), and hypertriglyceridemia are risk factors for progressive fibrosis in NAFLD. Importantly, Adams et al. [25] revealed that changes in aminotransferase paralleled those in steatosis and inflammatory features but not fibrosis stage, indicating that it is important to reduce body weight to within normal range. Argo et al. [27] reported a systematic analysis of 221 cases of biopsy-proven NASH. In total, 38% had progressive fibrosis, 21% improved, and 41% remained stable over a mean duration of follow-up of 5.3 years. Age and presence of inflammation on the initial biopsy were independent predictors of progression to advanced fibrosis (bridging fibrosis or cirrhosis) in patients with NASH.

Natural history of NAFLD and NASH

According to Japanese annual health checks, the incidence and remission rates of NAFLD were 10 and 16%, respectively for mean time of 1.1 years. The long-term prognoses of NAFLD and NASH have been reported in population-based studies as well as cohort studies of biopsy-proven cases, with a maximum follow-up of 28 years [14, 23, 24, 26, 28–34] (Table 2). It is important to consider that patients with NAFLD who underwent liver biopsy had a significantly shorter survival compared with NAFLD patients who did not [28]. This leads to referral bias. Compared with individuals of the general population of the same age and gender, those with NAFLD had lower than expected survival, at a standardized mortality ratio from 1.34 to 1.69 according to American and Swedish studies [26, 28, 30]. These studies also revealed that simple steatosis or non-NASH has a benign clinical course without excess mortality and that only the survival of patients with NASH was reduced [23, 24, 26, 29, 30]. The most common causes of death in NAFLD and NASH were coronary artery disease and malignancy, followed by liver-related death. In the general population of Minnesota, USA, liver-related death is the 13th leading cause of death. These studies confirmed increases in cardiovascular disease and liver-related death in patients with NASH. In NASH, the incidences of cirrhosis were 5–25% during around 7-year follow-up periods.

Several findings have revealed that individuals with NAFLD have a high risk of developing type 2 diabetes and cardiovascular diseases, possibly due to possession of the same risk factors for NAFLD and these other diseases, such as obesity and insulin resistance. However, recent findings have suggested that the association between NAFLD and cardiovascular disease appears to be independent of classical risk factors like type 2 diabetes [35]. Further studies are needed regarding this important evidence.

Natural history of cirrhotic NASH

To elucidate the natural history of cirrhotic NASH, we [14] analyzed the natural history of 68 cases of biopsy proven cirrhotic NASH and compared them with 69 cases of cirrhosis matched based on hepatitis C (LC-C). Mean NASH patient age was 62.7 years (range 16–89 years), with a median follow-up period of 41 months. Although the outcome of cirrhotic NASH was better than that for LC-C, cirrhotic NASH followed a similar course to that of LC-C (5-year cumulative HCC development rate 11.3% for cirrhotic NASH and 30.5% for LC-C, 5-year survival rates of 75.2 and 73.8%, respectively). A total of 18 patients with cirrhotic NASH died and 1 patient underwent liver transplantation. HCC was the leading cause of death (9 patients), followed by liver failure (6 patients). Occurrence of HCC and Child–Turcotte–Pugh score were significant risk factors for mortality in cirrhotic NASH patients on multivariate analysis. The survival of cirrhotic NASH patients with or without HCC showed significant difference (Fig. 4). Sanyal et al. [33] also compared 152 cirrhotic NASH patients with a median age of 55 years with 150 matched LC-C. They reported that there were no significant

Table 1 Paired biopsy studies on NAFLD and NASH

References	Number (*n*)	Diagnosis	Years of follow-up means (range)	Changes in fibrosis stage		
				Regressed (%)	No change (%)	Progressed (%)
Adams et al. [25]	103	NAFLD	3.2 (0.7–21.3)	29	34	37
Ekstedt et al. [26]	70	NAFLD	13.8 (10.3–16.3)	16	43	41
Argo et al. [27]	221	NASH	5.3 (1.0–21.3)	21	42	38

Table 2 Studies on long-term mortality in NAFLD and NASH

References	Diagnosis	Number (*n*)	Average follow-up (years)	Cirrhosis prevalence *n* (%)[a]	HCC *n*[a]	Death	
						Overall *n* (%)	Liver-related/ overall (%)
Adams et al. [28]	NAFLD[b]	420	7.6	21 (5)	2 (0)	53 (12.6)	13.2
Ekstedt et al. [26]	NAFLD[c]	129	13.7	10 (7.8)	3 (0)	26 (20.2)	7.7
Rafiq et al. [29]	NAFLD[c]	131	18.5	NR	1 (0)	78 (59.5)	15.4
Söderberg et al. [30]	NAFLD[c]	118	21	9 (7.6)	5 (0)	47 (39.8)	19.1
Teli et al. [23]	Simple steatosis[c]	40	9.6	0	0 (0)	14 (35)	0.0
Dam-Larsen et al. [24]	Simple steatosis[c]	170	20.4	2 (1.2)	0 (0)	48 (28.2)	2.1
Evans et al. [31]	NASH[c]	26	8.7	1 (4)	0 (0)	4 (15)	0.0
Hui et al. [32]	Cirrhotic NAFLD[c]	23	7.0	100	0 (0)	6 (26)	83.3
Sanyal et al. [33]	Cirrhotic NAFLD[c]	152	10	100	13 (3)	29 (19.1)	69.0
Yatsuji et al. [14]	Cirrhotic NAFLD[c]	68	3.4	100	21 (14)	19 (27.9)	78.9
Söderberg et al. [30]	Cirrhotic NAFLD[c]	9	21	100	3 (0)	8 (88.9)	50.0
Ascha et al. [34]	Cirrhotic NAFLD[b]	195	3.2	100	25 (0)	NR	NR

NR not reported

[a] Data are presented as the number at the end of the follow-up period with the number at baseline in parentheses

[b] The diagnosis was made by imaging or liver biopsy

[c] The diagnosis was made by liver biopsy

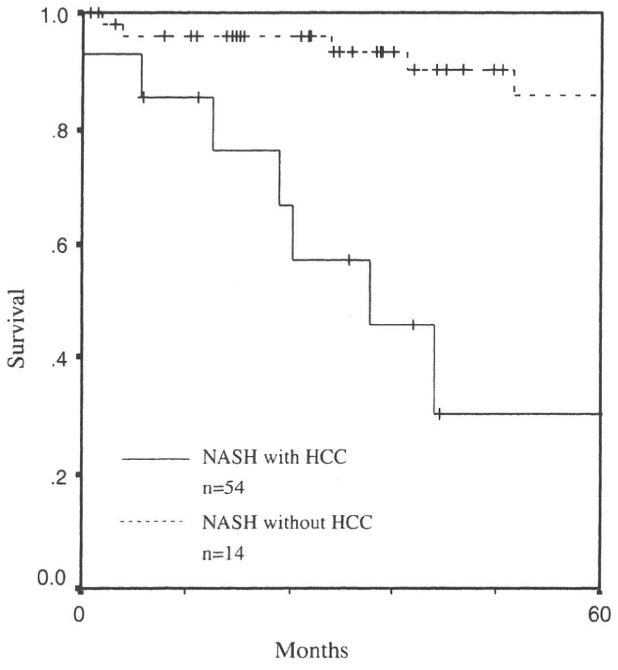

Fig. 4 The survival of cirrhotic NASH patients with or without HCC. HCC was a significant risk factor for death of cirrhotic NASH patients

across-group differences in mortality in patients with Child class B or C cirrhosis, except that cirrhotic NASH had a significantly lower risk of development of HCC (10/149 vs. 25/147 patients at risk; $P < 0.01$). As expected, cardiovascular mortality was significantly higher in patients with NASH. In contrast, of our cirrhotic NASH patients, only one died of a cardiovascular event.

Recently, Ascha et al. [34] compared 195 cirrhotic NASH patients with 315 LC-C patients. During follow-up periods lasting on average 3.2 years, 25/195 (12.8%) of cirrhotic NASH and 64/315 (20.3%) of LC-C patients developed HCC ($P = 0.03$). The yearly cumulative incidence of HCC was found to be 2.6% in patients with cirrhotic NASH, compared with 4.0% in patients with LC-C ($P = 0.09$). The incidence of HCC in their study was similar to our results.

All previous studies confirmed that cirrhotic NASH has a similar course to LC-C [14, 32–34] (Table 2). In the natural history of cirrhotic NASH, liver disease is the main cause of morbidity and mortality. Regular screening for complications of liver cirrhosis and HCC is thus extremely important for cirrhotic NASH patients.

NASH and HCC

Concerning underlying liver disease in HCC, hepatitis C accounts for 70% of all cases of HCC, followed by hepatitis B at 16% in Japan. The incidence of HCC of non-viral cause has gradually increased. According to a nationwide survey of the etiology of liver cirrhosis [8], HCC caused by cirrhotic NAFLD accounted for 1.6%. Malik et al. [36] reviewed the records of cirrhotic NASH patients who underwent liver transplantation by using a prospectively collected database from a single center. Of 98 NASH

patients 17 (17.3%) were diagnosed as having HCC. NASH-related HCC accounted for 3.8% of all cases of HCC. Malik et al. concluded that patients with cirrhotic NASH are at risk for developing HCC. The exact mechanism of the development of HCC in NASH remains unclear; however, the pathophysiological mechanisms related to the development to NASH, such as insulin resistance, oxidative stress, and inflammatory cytokines, likely contribute to the carcinogenic potential of NASH [37–39].

According to several case reports of HCC in NASH patients, the median age of NASH patients with HCC was around 70 years [15, 40, 41]. We [15] found that older age and histological severe fibrosis were the most important risk factors for the development of HCC in NASH. This is true of any underlying liver disease, and fibrosis is the single most important risk factor for HCC. It is known that cirrhosis is present in about 80% of HCC patients with NASH.

We also assessed the outcomes and disease recurrence in 34 NASH patients with HCC [42]. The 5-year survival rate was 55.2% and the cumulative recurrence of HCC at 5 years was 69.8% in curatively treated cases of HCC in NASH. Concerning high HCC recurrence, the HCC may be of multicentric origin. Zen et al. [43] reported a NASH patient with HCC for whom liver histology suggested a multicentric origin. These features are similar to HCC based on viral hepatitis. Regular screening for HCC is thus needed for NASH patients with HCC even if they receive curative treatment.

Burned-out NASH

End-stage cirrhotic NASH patients usually exhibit 'burned-out NASH', in which steatosis disappears with necroinflammatory changes, leading to the diagnosis of end-stage NASH as cryptogenic cirrhosis [44]. It has also been acknowledged that a substantial proportion of patients with cryptogenic cirrhosis have previously unrecognized NAFLD, since patients with cryptogenic cirrhosis have high prevalences of obesity and/or type 2 diabetes and after liver transplant some patients develop steatosis and steatohepatitis in sequence. Prospective cohort studies of biopsy-proven NASH will reveal the clinical features of end-stage cirrhotic NASH.

Summary

The true prevalence and natural history of NAFLD and NASH are still unclear. However, accumulating evidence suggests that the prevalence of NAFLD in Japanese general populations ranges from 9 to 30%, and those of cirrhotic NASH and NASH-related HCC have been increasing. Among premenopausal women, NASH is relatively uncommon. The survival rate of patients with NASH may be slightly less than that of the general population, because cardiovascular mortality and liver-related mortality are higher than those in the general population. In cirrhotic NASH, HCC, and liver failure are the main causes of morbidity and mortality. Regular screening for complications of liver cirrhosis and HCC is extremely important for cirrhotic NASH patients.

The high prevalence of NAFLD indicates that it can complicate other chronic liver diseases and contribute to progression of these diseases. The effects of NAFLD on other liver diseases and the association between NAFLD and cardiovascular disease also need to be studied.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Prospective study of hepatocellular carcinoma in nonalcoholic steatohepatitis in comparison with hepatocellular carcinoma caused by chronic hepatitis C

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Abstract
Background This study was performed to clarify the outcomes and recurrence of hepatocellular carcinoma (HCC) in nonalcoholic steatohepatitis (NASH) in comparison with the data for HCC caused by hepatitis C virus (HCV) infection.
Methods Data for 34 NASH patients with HCC (NASH-HCC) were analyzed prospectively, and data for 56 age- and sex-matched patients with HCC due to HCV chronic liver disease (HCV-HCC) were collected retrospectively. After the initial treatment for HCC, patients were followed regularly at least every 4 months by performing clinical examinations, serum liver function tests, monitoring alpha-fetoprotein and des-gamma-carboxy prothrombin, and utilizing various imaging modalities.
Results The five-year survival rate was 55.2% and the cumulative recurrence of HCC at 5 years was 69.8% in treated cases of NASH-HCC. The NASH-HCC and HCV-HCC groups showed similar survival and recurrence rates. Of the 16 NASH-HCC patients curatively treated, recurrence was detected more than 2 years after the initial treatment in 9. Three patients showed intrahepatic recurrences away from the initial HCC, and 3 patients showed a change in tumor marker production after treatment of the initial HCC. The size of the HCC and the stage of fibrosis were significant risk factors for HCC recurrence in NASH-HCC.
Conclusions HCC recurrence was very high in NASH, and the HCC may be of multicentric origin, similar to HCC based on viral hepatitis. Regular screening for HCC is extremely important for NASH patients with HCC, even after curative treatment. This study confirmed that NASH-HCC has a similar course to that of HCV-HCC.

Keywords Nonalcoholic steatohepatitis · Hepatocellular carcinoma · Natural history · Recurrence

Abbreviations

HCC	Hepatocellular carcinoma
NASH	Nonalcoholic steatohepatitis
HCV	Hepatitis C virus
NASH-HCC	NASH patients with HCC
HCV-HCC	HCC due to HCV chronic liver disease

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer-related mortality [1]. According to the most recent nationwide Japanese registration data, primary liver cancer ranks third for men and fifth for women as causes of death from malignant neoplasm [2]. The latest nationwide report registered every two years by the Liver Cancer Study Group of Japan showed that hepatitis C virus (HCV) is the most common underlying liver disease in HCC [3]. HCV-related HCC accounts for 70% of all cases of HCC, followed by hepatitis B virus (HBV) at 16%. However, the incidence of HCV-related HCC has gradually decreased in recent years, and that of HCC in cases of nonviral liver

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disease has gradually increased. According to our hospital database, increasing numbers of HCC cases arising from nonalcoholic steatohepatitis (NASH) have been seen, and such cases have accounted for 4% of all cases of HCC each year since 2000. Because of the dramatic increase in cases of NASH in Japan, it is logical that the incidence of HCC in patients with NASH has also increased.

NASH shows a wide range of severities, from minimal fibrosis to cirrhosis, so it is important to clarify the natural history of each stage (especially cirrhotic NASH) in order to assess the severity of liver disease and to determine how to manage these patients. A prospective longitudinal study of NASH patients with advanced fibrosis (bridging fibrosis and cirrhosis) was started at Tokyo Women's Medical University Hospital in 1990. We have previously provided case reports describing the characteristic features of HCC in NASH and the natural history of NASH with advanced fibrosis, as well as comparing the clinical features between cirrhotic NASH and cirrhosis caused by HCV [4–6]. These studies have shown that older age, low levels of AST, a histologically low grade of activity, and advanced fibrosis are significant risk factors for the development of HCC based on multivariate logistic regression analysis. HCC is a leading cause of mortality in NASH with cirrhosis. Several other studies have provided data regarding the natural history of NASH [7–9]. These data appear to confirm the eventual development of HCC in significant numbers of NASH patients.

Unfortunately, there have been no reports regarding outcomes and recurrence of HCC in NASH patients. Accordingly, the present study was performed to clarify the outcomes and recurrence of HCC in NASH by comparing the clinical features of HCC due to NASH with those of HCC caused by HCV infection, as well as to define the risk factors for the recurrence of HCC and the mortality of HCC in NASH patients undergoing curative treatment.

Patients and methods

Subjects

From 1990 to 2007, 412 Japanese patients at Tokyo Women's Medical University were diagnosed as having biopsy-proven nonalcoholic fatty liver disease. Among them, 34 NASH patients had HCC (NASH-HCC). All 34 patients gave informed consent to participate in a study examining the natural history of HCC in NASH. Data on age- and sex-matched patients with HCC due to HCV (HCV-HCC) who had almost the same treatment modality and were concurrently managed at our hospital were also collected retrospectively as a control. Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. The clinical data for the NASH patients were collected prospectively.

Definition

Diagnosis of NASH was based on the following criteria: [1] detection of steatohepatitis on liver biopsy [2], intake of <100 g of ethanol per week (as confirmed by the attending physician and family members in close contact with the patient), and [3] appropriate exclusion of other liver diseases [10–12]. All patients were negative for hepatitis B (HB) surface antigen and antibody to HCV and/or HCV RNA by polymerase chain reaction analysis. The serum alpha fetoprotein (AFP) levels were determined by enzyme-linked immunosorbent assay with a commercially available kit (ELISA-AFP, International Reagents, Kobe, Japan). The serum des-gamma-carboxy prothrombin (DCP) levels were determined by sensitive enzyme-linked immunoassay (Eitest PIVKA-II kit, Eisai Co., Tokyo, Japan) according to the manufacturer's instructions.

To evaluate the liver specimens, hematoxylin and eosin, silver impregnation for reticulin fiber, Mallory stain for collagen fiber, Victoria blue for copper-binding protein and elastic fiber, and Perls' Prussian blue stain for iron were carried out. All liver biopsy specimens were examined for fibrosis, and the NAFLD activity score (NAS) was calculated [13, 14]. An NAS of more than 4 resulted in a diagnosis of NASH. Staging of fibrosis and grading were also assessed. Patients in the HCV group were shown to be positive for HCV RNA by a quantitative polymerase chain reaction assay. They had either not been treated with interferon or were virological nonresponders to interferon therapy.

HCC was diagnosed histologically or by the detection of consistent findings using at least two imaging techniques: ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and selective hepatic arteriography [15]. Vascular invasion was assessed by US, dynamic CT, and angiography. The clinical stage (TNM classification) was defined according to the Liver Cancer Study Group of Japan [16]. HCC was pathologically graded based on Edmondson–Steiner criteria [17].

Obesity was defined as a body mass index of more than 25 according to the Japanese criteria. The diagnosis of type II diabetes mellitus was based on the WHO criteria, or current treatment for type II diabetes. Hyperlipidemia was diagnosed if the patient was being treated with lipid-lowering medications or had elevated levels of total cholesterol (>220 mg/dL) and/or triglycerides (>150 mg/dL) on at least three occasions. Hypertension was diagnosed if the patient was undergoing antihypertensive therapy or had a blood pressure of more than 140/90 mmHg on at least three occasions.

Management of the patients

A complete history was obtained, and a physical examination was carried out for all patients. None of the patients were undergoing hormone replacement therapy for menopause or had undergone liver transplantation. The following laboratory parameters were measured at the time of the diagnosis of HCC and during the follow-up period: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase, gamma-glutamyl transpeptidase, albumin, white blood cell count, red blood cell count, hemoglobin, platelet count, prothrombin time, hepaplastin test, AFP, and DCP.

The first choice for treatment is usually hepatectomy or radiofrequency ablation (RFA). Surgical treatment was decided upon by assessing resectability based on both tumor progression and liver functional reserve. RFA was generally performed for small tumors <3 cm in size and <3 in number. Transcatheter arterial chemoembolization (TACE) was selected for the other HCC patients. Curative treatment was defined as complete macroscopic and/or imaging modalities.

After the initial treatment for HCC, patients were scheduled to be monitored regularly at least every 4 months via clinical examinations, serum liver function tests, AFP, DCP, and US. CT, MRI, and/or selective hepatic angiography were performed in patients with suspected recurrence of HCC based on tumor markers and/or US.

We did not perform any special therapy for NASH. Advice regarding diet was given to the NASH patients who were obese, and several drugs were given to the patients with DM and/or HT and/or hyperlipidemia.

Statistical analysis

Analysis was performed with the SPSS statistical software package (SPSS Inc., Chicago, IL, USA). The Mann–Whitney test or the chi-square test was used to compare baseline variables between the NASH-HCC and HCV-HCC groups. The start date for analysis was the date of the initial diagnosis of HCC. The patients in both groups were followed until they died and were censored at the time of their last clinic visit. The primary outcomes measured were recurrence of HCC among patients after their initial curative treatments and overall survival. The time frame for each outcome was defined as the time from the first diagnosis of HCC until the onset of the relevant event. Time to failure analysis (Kaplan–Meier) was performed, and the log-rank test was used for comparison between the NASH and HCV groups. To clarify risk factors for the recurrence of HCC among NASH patients with HCC who had curative treatment, Cox's proportional hazards analysis was used. Age, obesity, diabetes, hyperlipidemia, hypertension, laboratory data, size of HCC, number of HCCs, and treatments for HCC were examined in the Cox proportional hazards model. A P value of 0.05 or less was considered statistically significant.

Results

Eleven NASH patients were diagnosed with HCC during follow-up for NASH. The other 23 patients, who were simultaneously diagnosed with NASH and HCC, were referred to our hospital from local hospitals for diagnosis and treatment of liver tumor. Baseline demographic, clinical, and laboratory data for the patients with NASH-HCC or HCV-HCC are shown in Table 1. The median age of the patients with NASH-HCC was 70.14 years, with a range of 54–89 years. The mean age of the patients with HCV-HCC was 71.69 years (range 51–85 years). The NASH-HCC patients had a higher prevalence of obesity and lifestyle-related diseases, and the between-group differences in the prevalence of obesity, diabetes mellitus, and hyperlipidemia were significant. Three NASH-HCC patients had complications of cardiovascular disease, including 1 patient with ischemic heart disease. Transaminases were significantly higher in HCV-HCC patients, while the γGTP level was significantly higher in NASH-HCC patients. Histologically, noncancerous areas showed mild fibrosis in 4 patients (1 with F1, 3 with F2), bridging fibrosis in 6, and cirrhosis in 24, but activity varied among the patients with NASH-HCC.

Concerning tumor markers, 12 patients with NASH-HCC (35.3%) exhibited elevated AFP levels, and 18 patients (56.3%) showed elevated DCP levels, including 8 who had elevated DCP levels with normal levels of AFP. In NASH-HCC, the positive rate of DCP tended to be higher than that of AFP. Altogether, 20 patients (58.8%) showed elevations of at least one tumor marker. In contrast, 39 patients (69.6%) with HCV-HCC showed elevation of AFP and 19 (41.3%) showed elevation of DCP. Twenty-four NASH-HCC patients (70.6%) had a single nodule; there were 2 nodules in 3 patients (8.8%) and more than 3 in 7 patients (20.6%). The median diameter was 24 (8–83) mm: <20 mm in 17 patients, 20–50 mm in 11 patients, and more than 50 mm in 6 patients. We had measured anti-HBc antibody in 32 NASH-HCC patients and 41 HCV-HCC patients. The prevalence of anti-HBc antibody was 28% in NASH-HCC and 39% in HCV-HCC, and the difference between the groups was not significant. In addition, none of the NASH-HCC patients had a high HBc antibody titer.

Treatment for NASH-HCC consisted of surgery for 15 patients (48%), RFA for 3 (10%), and TACE for 13 (42%). Three patients did not receive any treatment; this lack of

Table 1 Patient profile

	NASH-HCC	HCV-HCC	*P* value
No.	34	56	
Gender (F/M)	13/21 (38/62%)	23/33 (41/59%)	NS
Age	70.14 ± 7.59	71.69 ± 8.34	NS
HCC (single nodule)	23 (68%)	30 (55%)	NS
Tumor size (mm)	21.37 ± 20.2	23.88 ± 18.0	NS
TNM (1/2/3)	13/15/6 (38/44/18%)	19/21/16 (34/38/28%)	NS
Therapy (S/R/T)	15/3/13 (48/10/42%)	26/6/24 (46/11/43%)	NS
Child (A/B or C)	24/10 (71/29%)	40/16 (71/29%)	NS
Obesity	22 (65%)	19 (34%)	0.004
Diabetes mellitus	25 (74%)	13 (23%)	0.000
Hyperlipidemia	10 (29%)	2 (8%)	0.008
Hypertension	14 (41%)	17 (30%)	NS
BMI (kg/m^2)	26.1 ± 3.8	24.2 ± 4.1	0.001
Alb (g/dL)	3.8 ± 0.44	3.58 ± 0.50	0.052
T-Bil (mg/dL)	0.825 ± 0.50	0.627 ± 0.38	NS
AST (IU/L)	42.0 ± 27.0	61.5 ± 30.4	0.004
ALT (IU/L)	37.5 ± 25.4	61.5 ± 30.8	0.008
ALP (IU/L)	242 ± 233.6	338 ± 232.1	NS
γ-GTP (IU/L)	102 ± 158.8	55 ± 92.5	0.02
Platelet (×10^4/μL)	11.7 ± 5.9	10.1 ± 5.5	0.09
Prothrombin time (%)	82.0 ± 16.1	82.9 ± 15.4	NS
AFP (ng/mL)	7.0 ± 283	24 ± 19626	0.007
DCP (mAU/mL)	50.5 ± 4424	29.5 ± 2108	NS

Data are expressed as median ± standard deviation (SD) or number of patients. Percentages are shown in parentheses

S/R/T surgery/radiofrequency ablation/transcatheter arterial chemoembolization, *Child* Child–Pugh classification

treatment was due to liver failure in 2 patients, and was the patient's decision in 1 patient. The difference in TNM classifications between NASH-HCC and HCV-HCC patients was not significant (TNM stage 1, 2, and 3 classifications comprised 38, 44, 18% of NASH-HCC, and 34, 33, 28% in HCV-HCC, respectively).

Liver specimens of NASH-HCC were obtained from 21 patients. Well-differentiated HCC was observed in 7 patients, moderately differentiated in 12, and poorly differentiated in 2. The histological pattern of HCC was evaluated in 16 patients, and the trabecular type was found to be the most common (81.3%).

According to macroscopic findings and/or imaging modalities, 16 NASH-HCC patients received curative treatment: 13 received surgery, and 3 RFA. All patients who had undergone curative treatment had HCC at an earlier stage, such as TNM stage 1 or 2. The median follow-up period was 35.4 (3–156) months after the initial diagnosis of HCC in NASH patients. One patient was lost to follow-up. Thirteen NASH-HCC patients died (12 due to HCC or hepatic failure and 1 due to gastric cancer). By contrast, 32 HCV-HCC patients received curative treatment and 15 of these HCV patients died (14 due to HCC or hepatic failure) during 2–151 months of follow-up. The five-year survival rate of HCC was 55.2% in all NASH-HCC versus 50.6% in all HCV-HCC patients (Fig. 1).

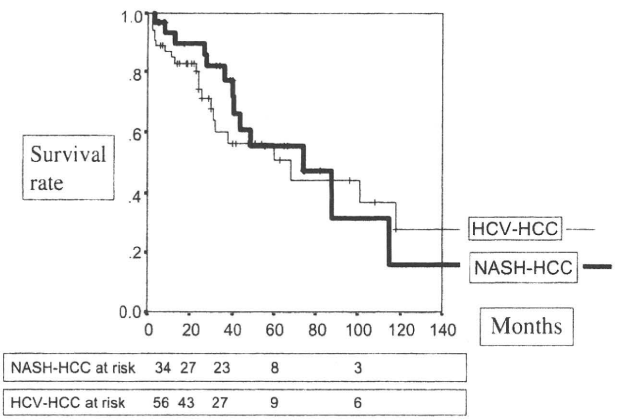

Fig. 1 Survival curves for the 34 NASH-HCC patients and 56 HCV-HCC patients. The survival rates at 1, 3, and 5 years were 93.5, 82.5, 55.2% in NASH-HCC and 83.0, 60.0, 50.6% in HCV-HCC, respectively

Kaplan–Meier analysis showed that there was no significant difference between the two groups.

Recurrence among patients receiving curative treatment

Among the 16 NASH-HCC patients who underwent curative treatment, recurrence during the follow-up period was detected in 14 (88%). Of these patients, recurrence was

detected more than 2 years after the initial treatment in 9 (Fig. 2a). The median duration between initial HCC and initial recurrence was 34.8 months (3–80 months). Eleven patients had their recurrences in the liver and 3 patients in other organs: brain in 1, bone in 1, and lymph nodes in 1. The longest duration between the initial HCC development and recurrence was 80 months. Figure 2b shows the interval between the initial diagnosis of the HCC and the diagnosis of the initial recurrence in the HCV-HCC cases. Recurrence during the follow-up period was detected in 22 (69%) of the HCV-HCC cases. In 10 of the HCV-HCC patients, recurrence was detected more than 2 years after the initial treatment.

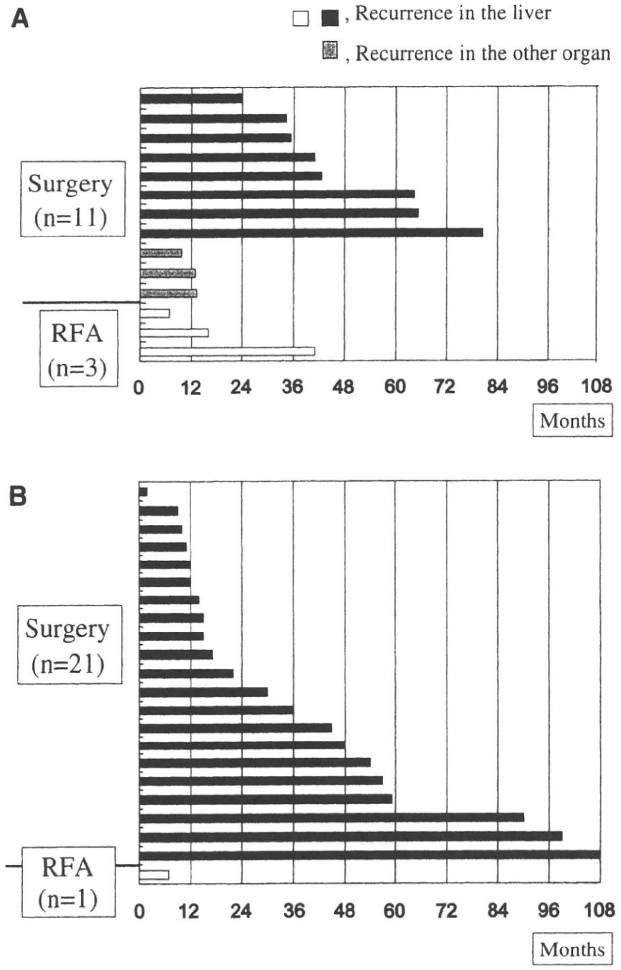

Fig. 2 The results for the interval between the initial diagnosis of HCC and the diagnosis of the initial recurrence according to treatment were as follows. **a** Sixteen NASH-HCC patients received curative treatment and recurrence was detected in 14 of them. Recurrence was detected more than 2 years after the initial treatment in 9 of the NASH-HCC patients. **b** Thirty-two of the HCV-HCC patients received curative treatment, and during the follow-up period recurrence was detected in 22 of them. Recurrence was detected more than 2 years after the initial treatment in 10 of the HCV-HCC patients. *RFA*, radiofrequency ablation

Concerning tumor marker data at the time of the initial recurrence of NASH-HCC, 5 patients had elevated AFP levels and 3 elevated DCP levels. Three patients showed elevation of different tumor markers at the time of initial HCC and at recurrence; i.e., they showed elevation of DCP (or AFP) without elevation of AFP (or DCP) at the time of initial HCC, and elevation of AFP (or DCP) without elevation of DCP (or AFP) at the time of recurrence (Fig. 3). With regard to the characteristic features of the 11 initial liver recurrence cases of HCC, 5 patients had a single nodule, 4 had 2 nodules, and the other 2 had more than 3. The median diameter of the HCC was 20 (15–40) mm. The recurrences were primarily located on the same side of the liver as the initial HCC: i.e., right (left) lobe in the initial HCC and right (left) lobe in the initial recurrence HCC. However, 3 patients showed recurrence in the opposite lobe.

The five-year recurrence rate for the 16 NASH-HCC patients who underwent curative treatment was 69.8, versus 83.1% in the 32 HCV-HCC patients (Fig. 4). Kaplan–Meier analysis showed that there was no significant difference between the two groups.

Risk factors for recurrence in HCC patients receiving curative treatment for HCC

Cox's proportional hazards analysis was used to identify risk factors for recurrence in the 16 NASH-HCC patients and the 32 HCV-HCC patients curatively treated by surgery or RFA. Table 2A shows the results in the NASH-HCC group, and tumor size ($P = 0.006$, HR 1.23) and fibrosis grade ($P = 0.002$, HR 91.8) were identified as risk factors for recurrence. Table 2B shows the results for HCV-HCC. Since we did not perform liver biopsy in the HCV-HCC cases, fibrosis grade was not included in the analysis. Obesity, DM, and number of HCCs were identified as risk factors for recurrence.

Discussion

There have been few reports regarding the natural history of NASH, and, as such, details related to progression of the disease remain unclear [4–9]. Moreover, there has been no prospective cohort study regarding the prognosis and disease recurrence of NASH-HCC patients. This report provides the first data from a prospective study of the outcomes and disease recurrence of biopsy-proven NASH-HCC patients who underwent follow-up using a predefined screening protocol for HCC at a single tertiary-care hospital. None of the patients underwent bariatric surgery or liver transplantation. Only 1 patient was lost to follow-up. Therefore, our study population was suitable for assessing

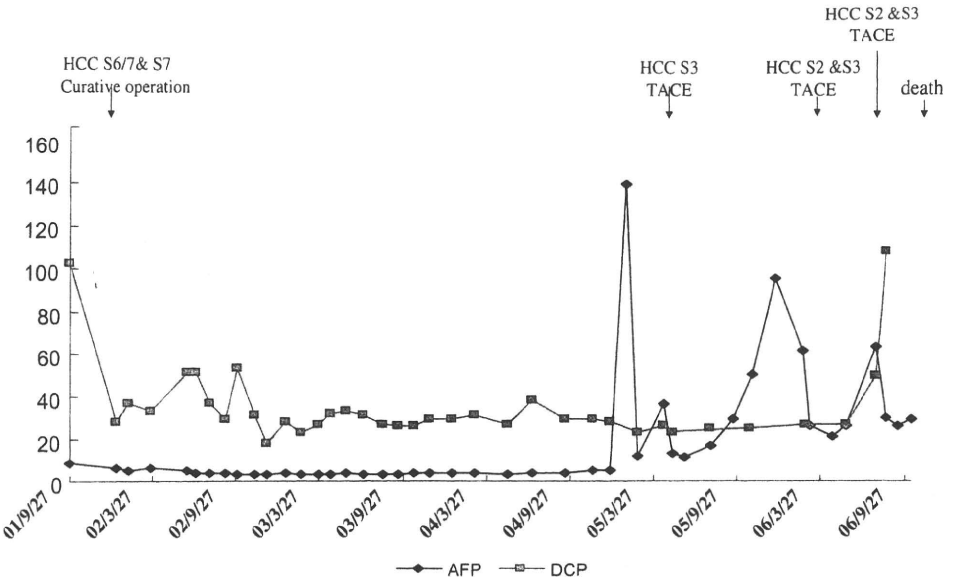

Fig. 3 An obese female NASH patient with diabetes and hypertension developed HCC at the age of 71 years. Curative surgical resection was performed. At that time, the DCP level was elevated. The initial recurrence of HCC was diagnosed three and a half years later. At that time, the AFP level was elevated

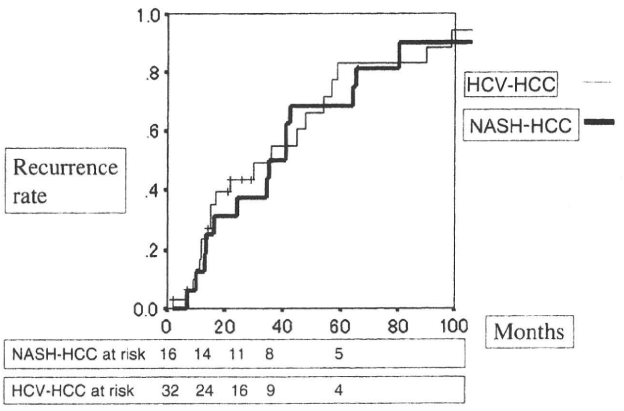

Fig. 4 The cumulative recurrence of HCC among the 16 NASH-HCC patients who received curative treatment. The cumulative recurrences at 1, 3, and 5 years were 12.5, 50, 69.8% in NASH-HCC, and 16.7, 54.9, 83.1% in HCV-HCC, respectively

Table 2 Risk factors for recurrence in the 16 NASH-HCC patients and the 32 HCV-HCC patients curatively treated by surgery or RFA (Cox's proportional hazards model was used)

	HR	*P* value	95% CI
(A) 16 NASH-HCC[a]			
HCC size	1.23	0.006	1.07–1.51
Fibrosis grade	91.8	0.002	1.37–6160
(B) 32 HCV-HCC[b]			
Number of HCCs	2.01	0.01	1.19–3.41
Obesity	3.77	0.009	1.38–10.3
DM	5.04	0.016	1.35–18.8

[a] Factors: gender, age, size of HCC, number of HCCs, fibrosis grade, obesity, DM, hyperlipidemia, hypertension, ALT, platelet, prothrombin time

[b] Factors: gender, age, size of HCC, number of HCCs, obesity, DM, hyperlipidemia, hypertension, ALT, platelet, prothrombin time

the prognosis and disease recurrence of NASH-HCC patients. Moreover, to clarify the outcomes and recurrence of NASH-HCC, we also compared the clinical features of NASH-HCC with retrospective data for HCV-HCC patients.

Recurrences in the early period after curative treatment were considered to be mostly attributable to intrahepatic metastasis, whereas recurrences during the later follow-up years after curative treatment can be presumed to have been of multicentric origin. The long duration between the initial HCC and the the initial recurrence, the location of the intrahepatic recurrence away from the initial HCC, and the change in tumor marker production after treatment of the initial HCC are consistent with the hypothesis that the HCC recurrence may have been, at least in some of our NASH patients, a new primary HCC. HCC in NASH may be of multicentric origin, similar to HCC based on viral hepatitis.

Cox's proportional hazards analysis of risk factors for HCC recurrence selected the stage of fibrosis of noncancerous areas and the size of the HCC as risk factors. The factor responsible for the development of the primary HCC, namely fibrosis stage, would affect de novo carcinogenesis, whereas the factor related to the severity of the primary HCC (i.e., its size) would likely affect the possibility of intrahepatic metastasis. It was thought that DM and obesity were not identified as risk factors for recurrence in NASH-HCC because of the higher prevalence of DM and obesity in NASH-HCC.

Zen et al. [18] have reported a NASH patient with HCC for whom liver histology suggested a multicentric origin. Their patient developed 4 nodules at different times after the diagnosis of NASH. Liver biopsies were performed from each nodule. The first nodule was pathologically diagnosed as a pseudolymphoma, the second a moderately

differentiated HCC, the third a well-differentiated HCC, and the fourth a dysplastic nodule. They provided histological data suggestive of multicentric carcinogenesis.

It has been reported that the recurrence rate of HCC at 5 years is 79% after hepatic surgery and 83% after percutaneous ethanol injection [19, 20]. Sasaki et al. [21] has attempted to clarify the difference in the risk of recurrence after curative hepatic resection between patients with HB and hepatitis C-related HCC. The five-year recurrence rate of hepatitis C-related HCC was reported to be 76%, and that of HB 66%. The risk of recurrence of the initial HCC was 1.93 times (95% confidence interval 1.27–2.97) greater in hepatitis C-related HCC than in HB-related HCC. Our NASH-HCC data suggest that the recurrence rate of NASH-HCC is similar to that of HCV-HCC, based on our retrospective data for HCV-HCC and Sasaki's reports of HCV.

The prognosis of HCC remains poor, due to the fact that HCC is often presented at an advanced stage, is associated with multicentric carcinogenesis, and usually arises in conjunction with advanced liver disease. According to the Liver Cancer Study Group of Japan, the overall survival rate after initial HCC diagnosis at 5 years is 35.4%, and the rates for those undergoing surgery and TACE are 53.4 and 22.6%, respectively [3]. Toyoda et al. [22] have reported that the survival rate for patients with viral marker-negative HCC is significantly lower than that for patients with viral HCC. Our studies of NASH-HCC showed a more favorable survival rate than theirs. However, histological diagnosis is required for the diagnosis of NASH; as such, our NASH patients with HCC consisted only of those with sufficient liver function for them to be able to undergo liver biopsy or surgery. In other words, these patients were in a relatively good condition. Further study is therefore required to clarify the survival of all patients with NASH-HCC. A study that includes only patients who have developed HCC during follow-up after a diagnosis of NASH would help to elucidate the natural history of NASH-HCC.

Taura et al. [23] have analyzed the reasons for the recent significant improvement in the survival of patients with HCC who have undergone hepatectomy. They found that the management of solitary intrahepatic recurrence is considered to be a major contributory factor to the improvement in survival after recurrence, as well as overall survival. The results of our present study clearly show that recurrence of HCC in NASH is very high, and that after curative HCC treatment, regular follow-up based on monitoring tumor markers of AFP and DCP and imaging is extremely important, since advances in HCC management can only come from treatment of small tumors that are diagnosed early.

It has been reported that the detection rate of elevated serum DCP for small liver cancer is approximately 44.3%, and that DCP appears to be a more useful tumor marker than AFP [24, 25]. In our study, the positive rate of DCP tended to be higher than that of AFP. This suggests that DCP is a more useful tumor marker in the management of NASH-HCC. However, there is no correlation between AFP and DCP. This finding indicates that the markers are complementary and would be useful for the diagnosis and evaluation of NASH-HCC when measured simultaneously.

Yatsuji et al. [6] reported a five-year HCC rate of 11.3% in NASH liver cirrhosis. This carcinoma incidence is not as high as the 8% rate in alcoholic cirrhosis [26]. Since we followed many cirrhotic NASH patients, the fact that 11 patients developed HCC was consistent with the report by Yatsuji et al. In addition, Caldwell reported that the incidence of HCC in various liver diseases was higher in Japan. These differences may be associated with ethnic differences. We measured anti-HBc antibody to investigate the influence of HBV on the carcinogenesis of NASH-HCC. The difference between the NASH-HCC group and HCV-HCC group was not significant, and none of the NASH-HCC patients had high HBc antibody titers which would have led us to suspect that they were HBV carriers. These findings suggest that if HBV does influence carcinogenesis in NASH, its influence is minimal.

The chief limitation of this study is that it was restricted to biopsy-proven NASH patients and thus does not reflect the entire population of patients with NASH-HCC. Our findings were thus affected by a certain degree of patient selection bias.

HCC recurrence was very high in patients with NASH. Regular screening for HCC is extremely important for NASH patients with HCC, even when they have received curative treatment. The risk factors for HCC recurrence are size of HCC and stage of liver fibrosis. HCC in NASH may be of multicentric origin, similar to HCC based on viral hepatitis. These findings should aid treatment selection for NASH patients and their daily management.

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