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TOPIC HIGHLIGHT

2016 Nonalcoholic Fatty Liver Disease: Global view

Genetics of non-alcoholic fatty liver disease: From susceptibility and nutrient interactions to management

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Abstract

Genetics plays an important role in determining the susceptibility of an individual to develop a disease. Complex, multi factorial diseases of modern day (diabetes, cardiovascular disease, hypertension and obesity) are a result of disparity between the type of food consumed and genes, suggesting that food which does not match the host genes is probably one of the major reasons for developing life style diseases. Non-alcoholic fatty liver is becoming a global epidemic leading to substantial morbidity. While various genotyping approaches such as whole exome sequencing using next generation sequencers and genome wide association studies have identified susceptibility loci for non-alcoholic fatty liver disease (NAFLD) including variants in patatin-like phospholipase domain containing 3 and transmembrane 6 superfamily member 2 genes apart from others; nutrient based studies emphasized on a combination of vitamin D, E and omega-3 fatty acids to manage fatty liver disease. However majority of the studies were conducted independent of each other and very few studies explored the interactions between the genetic susceptibility and nutrient interactions. Identifying such interactions will aid in optimizing the nutrition tailor made to an individual's genetic makeup, thereby aiding in delaying the onset of the disease and its progression. The present topic focuses on studies that identified the genetic susceptibility for NAFLD, nutritional recommendations, and their interactions for better management of NAFLD.

Key words: Transmembrane 6 superfamily member 2 gene; Patatin-like phospholipase domain containing 3 gene; Genotyping; Nutrient interactions; Non-alcoholic fatty liver disease; Genetic susceptibility

Core tip: Various genome wide association and replication studies across ethnicities have consistently associated variants in patatin-like phospholipase domain containing 3 gene with a higher risk of non-alcoholic fatty liver disease (NAFLD). More recently a variant in transmembrane 6 superfamily member 2 gene was also associated with susceptibility to the disease. Functional studies have established the role of these genes in NAFLD. Gene and nutrient interactions should be the focus of future research in the management of NAFLD.

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INTRODUCTION

Genetic susceptibility carried by an individual determines the risk of developing a disease. However, not all individuals who carry the risk manifest with the disease, suggesting that, most of the complex multifactorial diseases are the result of interactions between genes and environment. Disease occurrence (onset) or severity may differ in individuals with same genotype exposed to different environmental conditions or vice versa, reiterating the fact that phenotype is the consequence of genotype and environment interactions (Figure 1). Diet, life style, exposure to chemicals and toxins form the major part of environmental risks. Majority of the modern day life style diseases such as diabetes, cardiovascular disease, hypertension, obesity are typically inherited with multifactorial mode of inheritance. It refers to a complex pattern of inheritance where a combination of both genetic and other factors including environmental are involved. Multifactorial conditions do not always manifest despite the fact that the individual carries a genetic variant that increases the risk of disease, putting the emphasis on favorable environment.

Non-alcoholic fatty liver disease (NAFLD) is one of the most important lifestyle based complex and multifactorial diseases. The prevalence of the disease varies markedly in various populations. It ranges between 20%-30% in the Western countries[1], 20%-30% in Europeans[2], 8% in Japanese[3] and 25%-30% in Indians[4]. The spectrum of the disease ranges between steatosis alone on one hand and non-alcoholic steatohepatitis (NASH)/cirrhosis/hepatocellular carcinoma on the other. However, the progression through the spectrum involves multiple risks including genetic and environmental interactions, in addition to other risk factors (Obesity, advancing age, diabetes, hypertension, and hypertriglyceridemia). Therefore, understanding genetic susceptibility has been the major focus of recent research in addition to alterations in dietary habits and life style modifications which have been demonstrated to benefit the patients and aid in better management of the disease.

It is imperative for organisms from bacteria to humans to regulate their metabolism vis a vis availability of nutrients for better survival of the species. Nutrient-gene interactions have therefore been an ancient and omnipresent mechanism across species. However research started to explore these interactions only lately and the topic has been of prime importance in the context of disease including NAFLD. This review focuses on the genetic susceptibility identified till date employing various approaches (Exome sequencing, GWAs, candidate gene) thus far and the nutrient risks and the interactions between the two wherever studies are available.

WHOLE EXOME SEQUENCING AND NAFLD

Recent advances in sequencing the human genome have transformed methods of identifying genetic susceptibility for complex, multifactorial diseases. With whole exome sequencing studies, it is now possible to sequence protein coding regions of the genome and identify genetic susceptibility for complex diseases in an unbiased manner. Although very few studies are available that exploited this technology, important loci have been identified. A quick search on PubMed revealed a single whole exome sequencing study in morbidly obese patients of Caucasian origin with NAFLD, that revealed novel damaging mutations in Bardet-Biedl syndrome 1 gene and Melanocortin 3 receptor gene (*MC3R*). *MC3R* gene encodes MC3 a G-protein coupled receptor for melanocyte stimulating hormone and adenocorticotropic hormone. Studies have identified that mice deficient for this gene product have increased fat and play a critical role in weight regulation ("Entrez gene: *MC3R* melanocortin 3 receptor"). Further another patient with NAFLD-related cirrhosis was compound heterozygous for rare and damaging mutations in patatin-like phospholipase domain containing 3 (PNPLA3)[5]. It is only recently that researchers have started to harness NGS technology to identify genetic susceptibility for complex diseases. In future by exploiting this technology many more important loci may be identified for NAFLD that may aid in better management of the disease.

GENOME WIDE ASSOCIATION STUDIES AND NAFLD

Genome wide association studies (GWAS) are employed to identify genetic susceptibility for complex diseases in an unbiased way. One of the first GWA studies for NAFLD was performed by Romeo *et al*[6] that used a custom chip of approximately 9000 non-synonymous variants across

Table 1 List of genome/exome wide association studies and loci identified for non-alcoholic fatty liver disease

Ref.	Phenotype associated with	Ancestry of samples included	Genotyping platform	Discovery sample size	Replication sample size	Genes
Romeo *et al*[6], 2008	Increased hepatic fat levels and inflammation	Hispanic, African American and European American individuals	Perlegen Sciences Custom array (12138 NS variation)	2111 individuals with MRS measured hepatic steatosis	None	*PNPLA3*
Chalasani *et al*[15], 2010	Features of hepatic histology	Non-Hispanic white women	Illumina (324,623 SNPs)	236, non-Hispanic white women	None	*FDFT1, rs343062 (Chr 7), COL13A1, rs6591182 (Chr 11), EFCAB4B, rs2499604 (chr 1), PZP, rs1421201 (Chr 18) rs2710833 (Chr 4)*
Speliotes *et al*[16], 2011	CT measured hepatic steatosis	European american including Amish	Affymetrix, Illumina	7126 with CT measured hepatic steatosis	592/1405	*PNPLA3, NCAN, PPP1R3B, GCKR, LYPLAL1*
Kawaguchi *et al*[3], 2012	NAFLD	Japanese	Illumina	529 patients consisting of four NAFLD subgroups (Matteoni's classification)	None	*PNPLA3, SAMM50, PARVB, HS3ST1-HSP90AB2P, YIPF1*
Kitamoto *et al*[66], 2013	NAFLD	Japanese	Illumina	392 NAFLD and 934 controls	172 NAFLD and 1012 controls	*PNPLA3, SAMM50, PARVB* gene
Kozlitina *et al*[19], 2014	MRS measured hepatic steatosis	Hispanic, African, American and European	Illumina	2,736	None	*PNPLA3* and *TM6SF2*

NAFLD: Non-alcoholic fatty liver disease; CT: Computed tomography; SNPs: Single nucleotide polymorphisms; MRS: Magnetic resonance spectroscopy.

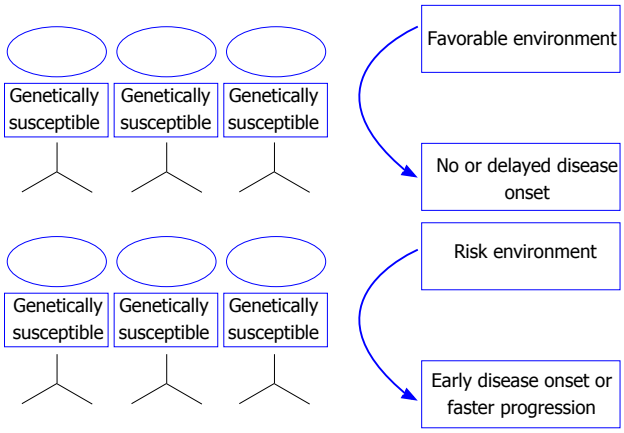

Figure 1 Genetic and environmental interactions to produce a phenotype.

the genome. The sample included patients with and without NAFLD of various ethnicities including European, Hispanic and African-American. The liver fat was measured by proton magnetic resonance spectroscopy. One variant (rs738409), a G allele encoding I148M in *PNPLA3* gene was associated with increased fat level in the liver across all the ethnicities. A list of various GWA studies and the variants identified are given in Table 1. Subsequently various groups have replicated the association of this variant in different ethnicities including Japanese[3,7], Indian[8,9], Chinese[10,11]. Further the variant was also associated with higher levels of ALT, histologic NAFLD including steatosis[7,8].

A meta-analysis of 24 studies that included 9915 patients from different ethnicities, identified that PNPLA3 rs738409 variant was associated with fibrosis severity (OR = 1.32, 95%CI: 1.20-1.45)[12]. Another meta-analysis of 16 studies[13], showed that rs738409 had a strong influence on liver fat accumulation. Individuals with GG homozygous genotype showed 77% higher lipid fat content compared to CC genotype and were susceptible to 3.24 fold aggressive disease and NASH. Further, when the risk associated with heterozygosity was evaluated for the variant, additive genetic model was better at explaining the effect of the variant on the susceptibility to develop NAFLD. However the analysis suggested that carrying two G alleles did not seem to increase the risk of severe histological features. Also, meta-regression showed a negative correlation between male sex and the effect of rs738409 on liver fat content (slope: -2.45 ± 1.04; $P < 0.02$). Importantly, the rs738409 GG genotype *vs* the CC genotype was associated with a 28% increase in serum alanine amino-transferase levels. Xu *et al*[14] recent meta analysis of the rs738409 variant that included 23 case-control studies (6071 NAFLD and 10366 controls) showed a significant association of the variant with NAFLD, NASH. The subgroup and sensitivity analysis revealed that the changes were not influenced by the ethnicities and age of the subjects.

A GWA study conducted[15] in 236 non-Hispanic white woman who were genotyped for 3,24,623 single nucleotide polymorphisms (SNPs) on the Illumina platform and were assessed for various histologic parameters revealed that NAFLD activity score was associated with

rs2645424 in farnesyl diphosphate farnesyl transferase 1. Further analysis revealed that degree of fibrosis was associated with rs343062, lobular inflammation with rs1227756 in COL13A1), rs6591182, and rs887304 in EFCAB4B. SNPs associated with serum levels of alanine aminotransferase included rs2499604, rs6487679, rs1421201 and rs2710833. However, no significant associations were found between genotypes and steatosis, ballooning degeneration, portal inflammation, or other features of NAFLD.

A meta-analysis[16] carried out across four groups of European ancestry and one of the largest GWA studies for NAFLD was tested for associations with computed tomography (CT) measured steatosis initially in the 4 groups independently followed by a meta-analysis. The study involved 7176 individuals that were controlled for age, gender and all the principal components. Variants in or near *PNPLA3, LYPLAL1, PPP1R3B, NCAN/*transmembrane 6 superfamily member 2 (*TM6SF2*) and *GCKR* genes were found to be associated with hepatic steatosis. These above variants but for *PPP1R3B* were also associated with NASH and fibrosis.

Few GWA studies identified loci associated with the associated parameters of NAFLD and most importantly the liver function tests. Two such studies[17,18] have identified four loci namely SNPs in or near *PNPLA3* (rs2281135, rs738409), *SAMM50* (rs2143571, *CPN1-ERLIN1-CHUK* gene cluster (rs10883437, rs11597390, rs11591741, rs11597086), *TRIB1* (rs2954021) and near *HSD17B13/MAPK10* (rs6834314) that were associated with elevated levels of ALT.

Our pooled genetic study[8], where 19 variants were selected that were associated with NAFLD from 4 GWA studies conducted until 2013 and replicated in patients with and without ultrasound detected NAFLD in Indians. The study identified variants in *PNPLA3, PZP, SAMM50* and *PARVB* were associated with NAFLD. Furthermore, the haplotype data suggested that variants in *PNPLA3, SAMM50* and *PARVB* on chromosome 22 were linked, suggesting that this loci is very important in Indian context. Studies from Japan[3] also associated these loci with NAFLD suggesting that it is an important loci conferring susceptibility in Asian population.

WHOLE EXOME ASSOCIATION STUDY AND NAFLD

Two studies published during the same time reported the association of a variant (rs58542926) in *TM6SF2* gene with susceptibility to NAFLD[19] and influencing total cholesterol and myocardial infarction risk[20]. The first study identified that the variant in *TM6SF2* gene was associated with hepatic triglyceride content (HTGC) and is a adenine to guanine substitution in coding nucleotide 499, replacing glutamate with lysine at position 167 (c.499A > G; p.Glu167Lys). The frequency of this variant was higher in three ancestries studied (European, African-American and Hispanics). The study suggested that the variant carriers had elevated mean and median HTGC in European and African-American ancestries. The study also identified that there was a reduction in the expression of recombinant protein in cultured hepatocytes by almost 50% by the Glu167Lys *TM6SF2* variant compared to the wild type. Further knockdown of the gene by Adeno-associated virus-mediated short hairpin RNA in mice increased the liver triglyceride content by threefold and decreased very-low-density lipoprotein (VLDL) secretion by half. Based on the above, the study suggested that TM6SF2 activity may be required for normal VLDL secretion and that impaired function of the *TM6SF2* gene causally contributes to NAFLD[19]. Further, the function of the gene was also clearly established, where it is now known to be a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content[21]. The second study[20], systematically assessed coding variants at the genome-wide level to identify novel lipid genes and also evaluate whether low frequency variants with large effect exist, identified a coding variant (p.Glu167Lys) in *TM6SF2* gene that modifies total cholesterol levels and further was associated with myocardial infarction. The functional role of few of the genes identified employing GWAS and exome wide association studies are as given in Table 2.

In an ongoing study at our center, we have replicated these variants (rs58542926 in *TM6SF2* and rs2281135 in *PNPLA3* genes) in 220 patients with NAFLD and 185 controls to date. Both the variants are significantly associated with the disease (*TM6SF2 P* = 0.00008; *PNPLA3 P* = 0.002) with a higher risk of the disease (Odds - 2.17, 95%CI: 1.34-3.52 and 1.85, 95%CI: 1.24-2.76). Further both the variants were significantly associated ($P > 0.05$) with higher ALT and AST levels (data unpublished).

CANDIDATE GENE STUDIES (OTHER GENES) AND NAFLD

Based on the two hit hypothesis as discussed earlier[22], studies have explored genes that have an important role in mechanisms related to lipid metabolism, insulin signaling, oxidative stress, inflammation and fibrogenesis.

While *APCO3* gene is the major gene studied for its role in lipid metabolism (association with higher triglyceride levels), *MTP* gene was studied for its role in regulating synthesis, storage and export of hepatic triglyceride content. A loci on the long arm of chromosome 11 (*11q23*) harbors genes coding for apolipoproteins, including apolipoprotein *A1 (APOAJ), A4 (APOA4* and *APOC3*[23]). Two polymorphisms T455C (rs2854117) and C482T (rs2854116) in the *APOC3* gene either singly or in combination had 30% higher levels of fasting plasma APOC3 and triglyceride levels as compared to the wild type[24]. Subsequent studies failed to replicate these associations[25,26], including our own study[27]. However we found that the SNPs were associated with higher triglyceride levels. *MTP* gene (microsomal triglyceride

Table 2 Functional role of major genes associated with non-alcoholic fatty liver disease identified by genome/exome wide association studies

Gene	Protein	Cellular location[1]	Function[1]	Chromosome location[2]	No. of exons and size[2]	Pathway/biologic function[1]	Tissues expressed[1]
PNPLA3	Patatin-like phospholipase domain-containing protein 3	Lipid droplets	Triacyl glycerol lipase and acylglycerol O-cyltransferase activities	Chromosome 22: 43,923,739-43,964,488 (forward strand)	9 (2805 bp)	Triacyl glycerol degradation and in glycerol-lipid metabolism	Liver, gall bladder, kidney, exocrine pancreas, seminal vesicles, intestine and salivary glands
TM6SF2	Transmembrane 6 superfamily member 2	ER and the ER-golgi intermediate compartment	Regulation of fat in liver influencing triglyceride secretion and lipid droplet content	Chromosome 19: 19,264,364-19,273,391 (reverse strand)	10 (1505 bp)	Promotes very low density lipoprotein export	Liver and intestine
SAMM50	Sorting and assembly machinery component 50 homolog	Outer mitochondrial membrane	Assembly of beta-barrel proteins	Chromosome 22: 43,955,421-44,010,531 (forward strand)	15 (1717)	Transport to the Golgi and subsequent modification and mitochondrial protein import	Liver, muscle, skeletal, lung adipocyte, colon and other tissues
PARVB	Parvin, beta	Cytoplasm	Involved in the reorganization of the actin cytoskeleton and formation of lamellipodia	Chromosome 22: 44,024,277-44,172,949 (forward strand)	13 (5429 bp)	ERK signaling and focal adhesion	Liver, muscle, skeletal, lung adipocyte, colon and other tissues
NCAN	Neurocan	Extracellular, Golgi lumen	Modulates neuronal adhesion and neurite growth during development	Chromosome 19: 19,211,973-19,252,233 (forward strand)	15 (6387 bp)	Developmental	Liver, muscle, skeletal, lung adipocyte, colon and other tissues
PPP1R3B	Protein phosphatase 1, regulatory subunit 3B	Glycogen granule	Acts as glycogen targeting subunit for phosphatase PP1	Chromosome 8: 9,136,255-9,151,574 (reverse strand)	2 (5548 bp)	Regulating glycogen synthesis	Liver, skeletal muscle
GCKR	GCKR	Cytoplasm, nucleus	Inhibits glucokinase by forming an inactive complex with this enzyme. The affinity of GCKR for GK is modulated by fructose metabolites	Chromosome 2: 27,496,842-27,523,684 (forward strand)	19 (2186 bp)	Carbohydrate metabolism	Liver, pancreas, colon and other tissues
LYPLAL1	Lysophospholipase-like 1	Cytoplasm	Depalmitoylating activity	Chromosome 1: 219,173,844-219,212,865 (forward strand)	5 (1898 bp)	Negative regulation of golgi to plasma membrane protein transport	Liver, muscle, skeletal, lung adipocyte, colon and other tissues

[1]Data extracted from http://www.genecards.org/; [2]Data extracted from ENSEBL http://asia.ensembl.org/index.html; ER: Endoplasmic reticulum; PP1: Protein phosphatase 1; GCKR: Glucokinase (Hexokinase 4) regulator; GK: Glucokinase; ERK: Extracellular signal–regulated kinase.

transfer protein), located at 4q24[28] is critical for the synthesis and secretion of VLDL (very low density lipoprotein) in the liver. A meta-analysis[29] of most studied polymorphism -493G > T (rs1800591 G > T) in the *MTP* gene suggested that the SNP was significantly associated with higher risk of NAFLD.

Genes influencing inflammation and immune responses are known to modify susceptibility to NAFLD. Cytokines not only play an active role in the development of disease, but also in the progression by regulating the inflammatory process[30]. Studies identified a positive correlation between increasing degree of liver fibrosis and levels of TNF-α[31-33] including pediatric NAFLD[34]. Further, polymorphism studies associated a promoter SNP (-238G > A) in *TNF-α* gene with susceptibility to NAFLD in Chinese

population[35]. transforming growth factor-beta (TGF-β), known to regulate cell death and lipid metabolism[36], has been shown to be up-regulated and is considered an early event in steatohepatitis that is progressive.

Expression of interleukin-6 (IL-6), a major pro-inflammatory cytokine was shown to be increased in animal models of NAFLD, while in mice, sustained selective up-regulation in the liver resulted in systemic insulin resistance[37]. This was subsequently confirmed in humans[38]. Further, a positive correlation was observed between the expression levels and degree of inflammation and stage of fibrosis. A study identified that -174G/C in the *IL-6* gene was involved in inflammation and insulin resistance and associated with NASH[39], chronic liver disease and Hepatocellular carcinoma[40].

Interleukin-10 (IL-10), an anti-inflammatory cytokine coded by *IL-10* gene[41] has a role in regulating inflammation and its anti-inflammatory properties are well known[42]. T cell, monocyte and macrophage mediated functions are inhibited by IL-10. Different types of the cells in liver including stellate cells, hepatocytes and kupffer cells have shown the presence of IL-10. Few studies that explored the role of the gene, identified the protective role of endogenous role of IL-10 against hepatic steatosis, however they suggested that it does not improve hepatic or whole body insulin sensitivity during high-fat feeding[43]. Furthermore, in an animal model of diet-induced fatty liver disease, inhibition of IL-10 promoted increased expression of inflammatory cytokines, worsened insulin signaling and activated gluconeogenic and lipidogenic pathways[44].

Hepatic insulin resistance is associated with NAFLD and is one of the contributory factors in the pathogenesis of metabolic syndrome. Genetic screening of insulin signaling cascade identified a substitution (Glycine-Arginine) at codon 972 of the insulin receptor substrate-1 gene with a prevalence of approximately 9% in Caucasians that was associated with reduced insulin sensitivity. Furthermore, obese individuals heterozygous for this mutation have 50% reduced insulin sensitivity as compared to wild type obese subjects[45]. This variant is known to affect insulin receptor activity predisposing to liver damage and decreased hepatic insulin signaling in patients with NAFLD. It is suggested that insulin signaling might play a causal role in the progression of liver damage in NAFLD[46].

NAFLD pathogenesis is a complex mechanism with involvement of free fatty acid (FFA) oxidation[47,48] and genes encoding proteins that are involved in the oxidation process of FFAs influence the oxidation load in individuals with obesity, insulin resistance and metabolic syndrome[22]. Genes harboring polymorphisms involved in generation and degradation of reactive oxygen species play a crucial role that could be due to excessive oxidation of FFA leading to oxidative stress casing apoptosis and liver injury[49]. Namikawa *et al*[50] reported that the TT genotype in the *MnSOD* gene, the main ROS scavenger in mitochondria, leads to decreased efficiency in the transport of MnSOD to the mitochondria and therefore confers susceptibility for NAFLD. Apart from the *MnSOD* gene, substantial evidence is now available on the role of polymorphisms in genes namely *GSTM1, GSTT1* and *GSTP1* genes that are involved in the generation or degradation of ROS. These genes are known to be involved in the progression to cirrhosis[51].

NUTRITIONAL RECOMMENDATIONS

Currently in clinical practice, a combination of vitamin D, vitamin E and omega-3 fatty acids have shown promise in the treatment of NAFLD and seem to be beneficial in patients with NAFLD. Studies further suggests that apart from nutritional counseling that includes a multi-disciplinary team (dietician, psychologist, and physical activity supervisor) aerobic exercises, gradual weight loss, management of NAFLD associated conditions namely diabetes, obesity and metabolic syndrome, nutritional recommendations namely use of 400-800 IU/d vitamin E, 1000 IU/d vitamin D, 1 g/d omega-3 fatty acids, and olive oil containing omega-9 fatty acids seem to benefit in reducing the severity of NAFLD. Also, restricting calorie intake to less than 30 kcal/kg per day and including a balanced diet with low levels of saturated and trans fats and simple sugars, avoiding soft drinks with high fructose corn syrup, fast food (trans fats, and reduce red and processed meats), and genetically modified crops seem to be beneficial[52].

MANAGEMENT OF NAFLD

Lifestyle modification usually by way of weight reduction through diet and exercises is currently the only proven strategy for managing NAFLD. As obesity is a strong risk and influencing factor for NAFLD, weight loss (⩾ 8% of body weight) is effective and is also the first line of therapy. A low caloric diet with reduction in the intake of total fat, saturated fatty acids, trans fatty acids and fructose, increase in physical activity and abstaining from smoking is advantageous and the patients are encouraged to follow these. Antioxidants, anti-inflammatory, insulin sensitizers, lipid lowering agents apart from wide range of drugs and supplements have been evaluated in various studies, both animal and human, however none of these have efficacy on long term use[53,54].

GENETIC AND NUTRIENT INTERACTIONS

It is almost imperative that all living organisms either simple or complex multicellular, regulate their metabolism vis a vis nutrient availability. Thus, interactions between nutrition and gene are widespread and an ancient feature across species. However, this aspect has not been explored and it is only recently that research started to uncover the mechanism. In view of the rapid advances made in sequencing human genome enormous amount of genetic data is being generated, particularly with

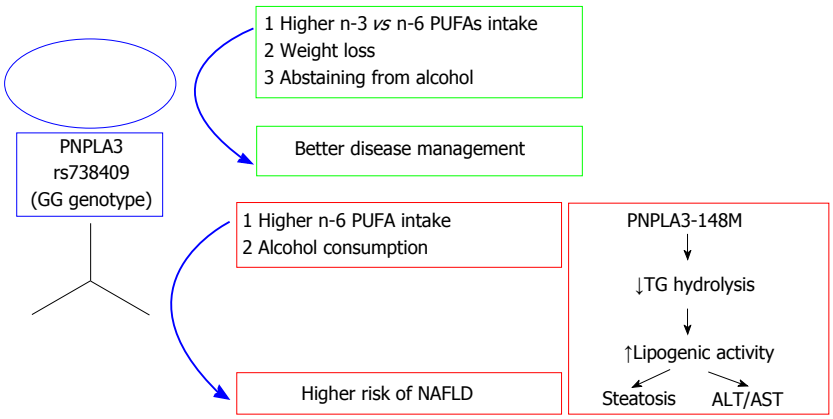

Figure 2 Genotype (rs738409) in patatin-like phospholipase domain containing 3 gene and its interactions. PNPLA3: Patatin-like phospholipase domain containing 3; PUFA: Polyunsaturated fatty acid; NAFLD: Non-alcoholic fatty liver disease; ALT/AST: Alanine aminotransferase/aspartate aminotransferase.

respect to common multigenic, multifactorial conditions including obesity, diabetes, NAFLD, *etc*. It is becoming more and more obvious that an individual's susceptibility to lifestyle disease represents a complex interaction between genetics and environmental interactions. Food and nutrient intake are the important environmental factors and their interactions with genes play a key role in the pathogenesis and progression of polygenic diseases. Therefore, research should be focused on identifying such interactions of genes with nutrients and identifying susceptible genotypes to particular nutrients. This will help us optimize nutrient/diet intake (personalized nutrition) to reduce disease risk[55]. However, there are challenges in analyzing these interactions in the form of genetic heterogeneity and complex nature of human genome, complexity of environmental factors including diet, *etc*[56].

NAFLD is considered to be the hepatic manifestation of the metabolic syndrome[57]. The "thrifty genotype" a possible explanation for the steep increase in obesity and diabetes, where periods of famine in the history of modern humans has exerted natural selection in favor of selecting genes favorable for fat storage and this is likely mediated through fertility and not viability selection[58].

Hepatic lipase gene (*HL*) is a lipolytic enzyme that regulates triglyceride levels. Insulin is known to up-regulate the activity of HL through the insulin-responsive elements in the promoter region. It is suggested that higher intake of total and saturated fat is associated with higher activity of *HL* gene. A study reported that this activity is influenced by the -514 C > T polymorphism in the *HL* gene, with significantly stronger associations noted between total dietary fat intake and HL activity in individuals with CT and TT genotypes as compared to the wild type (CC)[59]. Another study[60] that explored epidemiologic genotype-nutrient interactions in obesity, where a total of 42 SNPs in 26 candidate genes were genotyped identified an interaction between -514 C > T in *HL* gene and fiber intake. Further they also suggested that the -681 C > G polymorphism in *PPARG3* gene might interact with the percentage of energy derived from fat in the diet for the development of obesity. However, this was a case-only study with only adult obese women as part of the analysis. A study[61] that examined the interactions between the -514 C > T in *HL* gene, dietary fat and HDL-related measures in 1020 men and 1110 women from the farmingham study reported that individuals with the "TT" genotype may have an impaired adaptation to higher animal fat diets. Furthermore, they suggested that dietary fat intake modifies the effect of the polymorphism in *HL* gene on HDL-C concentrations and subclasses, where the T allele was significantly associated with greater HDL-C concentrations only in subjects consuming < 30% of energy from fat and the reverse is true when total fat intake was ⩾ 30% of energy.

It is well established that apolipoprotein A5 (*APOA5*) gene is an important determinant of plasma triglyceride levels. Further it is a component of several lipoprotein fractions including high density lipoprotein (HDL), VLDL[62]. A study[63], investigated the interaction between variants in *APOA5* gene and dietary fat in determining plasma fasting triglycerides, remnant-like particle concentrations and lipoprotein particle size in 1001 men and 1147 women from farmingham heart study reported significant gene-diet interactions between the -1131T > C polymorphism in *APOA5* gene and polyunsaturated fatty acid (PUFA) intake were found that determined fasting TGs, RLP concentrations and particle size. However, these interactions were not found for the other polymorphism (56C > G). Further they noted that the -1131C allele was associated with higher fasting TGs and RLP concentrations in only individuals who consumed a high-PUFA diet with > 6% of total energy. The study concluded that individuals who are carriers of -1131C polymorphism in *APOA5* gene and take higher n-6 but not n-3 PUFA, have increased fasting TGs, RLP concentrations, and VLDL size and decreased LDL size, suggesting a more atherogenic lipid profile in these individuals because of the n-6 PUFA-rich diet.

A study[64] that recruited 8 subjects with homozygous genotype for the rs738409G allele in *PNPLA3* gene and

10 with C allele, explored the influence of the variant on the ability to lose weight thereby reducing liver fat or change insulin sensitivity. The study identified that the fasting serum insulin and C-peptide concentrations were significantly lower in rs738409G as compared to rs738409C group. Although weight loss was not significantly different between the groups (approximately 3.1 kg), liver fat decreased by 45% in rs738409G as compared to 18% in the rs738409C group, suggesting weight loss is more effective in decreasing liver fat in rs738409G carriers. Another study[65] that explored the influence of PNPLA3 (rs738409) genotype on hepatic fat and modulation by dietary factors such as PUFAs identified that the ratio of n-6 to n-3 PUFs interacted with the GG genotype to promote hepatic steatosis (Figure 2).

FUTURE TRENDS

In future, testing for variants that pre-dispose to NAFLD along with their nutrient interactions would help identify the type of nutrition to be taken based on individual's genetic makeup thereby minimizing the risk of fatty infiltration.

CONCLUSION

Among all the loci identified thus far, there is a compelling evidence of the association of variants in PNPLA3 with NAFLD and functional role of TM6SF2 in the regulation of liver fat metabolism and hepatic lipid droplet content. It may be prudent to genotype these well characterized variants (PNPLA3) as part of the diagnostic workup for NAFLD, to assess the risk of an individual. Further genotyping in asymptomatic individuals will help in making lifestyle based recommendations, including nutrition to minimize the risk of future disease. As the genetic susceptibility risk cannot be changed, it is important to identify the risk at an early age and manage/lower the other modifiable risks/modifiable triggers to efficiently manage the disease without progressing to subsequent pathologies. Finally, the genetic information along with personalized environment exposures will help in stratifying risk of NAFLD in an individual.

REFERENCES

1 **Bellentani S**, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]

2 **Blachier M**, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol* 2013; **58**: 593-608 [PMID: 23419824 DOI: 10.1016/j.jhep.2012.12.005]

3 **Kawaguchi T**, Sumida Y, Umemura A, Matsuo K, Takahashi M, Takamura T, Yasui K, Saibara T, Hashimoto E, Kawanaka M, Watanabe S, Kawata S, Imai Y, Kokubo M, Shima T, Park H, Tanaka H, Tajima K, Yamada R, Matsuda F. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PLoS One* 2012; **7**: e38322 [PMID: 22719876 DOI: 10.1371/journal.pone.0038322]

4 **Amarapurkar D**, Kamani P, Patel N, Gupte P, Kumar P, Agal S, Baijal R, Lala S, Chaudhary D, Deshpande A. Prevalence of non-alcoholic fatty liver disease: population based study. *Ann Hepatol* 2007; **6**: 161-163 [PMID: 17786142]

5 **Gerhard GS**, Chu X, Wood GC, Gerhard GM, Benotti P, Petrick AT, Gabrielsen J, Strodel WE, Still CD, Argyropoulos G. Next-generation sequence analysis of genes associated with obesity and nonalcoholic fatty liver disease-related cirrhosis in extreme obesity. *Hum Hered* 2013; **75**: 144-151 [PMID: 24081230 DOI: 10.1159/000351719]

6 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]

7 **Akuta N**, Kawamura Y, Arase Y, Suzuki F, Sezaki H, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Suzuki Y, Ikeda K, Kumada H. Relationships between Genetic Variations of PNPLA3, TM6SF2 and Histological Features of Nonalcoholic Fatty Liver Disease in Japan. *Gut Liver* 2016; **10**: 437-445 [PMID: 26610348 DOI: 10.5009/gnl15163]

8 **Kanth VV**, Sasikala M, Rao PN, Steffie Avanthi U, Rao KR, Nageshwar Reddy D. Pooled genetic analysis in ultrasound measured non-alcoholic fatty liver disease in Indian subjects: A pilot study. *World J Hepatol* 2014; **6**: 435-442 [PMID: 25018854 DOI: 10.4254/wjh.v6.i6.435]

9 **Bhatt SP**, Nigam P, Misra A, Guleria R, Pandey RM, Pasha MA. Genetic variation in the patatin-like phospholipase domain-containing protein-3 (PNPLA-3) gene in Asian Indians with nonalcoholic fatty liver disease. *Metab Syndr Relat Disord* 2013; **11**: 329-335 [PMID: 23734760 DOI: 10.1089/met.2012.0064]

10 **Zhang Y**, Cai W, Song J, Miao L, Zhang B, Xu Q, Zhang L, Yao H. Association between the PNPLA3 I148M polymorphism and non-alcoholic fatty liver disease in the Uygur and Han ethnic groups of northwestern China. *PLoS One* 2014; **9**: e108381 [PMID: 25290313 DOI: 10.1371/journal.pone.0108381]

11 **Peng XE**, Wu YL, Lin SW, Lu QQ, Hu ZJ, Lin X. Genetic variants in PNPLA3 and risk of non-alcoholic fatty liver disease in a Han Chinese population. *PLoS One* 2012; **7**: e50256 [PMID: 23226254 DOI: 10.1371/journal.pone.0050256]

12 **Singal AG**, Manjunath H, Yopp AC, Beg MS, Marrero JA, Gopal P, Waljee AK. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 325-334 [PMID: 24445574 DOI: 10.1038/ajg.2013.476]

13 **Sookoian S**, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]

14 **Xu R**, Tao A, Zhang S, Deng Y, Chen G. Association between patatin-like phospholipase domain containing 3 gene (PNPLA3) polymorphisms and nonalcoholic fatty liver disease: a HuGE review and meta-analysis. *Sci Rep* 2015; **5**: 9284 [PMID: 25791171 DOI: 10.1038/srep09284]

15 **Chalasani N**, Guo X, Loomba R, Goodarzi MO, Haritunians T, Kwon S, Cui J, Taylor KD, Wilson L, Cummings OW, Chen YD, Rotter JI. Genome-wide association study identifies variants associated with histologic features of nonalcoholic Fatty liver disease. *Gastroenterology* 2010; **139**: 1567-1576, 1576.e1-e6 [PMID: 20708005 DOI: 10.1053/j.gastro.2010.07.057]

16 **Speliotes EK**, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, Gudnason V, Eiriksdottir G, Garcia ME, Launer LJ, Nalls MA, Clark JM, Mitchell BD, Shuldiner AR, Butler JL, Tomas M, Hoffmann U, Hwang SJ, Massaro JM, O'Donnell CJ, Sahani DV, Salomaa V, Schadt EE, Schwartz SM, Siscovick DS, Voight BF, Carr JJ, Feitosa MF, Harris TB, Fox CS, Smith AV, Kao WH, Hirschhorn JN, Borecki IB. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet* 2011; **7**:

e1001324 [PMID: 21423719 DOI: 10.1371/journal.pgen.1001324]

17 **Yuan X**, Waterworth D, Perry JR, Lim N, Song K, Chambers JC, Zhang W, Vollenweider P, Stirnadel H, Johnson T, Bergmann S, Beckmann ND, Li Y, Ferrucci L, Melzer D, Hernandez D, Singleton A, Scott J, Elliott P, Waeber G, Cardon L, Frayling TM, Kooner JS, Mooser V. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am J Hum Genet* 2008; **83**: 520-528 [PMID: 18940312 DOI: 10.1016/j.ajhg.2008.09.012]

18 **Chambers JC**, Zhang W, Sehmi J, Li X, Wass MN, Van der Harst P, Holm H, Sanna S, Kavousi M, Baumeister SE, Coin LJ, Deng G, Gieger C, Heard-Costa NL, Hottenga JJ, Kühnel B, Kumar V, Lagou V, Liang L, Luan J, Vidal PM, Mateo Leach I, O'Reilly PF, Peden JF, Rahmioglu N, Soininen P, Speliotes EK, Yuan X, Thorleifsson G, Alizadeh BZ, Atwood LD, Borecki IB, Brown MJ, Charoen P, Cucca F, Das D, de Geus EJ, Dixon AL, Döring A, Ehret G, Eyjolfsson GI, Farrall M, Forouhi NG, Friedrich N, Goessling W, Gudbjartsson DF, Harris TB, Hartikainen AL, Heath S, Hirschfield GM, Hofman A, Homuth G, Hyppönen E, Janssen HL, Johnson T, Kangas AJ, Kema IP, Kühn JP, Lai S, Lathrop M, Lerch MM, Li Y, Liang TJ, Lin JP, Loos RJ, Martin NG, Moffatt MF, Montgomery GW, Munroe PB, Musunuru K, Nakamura Y, O'Donnell CJ, Olafsson I, Penninx BW, Pouta A, Prins BP, Prokopenko I, Puls R, Ruokonen A, Savolainen MJ, Schlessinger D, Schouten JN, Seedorf U, Sen-Chowdhry S, Siminovitch KA, Smit JH, Spector TD, Tan W, Teslovich TM, Tukiainen T, Uitterlinden AG, Van der Klauw MM, Vasan RS, Wallace C, Wallaschofski H, Wichmann HE, Willemsen G, Würtz P, Xu C, Yerges-Armstrong LM; Alcohol Genome-wide Association (AlcGen) Consortium; Diabetes Genetics Replication and Meta-analyses (DIAGRAM+) Study; Genetic Investigation of Anthropometric Traits (GIANT) Consortium; Global Lipids Genetics Consortium; Genetics of Liver Disease (GOLD) Consortium; International Consortium for Blood Pressure (ICBP-GWAS); Meta-analyses of Glucose and Insulin-Related Traits Consortium (MAGIC), Abecasis GR, Ahmadi KR, Boomsma DI, Caulfield M, Cookson WO, van Duijn CM, Froguel P, Matsuda K, McCarthy MI, Meisinger C, Mooser V, Pietiläinen KH, Schumann G, Snieder H, Sternberg MJ, Stolk RP, Thomas HC, Thorsteinsdottir U, Uda M, Waeber G, Wareham NJ, Waterworth DM, Watkins H, Whitfield JB, Witteman JC, Wolffenbuttel BH, Fox CS, Ala-Korpela M, Stefansson K, Vollenweider P, Völzke H, Schadt EE, Scott J, Järvelin MR, Elliott P, Kooner JS. Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. *Nat Genet.* 2011; **43**: 1131-1138 [PMID: 22001757 DOI: 10.1038/ng.970]

19 **Kozlitina J**, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, Vogt TF, Hobbs HH, Cohen JC. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014; **46**: 352-356 [PMID: 24531328 DOI: 10.1038/ng.2901]

20 **Holmen OL**, Zhang H, Fan Y, Hovelson DH, Schmidt EM, Zhou W, Guo Y, Zhang J, Langhammer A, Løchen ML, Ganesh SK, Vatten L, Skorpen F, Dalen H, Zhang J, Pennathur S, Chen J, Platou C, Mathiesen EB, Wilsgaard T, Njølstad I, Boehnke M, Chen YE, Abecasis GR, Hveem K, Willer CJ. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet* 2014; **46**: 345-351 [PMID: 24633158 DOI: 10.1038/ng.2926]

21 **Mahdessian H**, Taxiarchis A, Popov S, Silveira A, Franco-Cereceda A, Hamsten A, Eriksson P, van't Hooft F. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. *Proc Natl Acad Sci USA* 2014; **111**: 8913-8918 [PMID: 24927523 DOI: 10.1073/pnas.1323785111]

22 **Day CP**. From fat to inflammation. *Gastroenterology* 2006; **130**: 207-210 [PMID: 16401483 DOI: 10.1053/j.gastro.2005.11.017]

23 **Karathanasis SK**, Oettgen P, Haddad IA, Antonarakis SE. Structure, evolution, and polymorphisms of the human apolipoprotein A4 gene (APOA4). *Proc Natl Acad Sci USA* 1986; **83**: 8457-8461 [PMID: 3095836]

24 **Petersen KF**, Dufour S, Hariri A, Nelson-Williams C, Foo JN, Zhang XM, Dziura J, Lifton RP, Shulman GI. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N Engl J Med* 2010; **362**: 1082-1089 [PMID: 20335584 DOI: 10.1056/NEJMoa0907295]

25 **Kozlitina J**, Boerwinkle E, Cohen JC, Hobbs HH. Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. *Hepatology* 2011; **53**: 467-474 [PMID: 21274868 DOI: 10.1002/hep.24072]

26 **TG and HDL Working Group of the Exome Sequencing Project**, National Heart, Lung, and Blood Institute, Crosby J, Peloso GM, Auer PL, Crosslin DR, Stitziel NO, Lange LA, Lu Y, Tang ZZ, Zhang H, Hindy G, Masca N, Stirrups K, Kanoni S, Do R, Jun G, Hu Y, Kang HM, Xue C, Goel A, Farrall M, Duga S, Merlini PA, Asselta R, Girelli D, Olivieri O, Martinelli N, Yin W, Reilly D, Speliotes E, Fox CS, Hveem K, Holmen OL, Nikpay M, Farlow DN, Assimes TL, Franceschini N, Robinson J, North KE, Martin LW, DePristo M, Gupta N, Escher SA, Jansson JH, Van Zuydam N, Palmer CN, Wareham N, Koch W, Meitinger T, Peters A, Lieb W, Erbel R, Konig IR, Kruppa J, Degenhardt F, Gottesman O, Bottinger EP, O'Donnell CJ, Psaty BM, Ballantyne CM, Abecasis G, Ordovas JM, Melander O, Watkins H, Orho-Melander M, Ardissino D, Loos RJ, McPherson R, Willer CJ, Erdmann J, Hall AS, Samani NJ, Deloukas P, Schunkert H, Wilson JG, Kooperberg C, Rich SS, Tracy RP, Lin DY, Altshuler D, Gabriel S, Nickerson DA, Jarvik GP, Cupples LA, Reiner AP, Boerwinkle E, Kathiresan S. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med* 2014; **371**: 22-31 [PMID: 24941081 DOI: 10.1056/NEJMoa1307095]

27 **Ravikanth VV**, Mitnala S, Avanthi US, Manjeera B, Aswini R, Mukherjee RM, Rao PN, Reddy DN. PNPLA3 gene polymorphism but not APOC3 enhances risk for nonalcoholic fatty liver disease in Indian subjects. *J Clin Exp Hepatol* 2013; **3**: S24 [DOI: 10.1016/j.jceh.2013.03.049]

28 **Narcisi TM**, Shoulders CC, Chester SA, Read J, Brett DJ, Harrison GB, Grantham TT, Fox MF, Povey S, de Bruin TW. Mutations of the microsomal triglyceride-transfer-protein gene in abetalipoproteinemia. *Am J Hum Genet* 1995; **57**: 1298-1310 [PMID: 8533758]

29 **Li L**, Wang SJ, Shi K, Chen D, Jia H, Zhu J. Correlation between MTP -493G& gt; T polymorphism and non-alcoholic fatty liver disease risk: a meta-analysis. *Genet Mol Res* 2014; **13**: 10150-10161 [PMID: 25501226 DOI: 10.4238/2014.December.4.9]

30 **Braunersreuther V**, Viviani GL, Mach F, Montecucco F. Role of cytokines and chemokines in non-alcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 727-735 [PMID: 22371632 DOI: 10.3748/wjg.v18.i8.727]

31 **Lesmana CR**, Hasan I, Budihusodo U, Gani RA, Krisnuhoni E, Akbar N, Lesmana LA. Diagnostic value of a group of biochemical markers of liver fibrosis in patients with non-alcoholic steatohepatitis. *J Dig Dis* 2009; **10**: 201-206 [PMID: 19659788 DOI: 10.1111/j.1751-2980.2009.00386.x]

32 **Hui JM**, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004; **40**: 46-54 [PMID: 15239085 DOI: 10.1002/hep.20280]

33 **Crespo J**, Cayón A, Fernández-Gil P, Hernández-Guerra M, Mayorga M, Domínguez-Díez A, Fernández-Escalante JC, Pons-Romero F. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology* 2001; **34**: 1158-1163 [PMID: 11732005 DOI: 10.1053/jhep.2001.29628]

34 **Manco M**, Marcellini M, Giannone G, Nobili V. Correlation of serum TNF-alpha levels and histologic liver injury scores in pediatric nonalcoholic fatty liver disease. *Am J Clin Pathol* 2007; **127**: 954-960 [PMID: 17509993 DOI: 10.1309/6VJ4DWGYDU0XYJ8Q]

35 **Hu ZW**, Luo HB, Xu YM, Guo JW, Deng XL, Tong YW, Tang X. Tumor necrosis factor--alpha gene promoter polymorphisms in Chinese patients with nonalcoholic fatty liver diseases. *Acta*

Gastroenterol Belg 2009; **72**: 215-221 [PMID: 19637776]
36 **Stärkel P**, Sempoux C, Leclercq I, Herin M, Deby C, Desager JP, Horsmans Y. Oxidative stress, KLF6 and transforming growth factor-beta up-regulation differentiate non-alcoholic steatohepatitis progressing to fibrosis from uncomplicated steatosis in rats. *J Hepatol* 2003; **39**: 538-546 [PMID: 12971963 DOI: 10.1016/S0168-8278(03)00360-X]
37 **Nieto-Vazquez I**, Fernández-Veledo S, de Alvaro C, Lorenzo M. Dual role of interleukin-6 in regulating insulin sensitivity in murine skeletal muscle. *Diabetes* 2008; **57**: 3211-3221 [PMID: 18796617 DOI: 10.2337/db07-1062]
38 **Wieckowska A**, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 2008; **103**: 1372-1379 [PMID: 18510618 DOI: 10.1111/j.1572-0241.2007.01774.x]
39 **Carulli L**, Canedi I, Rondinella S, Lombardini S, Ganazzi D, Fargion S, De Palma M, Lonardo A, Ricchi M, Bertolotti M, Carulli N, Loria P. Genetic polymorphisms in non-alcoholic fatty liver disease: interleukin-6-174G/C polymorphism is associated with non-alcoholic steatohepatitis. *Dig Liver Dis* 2009; **41**: 823-828 [PMID: 19403348 DOI: 10.1016/j.dld.2009.03.005]
40 **Giannitrapani L**, Soresi M, Balasus D, Licata A, Montalto G. Genetic association of interleukin-6 polymorphism (-174 G/C) with chronic liver diseases and hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 2449-2455 [PMID: 23674845 DOI: 10.3748/wjg.v19.i16.2449]
41 **Eskdale J**, Kube D, Tesch H, Gallagher G. Mapping of the human IL10 gene and further characterization of the 5' flanking sequence. *Immunogenetics* 1997; **46**: 120-128 [PMID: 9162098 DOI: 10.1007/s002510050250]
42 **Couper KN**, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol* 2008; **180**: 5771-5777 [PMID: 18424693 DOI: 10.4049/jimmunol.180.9.5771]
43 **den Boer MA**, Voshol PJ, Schröder-van der Elst JP, Korsheninnikova E, Ouwens DM, Kuipers F, Havekes LM, Romijn JA. Endogenous interleukin-10 protects against hepatic steatosis but does not improve insulin sensitivity during high-fat feeding in mice. *Endocrinology* 2006; **147**: 4553-4558 [PMID: 16709607 DOI: 10.1210/en.2006-0417]
44 **Cintra DE**, Pauli JR, Araújo EP, Moraes JC, de Souza CT, Milanski M, Morari J, Gambero A, Saad MJ, Velloso LA. Interleukin-10 is a protective factor against diet-induced insulin resistance in liver. *J Hepatol* 2008; **48**: 628-637 [PMID: 18267346 DOI: 10.1016/j.jhep.2007.12.017]
45 **Pedersen O**. Genetics of insulin resistance. *Exp Clin Endocrinol Diabetes* 1999; **107**: 113-118 [PMID: 10320051 DOI: 10.1055/s-0029-1212085]
46 **Dongiovanni P**, Valenti L, Rametta R, Daly AK, Nobili V, Mozzi E, Leathart JB, Pietrobattista A, Burt AD, Maggioni M, Fracanzani AL, Lattuada E, Zappa MA, Roviaro G, Marchesini G, Day CP, Fargion S. Genetic variants regulating insulin receptor signalling are associated with the severity of liver damage in patients with non-alcoholic fatty liver disease. *Gut* 2010; **59**: 267-273 [PMID: 20176643 DOI: 10.1136/gut.2009.190801]
47 **Sanyal AJ**, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; **120**: 1183-1192 [PMID: 11266382 DOI: 10.1053/gast.2001.23256]
48 **Day CP**. Pathogenesis of steatohepatitis. *Best Pract Res Clin Gastroenterol* 2002; **16**: 663-678 [PMID: 12406438 DOI: 10.1053/bega.2002.0333]
49 **Wang K**. Molecular mechanisms of hepatic apoptosis. *Cell Death Dis* 2014; 5: e996 [PMID: 24434519 DOI: 10.1038/cddis.2013.499]
50 **Namikawa C**, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M, Akisawa N, Saibara T, Hiroi M, Enzan H, Onishi S. Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. *J Hepatol* 2004; **40**: 781-786 [PMID: 15094225 DOI: 10.1016/j.jhep.2004.01.028]
51 **Ghobadloo SM**, Yaghmaei B, Bakayev V, Goudarzi H, Noorinayer B, Rad FH, Samiy S, Aghabozorghi S, Zali MR. GSTP1, GSTM1, and GSTT1 genetic polymorphisms in patients with cryptogenic liver cirrhosis. *J Gastrointest Surg* 2004; **8**: 423-427 [PMID: 15120366 DOI: 10.1016/j.gassur.2004.02.005]
52 **Assy N**. Nutritional recommendations for patients with non-alcoholic fatty liver diseases. *World J Gastroenterol* 2011; **17**: 3375-3376 [PMID: 21876629 DOI: 10.3748/wjg.v17.i29.3375]
53 **Eslamparast T**, Eghtesad S, Poustchi H, Hekmatdoost A. Recent advances in dietary supplementation, in treating non-alcoholic fatty liver disease. *World J Hepatol* 2015; **7**: 204-212 [PMID: 25729475 DOI: 10.4254/wjh.v7.i2.204]
54 **Dongiovanni P**, Lanti C, Riso P, Valenti L. Nutritional therapy for nonalcoholic fatty liver disease. *J Nutr Biochem* 2016; **29**: 1-11 [PMID: 26895659 DOI: 10.1016/j.jnutbio.2015.08.024]
55 **Phillips CM**, Tierney AC, Roche HM. Gene-nutrient interactions in the metabolic syndrome. *J Nutrigenet Nutrigenomics* 2008; **1**: 136-151 [PMID: 19776623 DOI: 10.1159/000112461]
56 **Wise C**, Kaput J. A strategy for analyzing gene-nutrient interactions in type 2 diabetes. *J Diabetes Sci Technol* 2009; **3**: 710-721 [PMID: 20144318 DOI: 10.1177/193229680900300416]
57 **Kim CH**, Younossi ZM. Nonalcoholic fatty liver disease: a manifestation of the metabolic syndrome. *Cleve Clin J Med* 2008; **75**: 721-728 [PMID: 18939388 DOI: 10.3949/ccjm.75.10.721]
58 **Prentice AM**, Hennig BJ, Fulford AJ. Evolutionary origins of the obesity epidemic: natural selection of thrifty genes or genetic drift following predation release? *Int J Obes* (Lond) 2008; **32**: 1607-1610 [PMID: 18852700 DOI: 10.1038/ijo.2008.147]
59 **Bos G**, Snijder MB, Nijpels G, Dekker JM, Stehouwer CD, Bouter LM, Heine RJ, Jansen H. Opposite contributions of trunk and leg fat mass with plasma lipase activities: the Hoorn study. *Obes Res* 2005; **13**: 1817-1823 [PMID: 16286530 DOI: 10.1038/oby.2005.221]
60 **Santos JL**, Boutin P, Verdich C, Holst C, Larsen LH, Toubro S, Dina C, Saris WH, Blaak EE, Hoffstedt J, Taylor MA, Polak J, Clement K, Langin D, Astrup A, Froguel P, Pedersen O, Sorensen TI, Martinez JA. Genotype-by-nutrient interactions assessed in European obese women. A case-only study. *Eur J Nutr* 2006; **45**: 454-462 [PMID: 17080261 DOI: 10.1007/s00394-006-0619-6]
61 **Ordovas JM**, Corella D, Demissie S, Cupples LA, Couture P, Coltell O, Wilson PW, Schaefer EJ, Tucker KL. Dietary fat intake determines the effect of a common polymorphism in the hepatic lipase gene promoter on high-density lipoprotein metabolism: evidence of a strong dose effect in this gene-nutrient interaction in the Framingham Study. *Circulation* 2002; **106**: 2315-2321 [PMID: 12403660 DOI: 10.1161/01.CIR.0000036597.52291.C9]
62 **Nilsson SK**, Christensen S, Raarup MK, Ryan RO, Nielsen MS, Olivecrona G. "Endocytosis of apolipoprotein A-V by members of the low density lipoprotein receptor and the VPS10p domain receptor families". *The Journal of Biological Chemistry* 2008; **283**: 25920-25927 [PMID: 18603531 DOI: 10.1074/jbc.M802721200]
63 **Lai CQ**, Corella D, Demissie S, Cupples LA, Adiconis X, Zhu Y, Parnell LD, Tucker KL, Ordovas JM. Dietary intake of n-6 fatty acids modulates effect of apolipoprotein A5 gene on plasma fasting triglycerides, remnant lipoprotein concentrations, and lipoprotein particle size: the Framingham Heart Study. *Circulation* 2006; **113**: 2062-2070 [PMID: 16636175 DOI: 10.1161/CIRCULATIONAHA.105.577296]
64 **Sevastianova K**, Kotronen A, Gastaldelli A, Perttilä J, Hakkarainen A, Lundbom J, Suojanen L, Orho-Melander M, Lundbom N, Ferrannini E, Rissanen A, Olkkonen VM, Yki-Järvinen H. Genetic variation in PNPLA3 (adiponutrin) confers sensitivity to weight loss-induced decrease in liver fat in humans. *Am J Clin Nutr* 2011; **94**: 104-111 [PMID: 21525193 DOI: 10.3945/ajcn.111.012369]
65 **Santoro N**, Savoye M, Kim G, Marotto K, Shaw MM, Pierpont B, Caprio S. Hepatic fat accumulation is modulated by the interaction between the rs738409 variant in the PNPLA3 gene and the dietary omega6/omega3 PUFA intake. *PLoS One* 2012; **7**: e37827 [PMID:

22629460 DOI: 10.1371/journal.pone.0037827]

66 **Kitamoto T**, Kitamoto A, Yoneda M, Hyogo H, Ochi H, Nakamura T, Teranishi H, Mizusawa S, Ueno T, Chayama K, Nakajima A, Nakao K, Sekine A, Hotta K. Genome-wide scan revealed that polymorphisms in the PNPLA3, SAMM50, and PARVB genes are associated with development and progression of nonalcoholic fatty liver disease in Japan. *Hum Genet* 2013; **132**: 783-792 [PMID: 23535911 DOI: 10.1007/s00439-013-1294-3]

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ORIGINAL ARTICLE

Basic Study

Lipogenesis in Huh7 cells is promoted by increasing the fructose: Glucose molar ratio

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Abstract

AIM: To determine whether hepatocyte lipogenesis, in an *in vitro* cell culture model, is modulated by adjusting culture media monosaccharide content and concentration.

METHODS: Hepatocytes (Huh7), demonstrating glucose and fructose uptake and lipid biosynthesis, were incubated in culture media containing either glucose alone (0.65-0.72 mmol/L) or isosmolar monosaccharide (0.72 mmol/L) comprising fructose:glucose (F:G) molar ratios ranging from 0.58-0.67. Following a 24-h incubation, cells were harvested and analyzed for total protein, triglyceride (TG) and cholesterol (C) content. Significant differences ($P < 0.05$) among groups were determined using analysis of variance followed by Dunnett's test for multiple comparisons.

RESULTS: After a 24 h incubation period, Huh7 cell mass and viability among all experimental groups were not different. Hepatocytes cultured with increasing concentrations of glucose alone did not demonstrate a significant change either in C or in TG content. However, when the culture media contained increasing F:G molar ratios, at a constant total monosaccharide

concentration, synthesis both of C and of TG increased significantly [F:G ratio = 0.58, C/protein (μg/μg) = 0.13; F:G = 0.67, C/protein = 0.18, $P < 0.01$; F:G ratio = 0.58, TG/protein (μg/μg) = 0.06; F:G ratio = 0.67, TG/protein = 0.11, $P < 0.01$].

CONCLUSION: In an *in vitro* hepatocyte model, glucose or fructose plus glucose support total cell mass and lipogenic activity. Increasing the fructose:glucose molar ratio (but not glucose alone) enhances triglyceride and cholesterol synthesis. These investigations demonstrate fructose promotes hepatocellular lipogenesis, and they provide evidence supporting future, *in vivo* studies of fructose's role in the development of hepatic steatosis and non-alcoholic fatty liver disease.

Key words: Hepatocytes; Cholesterol; Triglycerides; Fructose; Glucose

Core tip: Employing an *in vitro* hepatocyte culture model, these data demonstrate fructose promotes intracellular synthesis both of cholesterol and of triglyceride. The results support the requirement for future, *in vivo* investigations to determine whether diets high in fructose are risk factors for hepatic steatosis and development of non-alcoholic fatty liver disease.

Windemuller F, Xu J, Rabinowitz SS, Hussain MM, Schwarz SM. Lipogenesis in Huh7 cells is promoted by increasing the fructose: Glucose molar ratio. *World J Hepatol* 2016; 8(20): 838-843 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i20/838.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i20.838

INTRODUCTION

Fructose, a five-carbon monosaccharide, comprises an increasing component of the Western diet, particularly in the form of high fructose corn syrup (HFCS). In the United States, this sweetener is both readily available from domestically grown corn and inexpensive, when compared to imported, granulated sugar. It was introduced in the 1960's, with subsequent expansion into a vast array of foods and beverages. HFCS is made from corn starch, processed by glucose isomerase to convert a portion of its glucose fraction into fructose. HFCS preparations contain approximately 25% water, fructose (up to 55% of the water-free fraction), glucose and 0%-5% unprocessed glucose oligomers. HFCS's use as a commercial sweetener has doubled in the last decade and, as a consequence, fructose intake in developed countries has increased five-fold[1].

In several human studies to date, increased fructose intake has been linked both to abnormal plasma lipid profiles and to the development of non-alcoholic fatty liver disease (NAFLD)[2-4]. However, because the effects of fructose, *per se*, are difficult to distinguish from the influences of other dietary carbohydrates and fats, current clinical evidence does not establish the precise contribution of fructose-containing food and beverage products to the etiology and/or the exacerbation of specific biochemical, clinical and histopathologic abnormalities. Further, since the causes of dietary carbohydrate and lipid-related problems (*e.g.,* hyperlipidemia, type Ⅱ diabetes, NAFLD, metabolic syndrome) are multifactorial and also related to age, gender and lifestyle, additional investigations are required to determine the relative contributions of dietary and other co-factors.

In attempting to identify the importance of fructose in hepatocellular lipid metabolism, available *in vitro* studies indicate fructose may preferentially promote *de novo* lipogenesis[5]. Since fructose bypasses the glycolytic rate-limiting enzyme phosphofructokinase, it is metabolized efficiently and provides a readily available substrate for hepatic lipid synthesis[6]. In addition, fructose has been shown to inhibit peroxisome proliferator-activated receptor (PPAR)-alpha mediated hepatocellular fatty acid beta-oxidation and lipid clearance[7].

The potential lipogenic role of fructose also may be related to its relationship to endogenous insulin activity. Insulin insensitivity has been implicated in the pathogenesis and progression of NAFLD[8]. Impaired insulin responsiveness to circulating carbohydrate is associated both with increased adipocyte lipolysis and with increased levels of circulating free fatty acids (FFA). Thus, insulin resistance promotes lipolysis, particularly in intra-abdominal, white adipose tissue. This phenomenon occurs as a consequence of dysregulation of lipid regulatory transcription factors (*e.g.*, PPAR-gamma), instability of adipocyte lipid and impaired lipogenesis. Released FFA lead to upregulation of inflammatory cytokines and chemokines[8,9]. Subsequently, liberated adipocyte FFA are taken-up by the liver and re-esterified to triglyceride. As fructose does not promote insulin secretion, it may therefore exacerbate the effects of insulin resistance on hepatic lipid synthesis, steatosis and the development of NAFLD[10]. Further evidence of fructose' role in the development of NAFLD derives from a recently reported rat model of hepatic steatosis. A high fructose-containing diet promoted not only lipogenesis leading to steatosis, but also increased expression of lipocalin-2, a ubiquitous glycoprotein involved in the response to inflammation and oxidative stress[11].

Despite evidence linking fructose to the development of altered lipid dynamics, hepatic steatosis and NAFLD, a recent meta-analysis concluded that studies examining the hepatocellular effects of fructose were confounded by the co-stimulation of lipogenesis resulting from increased total energy intake[12]. This observation suggests the deleterious effects of HFCS are merely a reflection of overall calorie excess in the Western diet.

In light of the above clinical and experimental data, the present study seeks to establish the influence of fructose on hepatocyte lipogenesis and provide a basis for future, translational investigations of fructose-

Table 1 Hepatocellular protein content

Glucose (mmol/L)	0.65	0.68	0.72
Protein (μg/mL)	635.0 ± 52.7	608.6 ± 35.3	543.2 ± 126.6
Fructose:glucose (mmol/L: mmol/L)	0.58:1[1]	0.61:1[1]	0.67:1[1]
Protein (μg/mL)	626.6 ± 95.2	610.2 ± 30.6	521.9 ± 38.7

[1]Total monosaccharide concentration = 0.72 mmol/L, Huh7 cell homogenates, means ± SD after 24 h of incubation in culture media containing only glucose or glucose plus fructose as the nutrient monosaccharide(s). All experiments comprise samples at a fixed volume of 10 mL/plate.

mediated lipid biosynthesis. These experiments employ an established, immortal and metabolically active human hepatocellular carcinoma cell line, Huh7, used extensively in studies of hepatocyte metabolism[13-15]. Since facilitated uptake of glucose and fructose by the transmembrane GLUT2 transporter is demonstrated in Huh7 cells[16], these cells provide an excellent model for studies of carbohydrate-induced lipogenesis. Accordingly, the studies herein were carried out to determine whether hepatocyte lipogenesis, in an *in vitro* cell culture model, is modulated by adjusting culture media monosaccharide content and concentration.

MATERIALS AND METHODS

Cell culture

Huh7 cells (American Type Culture Collection, Manassas, VA) were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS), 1% penicillin-streptomycin and 1% L-glutamine, in a 37 ℃, 5% CO_2 cell culture incubator on 100 mm × 200 mm tissue culture dishes (BD Falcon Durham, NC). Once 80% confluence was achieved (cell count - 1 × 10^5), cells were incubated for 24 h in culture media (DMEM) with 10% FBS containing either glucose alone (0.65, 0.68 and 0.72 mmol/L) or isosmolar monosaccharide (0.72 mmol/L) comprising fructose:glucose (F:G) molar ratios of 0.58, 0.61, and 0.67. For all experiments, total media osmolality was 400 mOsm/L. All incubations were performed in triplicate. Following the 24 h incubation, as described above, culture media was removed, plates were washed with phosphate buffered saline, and cells were lysed and collected in 750 microliters isopropanol, as previously described[17]. The cell lysates were kept at 4 ℃ for 12 h. Each sample was then centrifuged at 4 ℃ for 10 min at 10000 rpm. Supernatants were removed for lipid studies and the remaining cellular precipitate was re-suspended in 0.1N NaOH for protein quantification, employing a previously validated method[17].

Protein and lipid analyses

Cholesterol and triglyceride were measured utilizing samples from the isopropanol supernatant[18]. The samples were placed in a 96 well plate for lipid quantification, using established spectrophotometric methods[19,20]. Spectrophotometric absorbancies of each cell lysate sample were compared to known lipid standards, and the concentrations of cholesterol and triglyceride were calculated from the derived measurements[19,20]. Protein was quantified in triplicate utilizing samples from the NaOH suspension, employing standard spectrophotometric methods[21].

Cell mass determination

For these cell culture studies, estimates of cell mass in all experimental groups were made using total protein measurements in Huh7 lysates[22]. All single cell-line incubations were performed concurrently, under the same environmental conditions, thereby synchronizing cell cycles among experimental groups. Accordingly, as previously described, calculation of total cell protein content was employed to estimate relative cell biomass among incubations[23].

Statistical analysis

Differences among all experimental groups were assessed by analysis of variance, followed by Dunnett's test for multiple comparisons. A *P*-value of < 0.05 was considered significant.

RESULTS

Cell mass

Cultured Huh7 cells incubated in glucose-only supplemented media (0.65, 0.68, and 0.72 mmol/L) and in media containing varying F:G molar ratios (total monosaccharide concentration 0.72 mmol/L), did not show any statistically significant differences in protein content among all study groups. These data indicate total cell mass was not affected by varying the monosaccharide concentration or distribution (Table 1).

Glucose-mediated lipogenesis

As shown in Figures 1 and 2, triglyceride and cholesterol content (μg/mg cell protein) did not differ significantly among Huh7 cells incubated for 24 h in media containing 0.65, 0.68 or 0.72 mmol/L glucose per plate. Further increases (> 0.72 mmol/L) in glucose molar concentration did not result in any additional enhancement in cellular lipid content (data not shown).

Fructose-mediated lipogenesis

For these experiments, all cells were incubated in media containing 0.72 mmol/L monosaccharide (total media osmolality = 400 mOsm/L), the maximum sugar concentration employed in the "glucose-only" experiments, described above. To determine the effects of fructose on lipogenesis, cells were incubated in the presence of increasing molar ratios of F:G (0.58, 0.61 and 0.67). Following a 24 h incubation, Huh7 cell cholesterol content (μg/μg cell protein) increased significantly at a F:G molar ratio of 0.67:1 (Figure 3), compared both to a 0.61:1 ratio (0.18 μg/μg *vs* 0.14 μg/μg protein, $P < 0.05$) and to a 0.58:1 ratio (0.18 μg/μg *vs* 0.13 μg/μg protein, $P < 0.01$). Triglyceride analyses (Figure 4) demonstrated fructose-mediated Huh7 triglyceride synthesis also increased significantly in stepwise fashion. Thus, increased triglyceride content was

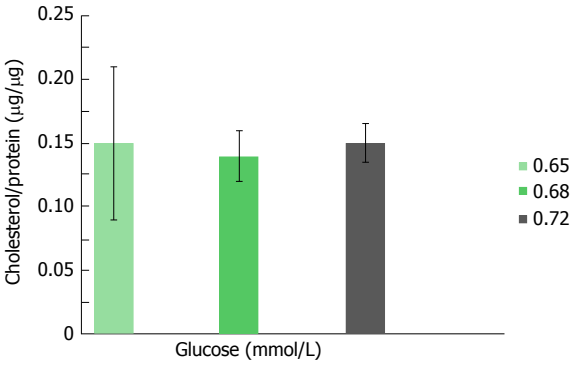

Figure 1 Cholesterol content of Huh7 cells (μg/μg cellular protein) following a 24-h incubation, in culture media containing 0.65, 0.68 and 0.72 mmol/L glucose.

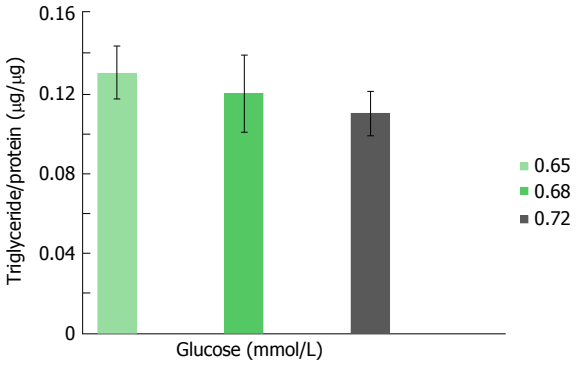

Figure 2 Triglyceride content of Huh7 cells (μg/μg cellular protein) following a 24-h incubation, in culture media containing 0.65, 0.68 and 0.72 mmol/L glucose.

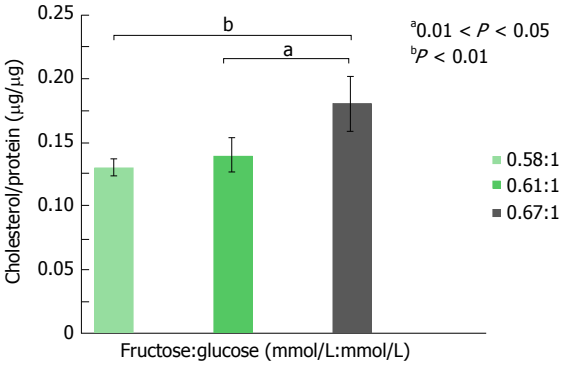

Figure 3 Cholesterol content of Huh7 cells (μg/μg cellular protein) following a 24-h incubation, in culture media containing fructose and glucose, at fructose:glucose molar ratios of 0.58:1, 0.61:1 and 0.67:1. All incubations were carried out in media containing 0.72 mmol/L total monosaccharide.

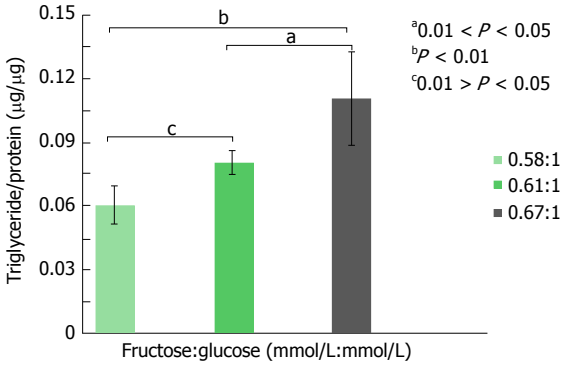

Figure 4 Triglyceride content of Huh7 cells (μg/μg cellular protein) following a 24-h incubation in culture media containing fructose and glucose, at fructose:glucose molar ratios of 0.58:1, 0.61:1 and 0.67:1. All incubations were carried out in media containing 0.72 mmol/L total monosaccharide.

noted at a F:G ratio of 0.61:1 (compared to the ratio of 0.58:1, 0.08 μg/μg *vs* 0.06 μg/μg protein, $P < 0.05$) and at a ratio of 0.67:1 (compared to the ratio of 0.61:1, 0.11 μg/μg *vs* 0.08 μg/μg protein, $P < 0.05$; to 0.67:1, 0.11 μg/μg *vs* 0.06 μg/μg protein, $P < 0.01$).

DISCUSSION

The results presented in this report demonstrate Huh7 cells, an immortal hepatocellular carcinoma cell line, grown in standard culture media containing increasing glucose concentrations (up to 0.72 mmol/L), exhibit no differences in relative cellular TG and C, as a consequence of increased media glucose content. However, when the nutrient monosaccharides comprise fructose plus glucose (at increasing F:G molar ratios), significant promotion of lipogenesis is demonstrated by increased hepatocellular TG and C concentrations. For these studies, the cell culture media monosaccharide content of 0.72 mmol/L (glucose alone or glucose plus fructose) was found to maximize hepatocellular lipogenesis. This molar amount was determined following a series of experiments, employing a step-wise increase in sugar content and based on previous human studies showing serum total monosaccharide concentrations of approximately 0.50 mmol/L following a fructose-rich meal[24]. Higher amounts of monosaccharide (> 0.72 mmol/L) in the Huh7 incubating media did not yield statistically significant increases either in cellular TG or in cellular C content, while further increases (> 400 mmol/L) in media osmolality resulted in decreased cell viability.

These results are consistent with a prior clinical report, suggesting hepatic fat (estimated by magnetic resonance imaging) in subjects fed fructose, in addition to a specific weight maintenance diet, was increased compared to a study group fed the same diet supplemented with glucose alone[4]. Conversely, another report failed to demonstrate any promotion of lipogenesis following four weeks of a fructose supplemented diet. As stated previously, a recent meta-analysis concluded the potential association between fructose and NAFLD was confounded by the concurrent consumption of hypercaloric diets[12]. The influence of fructose on lipogenesis, as a consequence of excess energy intake, may be mediated by this monosaccharide's direct attenuation of post-prandial ghrelin suppression[24]. On the other hand, another study examining the effects of dietary sucrose *vs* HFCS on endogenous hormone levels, failed to demonstrate any significant differences in serum insulin, leptin and ghrelin levels[25]. These combined *in vivo* studies therefore suggest the amount of carbohydrate taken up by hepatocytes is increased (thus leading

to enhanced lipogenesis) as a consequence of total calorie intake, rather than resulting directly from the lipogenic effects of any specific dietary substrate.

In contrast to available clinical data examining fructose mediated steatosis (often derived from imprecise imaging techniques), considerable biochemical evidence supports the role of fructose in promoting *de novo* lipogenesis. Since fructose is more efficiently utilized intracellularly, as compared with glucose, it may provide a more readily available substrate for lipid synthesis[26]. Interestingly, one recent study failed to demonstrate any effects of fructose on regulating hepatocellular lipogenic genes, thus providing further, indirect evidence linking the lipogenic role of this monosaccharide to its ability to provide increased amounts of carbon fragments for lipid synthesis[27]. The present results extend this observation by demonstrating that lipogenesis, in cultured hepatocytes, is not solely related to the provision of carbon precursors, since all incubations contained similar total monosaccharide concentrations. Thus, fructose, in this experimental model, appears to exert a direct effect on promotion of lipogenesis, and this effect is independent of carbon supply.

Because fructose metabolism is insulin-independent and does not stimulate pancreatic insulin secretion[28], this monosaccharide was previously thought to be a superior dietary substrate, as compared with other sugars[29]. However, more recent experimental data, while confirming these metabolic characteristics, also demonstrate fructose promotion of insulin resistance[12]. Fructose stimulates Jun-N-terminal kinase-1 (JNK-1), an intracellular mitogen activated protein kinase. JNK-1 in turn phosphorylates insulin receptor substrate-1 resulting in suppression of cellular glucose uptake, increased blood glucose levels and increased insulin secretion[30-32]. These findings, therefore, suggest another potential pathway for fructose-mediated steatosis.

Despite results presented in this report and published previously, the precise mechanisms by which fructose increases hepatic TG are not completely understood. Carbohydrate responsive element binding protein (ChREBP) and sterol regulatory element binding protein 1c (SREBP1c) are two transcription regulators of hepatic lipogenesis induced by glucose and insulin, respectively. Animal knockdown studies of peroxisome proliferator-activated receptor gamma co-activator-1 beta, a transcriptional co-activator of SREBP1c, have demonstrated improved hepatic lipid profiles in fructose fed rats. In a separate study[7], fructose fed rats showed increased expression of ChREBP hepatic mRNA, compared with a glucose fed group. Because recent data shows fetal bovine serum (FBS) contains factors that promote cellular lipogenesis, independent of insulin[33], and because FBS concentrations were held constant in our studies, stimulation of either SREBP1c or ChREBP represents an unlikely contributor to the observation of fructose-mediated *de novo* lipogenesis.

Our preliminary study certainly has several important limitations. These observations, in an *in vitro* cell culture model, cannot accurately predict the metabolic fate of fructose, in terms of its ability to enter lipogenic pathways. While the data clearly suggest direct effects of fructose on hepatocellular synthesis of triglyceride and cholesterol, they do not provide further elucidation of the underlying mechanisms leading to these findings. Nevertheless, the observation that fructose exerts a promoting influence on lipid synthesis, confirms prior studies suggesting the significant role of this monosaccharide in the development and/or exacerbation of hepatic steatosis. These data, therefore, warrant further investigations into the mechanisms and extent of fructose-mediated, *de novo* hepatic lipogenesis.

COMMENTS

Background

Available studies, both *in vitro* and in clinical trials, indicate the dietary monosaccharide, fructose, may be an important substrate for hepatic lipid synthesis, and may promote the development of hepatic steatosis. However, whether fructose-associated lipogenesis is related to dietary intake of fructose, *per se*, or merely reflects excess total energy consumption, remains unclear. The present study seeks to establish the effects of fructose on hepatocyte lipogenesis and provide a basis for future, translational investigations of fructose-mediated lipid biosynthesis. These experiments employ an established, immortal and metabolically active human hepatocellular carcinoma cell line, Huh7, used extensively in studies of hepatocyte metabolism. The studies herein were carried out to determine whether hepatocyte lipogenesis, in an *in vitro* cell culture model, is modulated by adjusting culture media monosaccharide content and concentration.

Innovations and breakthroughs

The results of these experiments clearly demonstrate, in a stable, *in vitro* hepatocyte culture model, at constant monosaccharide concentrations (glucose ± fructose), by increasing the culture medium fructose to glucose molar ratio, but not by increasing glucose alone, significant enhancement of lipogenesis.

Applications

The observation that fructose exerts a promoting influence on lipid synthesis, confirms prior studies suggesting the significant role of this monosaccharide in the development and/or exacerbation of hepatic steatosis. These studies, therefore, support the need for further investigations into the mechanisms and extent of fructose-mediated, *de novo* hepatic lipogenesis. Most important, these results provide a basis for future, clinical studies of fructose's role in development of hepatic steatosis and non-alcoholic liver disease.

Terminology

Huh7 cells, an immortal, stable hepatocyte line, derived from human hepatocellular carcinoma. These cells take-up both glucose and fructose, and have been used extensively in studies of hepatic metabolism.

Peer-review

This is an interesting study that reveals fructose is linked to lipogenesis in a concentration dependent way.

REFERENCES

1 **Lustig RH**. Fructose: metabolic, hedonic, and societal parallels with ethanol. *J Am Diet Assoc* 2010; **110**: 1307-1321 [PMID: 20800122 DOI: 10.1016/j.jada]

2 **Ouyang X**, Cirillo P, Sautin Y, McCall S, Bruchette JL, Diehl AM, Johnson RJ, Abdelmalek MF. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J Hepatol* 2008; **48**: 993-999 [PMID: 18395287 DOI: 10.1016/j.jhep]

3 **Silbernagel G**, Machann J, Unmuth S, Schick F, Stefan N, Häring HU, Fritsche A. Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. *Br J Nutr* 2011; **106**: 79-86 [PMID: 21396140

DOI: 10.1017/S000711451000574X]
4 **Lecoultre V**, Egli L, Carrel G, Theytaz F, Kreis R, Schneiter P, Boss A, Zwygart K, Lê KA, Bortolotti M, Boesch C, Tappy L. Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. *Obesity* (Silver Spring) 2013; **21**: 782-785 [PMID: 23512506 DOI: 10.1002/oby.20377]
5 **Spence JT**, Pitot HC. Induction of lipogenic enzymes in primary cultures of rat hepatocytes. Relationship between lipogenesis and carbohydrate metabolism. *Eur J Biochem* 1982; **128**: 15-20 [PMID: 6293823 DOI: 10.1111/j.1432-1033.1982.tb06924.x]
6 **Basciano H**, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab* (Lond) 2005; **2**: 5 [PMID: 15723702 DOI: 10.1186/1743-7075-2-5]
7 **Nagai Y**, Yonemitsu S, Erion DM, Iwasaki T, Stark R, Weismann D, Dong J, Zhang D, Jurczak MJ, Löffler MG, Cresswell J, Yu XX, Murray SF, Bhanot S, Monia BP, Bogan JS, Samuel V, Shulman GI. The role of peroxisome proliferator-activated receptor gamma coactivator-1 beta in the pathogenesis of fructose-induced insulin resistance. *Cell Metab* 2009; **9**: 252-264 [PMID: 19254570 DOI: 10.1016/j.cmet.2009.01.011]
8 **Delarue J**, Magnan C. Free fatty acids and insulin resistance. *Curr Opin Clin Nutr Metab Care* 2007; **10**: 142-148 [PMID: 17285001 DOI: 10.1097/MCO.0b013e328042ba90]
9 **Shah A**, Mehta N, Reilly MP. Adipose inflammation, insulin resistance, and cardiovascular disease. *JPEN J Parenter Enteral Nutr* 2008; **32**: 638-644 [PMID: 18974244 DOI: 10.1177/0148607108325251]
10 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850 [PMID: 11473047 DOI: 10.2337/diabetes.50.8.1844]
11 **Alwahsh SM**, Xu M, Seyhan HA, Ahmad S, Mihm S, Ramadori G, Schultze FC. Diet high in fructose leads to an overexpression of lipocalin-2 in rat fatty liver. *World J Gastroenterol* 2014; **20**: 1807-1821 [PMID: 24587658 DOI: 10.3748/wjg.v20.i7.1807]
12 **Bremer AA**, Mietus-Snyder M, Lustig RH. Toward a unifying hypothesis of metabolic syndrome. *Pediatrics* 2012; **129**: 557-570 [PMID: 22351884 DOI: 10.1542/peds.2011-2912]
13 **Chung M**, Ma J, Patel K, Berger S, Lau J, Lichtenstein AH. Fructose, high-fructose corn syrup, sucrose, and nonalcoholic fatty liver disease or indexes of liver health: a systematic review and meta-analysis. *Am J Clin Nutr* 2014; **100**: 833-849 [PMID: 25099546 DOI: 10.3945/ajcn.114.086314]
14 **Goto D**, Okimoto T, Ono M, Shimotsu H, Abe K, Tsujita Y, Kuwano M. Upregulation of low density lipoprotein receptor by gemfibrozil, a hypolipidemic agent, in human hepatoma cells through stabilization of mRNA transcripts. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2707-2712 [PMID: 9409246 DOI: 10.1161/01.ATV.17.11.2707]
15 **Yao H**, Ye J. Long chain acyl-CoA synthetase 3-mediated phosphatidylcholine synthesis is required for assembly of very low density lipoproteins in human hepatoma Huh7 cells. *J Biol Chem* 2008; **283**: 849-854 [PMID: 18003621 DOI: 10.1074/jbc.M706160200]
16 **Zhao Y**, Chen YQ, Bonacci TM, Bredt DS, Li S, Bensch WR, Moller DE, Kowala M, Konrad RJ, Cao G. Identification and characterization of a major liver lysophosphatidylcholine acyltransferase. *J Biol Chem* 2008; **283**: 8258-8265 [PMID: 18195019 DOI: 10.1074/jbc.M710422200]
17 **Tuch BE**, Szymanska B, Yao M, Tabiin MT, Gross DJ, Holman S, Swan MA, Humphrey RK, Marshall GM, Simpson AM. Function of a genetically modified human liver cell line that stores, processes and secretes insulin. *Gene Ther* 2003; **10**: 490-503 [PMID: 12621453 DOI: 10.1038/sj.gt.3301911]
18 **Egusa G**, Brady DW, Grundy SM, Howard BV. Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. *J Lipid Res* 1983; **24**: 1261-1267 [PMID: 6631250]
19 **Cramp DG**. Lipid methodology. *J Clin Pathol Suppl* (Assoc Clin Pathol) 1973; **5**: 17-21 [PMID: 4354843 DOI: 10.1136/jcp.s1-5.1.17]
20 **Roeschlau P**, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. *Z Klin Chem Klin Biochem* 1974; **12**: 226 [PMID: 4440114]
21 **Fossati P**, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 1982; **28**: 2077-2080 [PMID: 6812986]
22 **Spector T**. Refinement of the coomassie blue method of protein quantitation. A simple and linear spectrophotometric assay for less than or equal to 0.5 to 50 microgram of protein. *Anal Biochem* 1978; **86**: 142-146 [PMID: 655375 DOI: 10.1016/0003-2697(78)90327-5]
23 **Butler M**, Spearman M, Braasch K. Monitoring cell growth, viability, and apoptosis. *Methods Mol Biol* 2014; **1104**: 169-192 [PMID: 24297416]
24 **Teff KL**, Grudziak J, Townsend RR, Dunn TN, Grant RW, Adams SH, Keim NL, Cummings BP, Stanhope KL, Havel PJ. Endocrine and metabolic effects of consuming fructose- and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. *J Clin Endocrinol Metab* 2009; **94**: 1562-1569 [PMID: 19208729 DOI: 10.1210/jc.2008-2192]
25 **Melanson KJ**, Zukley L, Lowndes J, Nguyen V, Angelopoulos TJ, Rippe JM. Effects of high-fructose corn syrup and sucrose consumption on circulating glucose, insulin, leptin, and ghrelin and on appetite in normal-weight women. *Nutrition* 2007; **23**: 103-112 [PMID: 17234503 DOI: 10.1016/j.nut.2006.11.001]
26 **Basaranoglu M**, Basaranoglu G, Sabuncu T, Sentürk H. Fructose as a key player in the development of fatty liver disease. *World J Gastroenterol* 2013; **19**: 1166-1172 [PMID: 23482247 DOI: 10.3748/wjg.v19.i8.1166]
27 **Hirahatake KM**, Meissen JK, Fiehn O, Adams SH. Comparative effects of fructose and glucose on lipogenic gene expression and intermediary metabolism in HepG2 liver cells. *PLoS One* 2011; **6**: e26583 [PMID: 22096489 DOI: 10.1371/journal.pone.0026583]
28 **Curry DL**. Effects of mannose and fructose on the synthesis and secretion of insulin. *Pancreas* 1989; **4**: 2-9 [PMID: 2654926 DOI: 10.1097/00006676-198902000-00002]
29 **Schultz A**, Neil D, Aguila MB, Mandarim-de-Lacerda CA. Hepatic adverse effects of fructose consumption independent of overweight/obesity. *Int J Mol Sci* 2013; **14**: 21873-21886 [PMID: 24196354 DOI: 10.3390/ijms141121873]
30 **Hirosumi J**, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature* 2002; **420**: 333-336 [PMID: 12447443 DOI: 10.1038/nature01137]
31 **Lim JS**, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 251-264 [PMID: 20368739]
32 **Havel PJ**. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutr Rev* 2005; **63**: 133-157 [PMID: 15971409 DOI: 10.1111/j.1753-4887.2005.tb00132.x]
33 **Berenguer M**, Martinez L, Giorgetti-Peraldi S, Le Marchand-Brustel Y, Govers R. A serum factor induces insulin-independent translocation of GLUT4 to the cell surface which is maintained in insulin resistance. *PLoS One* 2010; **5**: e15560 [PMID: 21187969 DOI: 10.1371/journal.pone.0015560]

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ORIGINAL ARTICLE

Retrospective Study

Aluminum potassium sulfate and tannic acid sclerotherapy for Goligher Grades Ⅱ and Ⅲ hemorrhoids: Results from a multicenter study

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Author contributions: Miyamoto H designed the study; Miyamoto H, Hada T, Ishiyama G, Ono Y and Watanabe H acquired, analyzed and interpreted the data, and drafted the manuscript; Hada T contributed to statistical advice.

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Abstract

AIM: To show that aluminum potassium sulfate and tannic acid (ALTA) sclerotherapy has a high success rate for Grade Ⅱ and Ⅲ hemorrhoids.

METHODS: This study was based on the clinical data of 604 patients with hemorrhoids who underwent ALTA sclerotherapy between January 2009 and February 2015. The objective of this study was to assess the efficacy of this treatment for Grades Ⅱ and Ⅲ hemorrhoids. Preoperative and postoperative symptoms, complications and success rate were all assessed retrospectively. Follow-up consisted of a simple questionnaire, physical examination and an anoscopy. Patients were followed-up at one day, one week, two weeks, one month, one year, two years, three years, four years and five years after the ALTA sclerotherapy.

RESULTS: One hundred and sixty-nine patients were diagnosed with Grade Ⅱ hemorrhoids and 435 patients were diagnosed with Grade Ⅲ hemorrhoids. The one year, three year and five year cumulative success rates of ALTA sclerotherapy for Grades Ⅱ and Ⅲ hemo-

rrhoids were 95.9% and 93.1%; 89.3% and 83.7%; and 89.3% and 78.2%, respectively. No significant differences were observed in the cumulative success rates after ALTA sclerotherapy between Grades Ⅱ and Ⅲ hemorrhoids (P = 0.09). There were forty-seven post-operative complications (low grade fever; anal pain; urinary retention; rectal ulcer; and others). No serious or life-threatening complications occurred and all cases improved through conservative treatment. At univariate analysis there were no predictive factors of failure.

CONCLUSION: ALTA sclerotherapy has had a high success rate for Grade Ⅱ and Ⅲ hemorrhoids during five years of post-operative treatment. However, additional studies are needed to evaluate the efficacy of this ALTA sclerotherapy in the management of hemorrhoidal disease.

Key words: Sclerotherapy; Aluminum potassium sulfate and tannic acid; Goligher grade Ⅲ; Minimally invasive treatment; Hemorrhoid

Core tip: Since 2000, aluminum potassium sulfate and tannic acid (ALTA) sclerotherapy has been frequently performed in Japan for internal hemorrhoids as a minimally invasive treatment. This study affirms that ALTA sclerotherapy is an effective and safety treatment for Goligher Grades Ⅱ and Ⅲ hemorrhoids because of the high cumulative success rate and no serious complications during post-operative treatment.

Miyamoto H, Hada T, Ishiyama G, Ono Y, Watanabe H. Aluminum potassium sulfate and tannic acid sclerotherapy for Goligher Grades Ⅱ and Ⅲ hemorrhoids: Results from a multicenter study. *World J Hepatol* 2016; 8(20): 844-849 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i20/844.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i20.844

INTRODUCTION

Hemorrhoids are the most common anorectal disease world-wide. The term "hemorrhoidal disease" can be employed when hemorrhoidal tissue gives rise to symptoms such as bleeding, prolapse, or pruritus[1]. Etiologic factors are multi-factorial and include prolonged straining, irregular bowel habits and heredity. Supporting connective tissue degenerates and hemorrhoidal cushions slide as a consequence.

Conservative treatment based on lifestyle changes such as dietary and exercise and laxatives can help the majority of patients, and rubber band ligation, sclerotherapy and phlebotonic drugs can effectively treat Grades Ⅰ and Ⅱ hemorrhoids[2]. Surgical treatment is required for the most advanced stages, Grade Ⅲ or Ⅳ and bleeding. There are several methods of surgical treatment for hemorrhoids: Conventional hemorrhoidectomy (CH), stapled hemorrhoidopexy (PPH), and trans-anal hemorrhoidal dearterialization (THD)[1-4].

Among them, PPH and THD aim to correct the underlying pathophysiological mechanisms involved in the etiology of hemorrhoids. These treatments are painless treatment, because of the sparing of the anoderm. Although an increased risk of recurrence is the price to pay for these minimally invasive treatments, a rapid return to normal life without pain are greatly appreciated by patients[5].

On the other hand, new and effective sclerosant named aluminum potassium sulfate and tannic acid (ALTA) has been developed in Japan. ALTA sclerotherapy has been frequently performed in Japan for internal hemorrhoids as a minimally invasive treatment[6-9]. Nowadays in Japan, ALTA sclerotherapy for internal hemorrhoids has been performed in over 300000 cases.

In this report, we present effective results of ALTA sclerotherapy for Grades Ⅱ and Ⅲ hemorrhoids, according to the five year follow-up for this multi-center study.

Indication for ALTA sclerotherapy

ALTA sclerotherapy was performed on all patients with internal hemorrhoids except for the following: Patients with associated acute inflammatory internal hemorrhoids and acute irreducible hemorrhoids; patients with serious cardiac, hepatic, renal, and hematological diseases; pregnant women or women who may be pregnant; nursing mothers; and patients with a past history of hypersensitivity to local anesthetics.

MATERIALS AND METHODS

The medical records of 604 patients with hemorrhoidal disease who underwent ALTA sclerotherapy at four institutions from January 2009 to February 2015 were analyzed. All patients underwent clinical evaluation and physical examination including digital examination and proctoscopy for diagnosis of hemorrhoid engorgement and easy-bleeding, prolapsing hemorrhoids. The severity of hemorrhoidal disease was graded according to Goligher's classification.

Patients assumed the lithotomy position or Sims' position. Zero point five percent Lidocaine 10 mL was injected around sphincter muscle. The concentration of ALTA solution is set by, Mitsubishi Tanabe Pharma Corporation, Osaka, Japan. Procedures were undertaken under local anesthesia using the Z-type proctoscope (ARAKAWA SEISAKUJO, Tokyo, Japan) with a distally opening window that allowed for the application of an injection into the rectal mucosa. An ALTA four-step injection was performed[7]. The method used for the ALTA four-step injection was to inject four times, once into each of the following parts of the primary hemorrhoid: The superior part; the central deep and slight parts; and the inferior part above the dentate line.

Assessment and post-operative follow-up

All data were retrospectively collected from medical

Table 1 The characteristics of patients with Grades Ⅱ and Ⅲ hemorrhoids

Goligher classification	Grade Ⅱ	Grade Ⅲ	Statistical significance
Number	169	435	
Gender			
Male	103	231	NS
Female	66	204	
Age (yr)	58 ± 18	59 ± 16	NS
Injection dose of ALTA (mL)	13.4 ± 5.2	21.5 ± 6.8	$P < 0.0001$
Operative time	14 ± 8	16 ± 7	$P = 0.0020$

NS: Not significant; ALTA: Aluminum potassium sulfate and tannic acid.

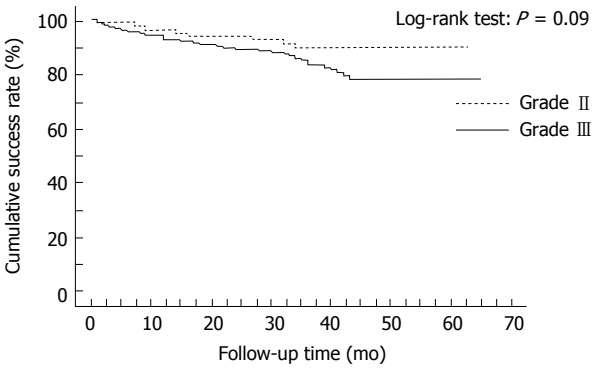

Figure 1 The cumulative success rate of aluminum potassium sulfate and tannic acid sclerotherapy. At one year, three years and five years after ALTA sclerotherapy, the cumulative success rate of Grades Ⅱ and Ⅲ were 95.9% and 93.1%; 89.3% and 83.7%; and 89.3% and 78.2%, respectively. ALTA: Aluminum potassium sulfate and tannic acid.

record. Patients took oral analgesia (loxoprofen or acetaminophen) at three days post operation. They were followed-up at one day, one week, two weeks, one month, one year, two years, three years, four years and five years after the ALTA sclerotherapy. Follow-up consisted of a simple questionnaire, physical examination and an anoscopy. Effects were defined as follows: (1) cure (after bowel movement there is no prolapse of the hemorrhoids, hemorrhage or other discomfort; and on examination with an anoscope, atrophied internal hemorrhoids have disappeared); (2) improvement (after bowel movement, some hemorrhoids prolapse but return into the anal canal spontaneously; occasional blood or hemorrhage with bowel movements; and an anoscopic examination reveals some internal hemorrhoids still visible); and (3) failure (no improvement, or some hemorrhoids prolapsed and did not return into the anal canal spontaneously).

The action mechanism of ALTA

ALTA compounds with aluminum potassium sulfate and tannic acid. The aluminum ion induces a strong local inflammatory reaction, resulting in fibrosis[10,11]. Tannic acid has a strong astringent effect on tissue, promoting protein coagulation and the contraction of blood vessels, while reducing exudation into tissue from the inflammatory reaction[10,11]. These actions tend to prevent tissue necrosis, and promote sclerosis, adhesion of hemorrhoidal tissue and immediate hemostasis and are also effective for the prolapse and bleeding of internal hemorrhoids early after injection.

Table 2 Prognostic factors associated with the recurrence of hemorrhoids after aluminum potassium sulfate and tannic acid sclerotherapy

Prognostic factor	Relative risk (95%CI)	*P* value
Age	0.997 (0.981-1.012)	0.6567
Gender	0.800 (0.469-1.365)	0.4124
Goligher grade	1.768 (0.897-3.483)	0.0997
Operation time	0.974 (0.941-1.010)	0.1542
ALTA injection dose	0.976 (0.941-1.012)	0.1901
Complication	1.293 (0.469-3.564)	0.6189

ALTA: Aluminum potassium sulfate and tannic acid.

RESULTS

The characteristics of the patients involved in this study are shown in Table 1. From January 2009 until February 2015 ALTA sclerotherapy was performed on 604 patients with second or third degree hemorrhoids. There were three 334 men and 270 women. The age range (mean ± SD) of the patients with Grades Ⅱ and Ⅲ hemorrhoids was 58 ± 18 years and 59 ± 16 years, respectively. Overall, 169 patients had Grade Ⅱ hemorrhoids and 435 had Grade Ⅲ hemorrhoids. The total injection dose of ALTA (mean ± SD) for Grades Ⅱ and Ⅲ was 13.4 ± 5.2 mL and 21.5 ± 6.8 mL, respectively. The operative time (mean ± SD) of Grades Ⅱ and Ⅲ was 14 ± 8 min and 16 ± 7 min, respectively. All the operations were performed under local anesthesia during either day surgery or a one day hospital stay.

Prolapses and bleeding disappeared immediately after ALTA sclerotherapy. All cases with bleeding or prolapses were cured or there was improvement after the first post-operative month. At one year after treatment, the rate of successful resolution of bleeding or prolapse of Grades Ⅱ and Ⅲ was 95.9% and 93.1%, respectively. The three year and five year cumulative success rates of ALTA sclerotherapy for Grades Ⅱ and Ⅲ hemorrhoids were 89.3% and 83.7%; and 89.3% and 78.2%, respectively. At one year after treatment, the rate of failure of Grades Ⅱ and Ⅲ was 4.1% and 6.9%, respectively. The three year and five year cumulative failure rates of ALTA sclerotherapy for Grades Ⅱ and Ⅲ hemorrhoids were 10.7% and 16.3%; and 10.7% and 21.8%, respectively. No significant differences were observed in the cumulative success rates after ALTA sclerotherapy between Grades Ⅱ and Ⅲ hemorrhoids ($P = 0.09$) (Figure 1). There were 47 post-operative complications (low grade fever; anal pain; urinary retention; rectal ulcer; and others). No serious or life-threatening complications occurred and all cases improved through conservative treatment. At univariate analysis there were no predictive factors of failure (Table 2). However, the only factor associated with a recurrence may be the grade of hemorrhoids ($P = 0.09$).

Table 3 Scheme of recurrence rate and/or additional operation rate of each hemorrhoid treatment

Ref.	Grade	Technique	*n*	Follow-up	Recurrence rate and/or additional surgery rate (%)
Giordano *et al*[4]	Ⅱ, Ⅲ	THD	28	3 yr	14.00
		SH	24		13.00
Giordano *et al*[17]	Ⅳ	THD + targeted mucopexy	31	32 mo	6.40
Ratto *et al*[18]	Ⅱ, Ⅲ, Ⅳ	THD	170	11.5 mo	4.10
Ratto *et al*[19]	Ⅱ, Ⅲ, Ⅳ	THD	803	11.1 mo	10.20
Zampieri *et al*[20]	Ⅲ, Ⅳ	THD	46	1-6 mo	0
		Ligasure	68		4.00
Theodoropoulos *et al*[21]	Ⅲ, Ⅳ	DGHAL + RAR	147	15 mo	4.00
Walega *et al*[22]	Ⅲ, Ⅳ	DGHAL + RAR	29	3 mo	10.34
Gravié *et al*[23]		SH	63	2 yr	7.50
		MMH	63		1.80
Ammaturo *et al*[24]	Ⅲ	SH	39	2 yr	13.00
		MMH	40		0
Hachiro *et al*[8]	Ⅲ, Ⅳ	ALTA	448	29 mo	3.60
Takano *et al*[6]	Ⅲ, Ⅳ	ALTA (OC-108)	80	1 yr	16.00
		CH	85		2.00
Miyamoto *et al*[7]	Ⅱ, Ⅲ, Ⅳ	ALTA	28	5 mo	10.70

THD: Trans-anal hemorrhoidal dearterialization; DGHAL: Doppler-guided hemorrhoidal arterial ligation; SH: Stapled hemorrhoidopexy; RAR: Rectoanal repair; CH: Conventional hemorrhoidectomy; ALTA: Aluminum potassium sulfate and tannic acid.

DISCUSSION

The ideal operation for hemorrhoids should be effective with a low rate of recurrence; minimal post-operative pain to allow early return to normal activities; and safe with minimal morbidity[12]. The treatment for internal hemorrhoids is gradually shifting to minimally invasive surgery. CH has been used to be the most widely performed surgical procedure till now. Although CH was very effective, it was painful and potentially affected the mechanism of anal continence. Over the years, alternative minimally invasive techniques have been developed including stapled hemorrhoidopexy, also known as PPH, and trans-anal hemorrhoidal dearterialization, also known as THD[1,3-5]. In Japan, new sclerosant, ALTA, has been developed. Takano *et al*[6] reported that ALTA (OC-108) sclerotherapy was effective for Grades Ⅱ, Ⅲ, and Ⅳ internal hemorrhoids. In recent years in Japan, ALTA sclerotherapy has become gradually recognized as minimally invasive treatment for internal hemorrhoids.

In Japan, ALTA sclerotherapy is popular with patients with symptomatic internal hemorrhoids, because unpleasant symptoms disappear immediately on the first post-operative day in almost all cases[7], and patients experience little post-operative pain and no serious complications[6-9]. ALTA sclerotherapy could be performed on patients with Grades Ⅱ and Ⅲ hemorrhoids in an outpatient clinic. Patients are highly satisfied with ALTA sclerotherapy as a treatment for internal hemorrhoids. Our previous study demonstrated that overall patient satisfaction at one month and one year after ALTA sclerotherapy was 97.7%[13]. In the Japanese literature, Matsuda *et al*[14] reported that the overall satisfaction of ALTA sclerotherapy was over 90% and concluded that ALTA sclerotherapy matched the needs of patients with symptomatic internal hemorrhoids. Minimally invasive treatments such as PPH, THD, or Doppler-guided hemorrhoidal arterial ligation (DGHAL) are acceptable treatments. But, if recurrence is the main consideration, CH is still considered the best[15]. A recent systematic review of 27 randomized controlled trials demonstrated that, compared with CH, PPH had less pain, shorter operative time, and quicker patient's recovery of patient, but a significantly higher rate of prolapse and reintervention for prolapse[16]. The recurrence rate and additional operation rate related to each technique were summarized in Table 3. Although the follow-up time was different, the recurrence rate and/or additional operation rate of CH, stapled hemorrhoidopexy, THD, DGHAL with rectoanal repair and ALTA was 0%-2%, 7.5%-13%, 0%-14%, 4%-10.34% and 3.6%-16%, respectively[4-7,17-24]. In this study, the one year cumulative rate for the successful resolution of bleeding or prolapse of Grades Ⅱ and Ⅲ was 95.9% and 93.1%, respectively. The five year cumulative success rate of ALTA sclerotherapy for Grades Ⅱ and Ⅲ hemorrhoids was 89.3% and 78.2%, respectively. ALTA sclerotherapy has had a high success rate for Grades Ⅱ and Ⅲ hemorrhoids during the five years post treatment.

Vidal *et al*[25] reported a new concept for the treatment of hemorrhoids with arterial embolization. Fourteen patients with disabling chronic rectal bleeding were treated using the emborrhoid technique. Although 10 patients had coagulation disorders (anticoagulants or cirrhosis), coil embolization of the superior rectal arteries is technically feasible, safe and well tolerated. Yano *et al*[26] reported that among patients with hemorrhoids receiving anticoagulant, ALTA sclerotherapy was recommended for those in whom it was difficult to discontinue anticoagulant. Miyamoto *et al*[27] reported the efficacy and safety of ALTA sclerotherapy for hemorrhoidal patients with liver cirrhosis.

In conclusion, ALTA sclerotherapy is an effective treatment for Grades Ⅱ or Ⅲ hemorrhoids. ALTA sclerotherapy might revolutionize the present state of hemo-

rrhoid treatment and be the ideal method for symptomatic internal hemorrhoids needed surgery. However, additional studies are needed to evaluate the efficacy of this ALTA sclerotherapy in the management of hemorrhoidal disease.

COMMENTS

Background

Aluminum potassium sulfate and tannic acid (ALTA) sclerotherapy is intended to shrink and harden internal hemorrhoids to eliminate hemorrhoidal prolapse and bleeding. ALTA sclerotherapy has been recognized as minimally invasive treatment for symptomatic internal hemorrhoid in Japan. The effectiveness of ALTA sclerotherapy, which shrinks and hardens internal hemorrhoids, is permanent.

Research frontiers

There are few reports of clarifying long time follow-up after ALTA sclerotherapy. The results of this study contribute to clarifying the effectiveness of ALTA sclerotherapy for Grade Ⅱ or Ⅲ hemorrhoids with five-year follow-up.

Innovations and breakthroughs

In this study, ALTA sclerotherapy is an effective treatment for Grades Ⅱ or Ⅲ hemorrhoids. The one-year, three-year and five-year cumulative success rates of ALTA sclerotherapy for Grades Ⅱ and Ⅲ hemorrhoids were 95.9% and 93.1%; 89.3% and 83.7%; and 89.3% and 78.2%, respectively. ALTA is an epoch-making sclerosant. ALTA sclerotherapy might be an effective sclerotherapy for symptomatic internal hemorrhoids needed surgery.

Applications

This study suggests that ALTA sclerotherapy is an effective treatment for Grades Ⅱ or Ⅲ hemorrhoids. If a patient is diagnosed with Grade Ⅱ or Ⅲ hemorrhoids, ALTA sclerotherapy can be chosen.

Peer-review

This is an interesting manuscript describing a relatively new minimally invasive treatment of haemorrhoids. The paper has a good number of cases and it is well designed.

REFERENCES

1 **Festen S**, van Hoogstraten MJ, van Geloven AA, Gerhards MF. Treatment of grade III and IV haemorrhoidal disease with PPH or THD. A randomized trial on postoperative complications and short-term results. *Int J Colorectal Dis* 2009; **24**: 1401-1405 [PMID: 19798507 DOI: 10.1007/s00384-009-0803-2]

2 **Acheson AG**, Scholefield JH. Management of haemorrhoids. *BMJ* 2008; **336**: 380-383 [PMID: 18276714 DOI: 10.1136/bmj.39465.674745.80]

3 **Dal Monte PP**, Tagariello C, Sarago M, Giordano P, Shafi A, Cudazzo E, Franzini M. Transanal haemorrhoidal dearterialisation: nonexcisional surgery for the treatment of haemorrhoidal disease. *Tech Coloproctol* 2007; **11**: 333-338; discussion 338-339 [PMID: 18060529 DOI: 10.1007/s10151-007-0376-4]

4 **Giordano P**, Nastro P, Davies A, Gravante G. Prospective evaluation of stapled haemorrhoidopexy versus transanal haemorrhoidal dearterialisation for stage II and III haemorrhoids: three-year outcomes. *Tech Coloproctol* 2011; **15**: 67-73 [PMID: 21318581 DOI: 10.1007/s10151-010-0667-z]

5 **Altomare DF**, Giuratrabocchetta S. Conservative and surgical treatment of haemorrhoids. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 513-521 [PMID: 23752820 DOI: 10.1038/nrgastro.2013.91]

6 **Takano M**, Iwadare J, Ohba H, Takamura H, Masuda Y, Matsuo K, Kanai T, Ieda H, Hattori Y, Kurata S, Koganezawa S, Hamano K, Tsuchiya S. Sclerosing therapy of internal hemorrhoids with a novel sclerosing agent. Comparison with ligation and excision. *Int J Colorectal Dis* 2006; **21**: 44-51 [PMID: 15843937 DOI: 10.1007/s00384-005-0771-0]

7 **Miyamoto H**, Asanoma M, Miyamoto H, Shimada M. ALTA injection sclerosing therapy: non-excisional treatment of internal hemorrhoids. *Hepatogastroenterology* 2012; **59**: 77-80 [PMID: 22260824 DOI: 10.5754/hge11089]

8 **Hachiro Y**, Kunimoto M, Abe T, Kitada M, Ebisawa Y. Aluminum potassium sulfate and tannic acid (ALTA) injection as the mainstay of treatment for internal hemorrhoids. *Surg Today* 2011; **41**: 806-809 [PMID: 21626327 DOI: 10.1007/s00595-010-4386-x]

9 **Tokunaga Y**, Sasaki H, Saito T. Evaluation of sclerotherapy with a new sclerosing agent and stapled hemorrhoidopexy for prolapsing internal hemorrhoids: retrospective comparison with hemorrhoidectomy. *Dig Surg* 2010; **27**: 469-472 [PMID: 21063123 DOI: 10.1159/000320321]

10 **Ono T**, Goto K, Takagi S, Iwasaki S, Komatsu H. Sclerosing effect of OC-108, a novel agent for hemorrhoids, is associated with granulomatous inflammation induced by aluminum. *J Pharmacol Sci* 2005; **99**: 353-363 [PMID: 16314689 DOI: 10.1254/jphs.FPJ05026X]

11 **Ono T**, Nakagawa H, Fukunari A, Hashimoto T, Komatsu H. Hemostatic action of OC-108, a novel agent for hemorrhoids, is associated with regional blood flow arrest induced by acute inflammation. *J Pharmacol Sci* 2006; **102**: 314-320 [PMID: 17072100 DOI: 10.1254/jphs.FP0060583]

12 **Yeo D**, Tan KY. Hemorrhoidectomy - making sense of the surgical options. *World J Gastroenterol* 2014; **20**: 16976-16983 [PMID: 25493010 DOI: 10.3748/wjg.v20.i45.16976]

13 **Miyamoto H**. ALTA sclerotherapy: the new sclerotherapy for curing advanced internal hemorrhoids, in Sclerotherapy: Procedures, Potential Complications and Clinical Outcomes (editor Brown ER), Nova Science Publishers, Inc. New York, USA, 2014: 149-163

14 **Matsuda Y**, Kawakami K, Nakai K, Asano M, Tanaka S, Nonaka M, Yano T, Ishimaru K, Yano Y, Ishii S, Kimura K. Recent evidence for operation versus non-operation of the anal treatment. *Geka* 2010; **72**: 1515-1520

15 **Giordano P**, Gravante G, Sorge R, Ovens L, Nastro P. Long-term outcomes of stapled hemorrhoidopexy vs conventional hemorrhoidectomy: a meta-analysis of randomized controlled trials. *Arch Surg* 2009; **144**: 266-272 [PMID: 19289667 DOI: 10.1001/archsurg.2008.591]

16 **Burch J**, Epstein D, Sari AB, Weatherly H, Jayne D, Fox D, Woolacott N. Stapled haemorrhoidopexy for the treatment of haemorrhoids: a systematic review. *Colorectal Dis* 2009; **11**: 233-243; discussion 243 [PMID: 18637932 DOI: 10.1111/j.1463-1318.2008.01638.x]

17 **Giordano P**, Tomasi I, Pascariello A, Mills E, Elahi S. Transanal dearterialization with targeted mucopexy is effective for advanced haemorrhoids. *Colorectal Dis* 2014; **16**: 373-376 [PMID: 24460621 DOI: 10.1111/codi.12574]

18 **Ratto C**, Donisi L, Parello A, Litta F, Doglietto GB. Evaluation of transanal hemorrhoidal dearterialization as a minimally invasive therapeutic approach to hemorrhoids. *Dis Colon Rectum* 2010; **53**: 803-811 [PMID: 20389215 DOI: 10.1007/DCR.0b013e3181cdafa7]

19 **Ratto C**, Parello A, Veronese E, Cudazzo E, D'Agostino E, Pagano C, Cavazzoni E, Brugnano L, Litta F. Doppler-guided transanal haemorrhoidal dearterialization for haemorrhoids: results from a multicentre trial. *Colorectal Dis* 2015; **17**: O10-O19 [PMID: 25213152 DOI: 10.1111/codi.12779]

20 **Zampieri N**, Castellani R, Andreoli R, Geccherle A. Long-term results and quality of life in patients treated with hemorrhoidectomy using two different techniques: Ligasure versus transanal hemorrhoidal dearterialization. *Am J Surg* 2012; **204**: 684-688 [PMID: 23140829 DOI: 10.1016/j.amjsurg.2012.01.014]

21 **Theodoropoulos GE**, Sevrisarianos N, Papaconstantinou J, Panoussopoulos SG, Dardamanis D, Stamopoulos P, Bramis K, Spiliotis J, Datsis A, Leandros E. Doppler-guided haemorrhoidal artery ligation, rectoanal repair, sutured haemorrhoidopexy and minimal mucocutaneous excision for grades III-IV haemorrhoids:

a multicenter prospective study of safety and efficacy. *Colorectal Dis* 2010; **12**: 125-134 [PMID: 19055522 DOI: 10.1111/j.1463-1318.2008.01739.x]

22 **Walega P**, Krokowicz P, Romaniszyn M, Kenig J, Sałówka J, Nowakowski M, Herman RM, Nowak W. Doppler guided haemorrhoidal arterial ligation with recto-anal-repair (RAR) for the treatment of advanced haemorrhoidal disease. *Colorectal Dis* 2010; **12**: e326-e329 [PMID: 19674029 DOI: 10.1111/j.1463-1318.2009.02034.x]

23 **Gravié JF**, Lehur PA, Huten N, Papillon M, Fantoli M, Descottes B, Pessaux P, Arnaud JP. Stapled hemorrhoidopexy versus milligan-morgan hemorrhoidectomy: a prospective, randomized, multicenter trial with 2-year postoperative follow up. *Ann Surg* 2005; **242**: 29-35 [PMID: 15973098 DOI: 10.1097/01.sla.0000169570.64579.31]

24 **Ammaturo C**, Tufano A, Spiniello E, Sodano B, Iervolino EM, Brillantino A, Braccio B. Stapled haemorrhoidopexy vs. Milligan-Morgan haemorrhoidectomy for grade III haemorrhoids: a randomized clinical trial. *G Chir* 2012; **33**: 346-351 [PMID: 23095566]

25 **Vidal V**, Sapoval M, Sielezneff Y, De Parades V, Tradi F, Louis G, Bartoli JM, Pellerin O. Emborrhoid: a new concept for the treatment of hemorrhoids with arterial embolization: the first 14 cases. *Cardiovasc Intervent Radiol* 2015; **38**: 72-78 [PMID: 25366092 DOI: 10.1007/s00270-014-1017-8]

26 **Yano T**, Nogaki T, Asano M, Tanaka S, Kawakami K, Matsuda Y. Outcomes of case-matched injection sclerotherapy with a new agent for hemorrhoids in patients treated with or without blood thinners. *Surg Today* 2013; **43**: 854-858 [PMID: 23052752 DOI: 10.1007/s00595-012-0365-8]

27 **Miyamoto H**, Nakagawa T, Miyamoto H, Takata A. Sclerotherapy Using Aluminum Potassium Sulfate and Tannic Acid (ALTA) for Haemorrhoids in Patients With Liver Cirrhosis. *Ann Colorectal Res* 2015; **3**: e32980 [DOI: 10.19975/acr-32980]

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ORIGINAL ARTICLE

Clinical Trials Study

Transjugular intrahepatic portosystemic shunt combined with esophagogastric variceal embolization in the treatment of a large gastrorenal shunt

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Author contributions: Jiang Q was involved in analysis and interpretation of data, as well as drafting the manuscript; Jiang Q and Kong DR designed the research; Jiang Q and Wu Q performed the research; Wang MQ, Zhang GB, and Xu JM provided TIPS technical support and were involved in study supervision; Xu JM and Kong DR were involved in study design, analysis and interpretation of data, critical revision of the manuscript, and study supervision.

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Informed consent statement: All involved patients gave their informed written consent prior to study inclusion.

Conflict-of-interest statement: We declare that we have no financial or personal relationships with other individuals or organizations that can inappropriately influence our work. We have no professional or personal interests of any nature related to any product, service, and/or company that could be construed as influencing the position presented within, or the review of, the entitled manuscript.

Data sharing statement: Technical appendix, statistical code, and dataset are available from the corresponding author at kdr168@sohu.com. Participants gave informed consent for data sharing.

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Abstract

AIM: To evaluate the efficacy and safety of transjugular intrahepatic portosystemic shunt (TIPS) combined with stomach and esophageal variceal embolization (SEVE) in cirrhotic patients with a large gastrorenal vessel shunt (GRVS).

METHODS: Eighty-one cirrhotic patients with gastric variceal bleeding (GVB) associated with a GRVS were enrolled in the study and accepted TIPS combined with SEVE (TIPS + SEVE), by which portosystemic pressure

gradient (PPG), biochemical, TIPS-related complications, shunt dysfunction, rebleeding, and death were evaluated.

RESULTS: The PPGs before TIPS were greater than 12 mmHg in 81 patients. TIPS + SEVE treatment caused a significant decrease in PPG (from 37.97 ± 6.36 mmHg to 28.15 ± 6.52 mmHg, t = 19.22, P < 0.001). The percentage of reduction in PPG was greater than 20% from baseline. There were no significant differences in albumin, alanine aminotransferase, aspartate aminotransferase, bilirubin, prothrombin time, or Child-Pugh score before and after operation. In all patients, rebleeding rates were 3%, 6%, 12%, 18%, and 18% at 1, 3, 6, 12, and 18 mo, respectively. Five patients (6.2%) were diagnosed as having hepatic encephalopathy. The rates of shunt dysfunction were 0%, 4%, 9%, 26%, and 26%, at 1, 3, 6, 12, and 18 mo, respectively. The cumulative survival rates in 1, 3, 6, 12, and 18 mo were 100%, 100%, 95%, 90%, and 90%, respectively.

CONCLUSION: Our preliminary results indicated that the efficacy and safety of TIPS + SEVE were satisfactory in cirrhotic patients with GVB associated with a GRVS (GVB + GRVS).

Key words: Transjugular intrahepatic portosystemic shunt; Cirrhosis; Gastric varices; Variceal embolization; Gastrorenal shunt

Core tip: The optimal treatment of gastric variceal bleeding (GVB) + gastrorenal vessel shunt (GRVS) remains uncertain. Transjugular intrahepatic portosystemic shunt (TIPS) alone cannot be widely used in the treatment of GVB + GRVS. Some studies have evaluated the short-term outcomes of cirrhosis treated with TIPS combined with variceal embolization. In this study, we found that the efficacy and safety of TIPS + stomach and esophageal variceal embolization were satisfactory for patients with GVB + GRVS.

Jiang Q, Wang MQ, Zhang GB, Wu Q, Xu JM, Kong DR. Transjugular intrahepatic portosystemic shunt combined with esophagogastric variceal embolization in the treatment of a large gastrorenal shunt. *World J Hepatol* 2016; 8(20): 850-857 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i20/850.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i20.850

INTRODUCTION

Although the rate of gastric variceal bleeding (GVB) is significantly lower than that of esophageal variceal bleeding (EVB)[1,2], it is usually more severe, requires more transfusions, and is associated with higher mortality than EVB[1-3]. Currently, the optimal treatment of GVB remains a difficult issue for clinicians. In terms of recommendatory therapy for gastric varices, there are various primary options, including surgery, endoscopic variceal obturation with tissue adhesive, Transjugular intrahepatic portosystemic shunt (TIPS) placement, and balloon-occluded retrograde transvenous obliteration[4,5]. First-line therapies for gastric varices are endoscopically administered tissue adhesives and TIPS placement.

GVB is often associated with a gastrorenal vessel shunt (GRVS)[6]. The safety of endoscopically-administered tissue adhesives in patients with GVB + GRVS is controversial, due to potential cerebral or pulmonary embolism secondary to migration of cyanoacrylate into the systemic circulation through GRVS[7]. TIPS placement has been widely accepted as an effective and safe treatment for GVB in cirrhotic patients[4,8]. However, because the portosystemic pressure gradient (PPG) in patient with GVB + GRVS is lower than that in patient with EV, TIPS placement alone is seldom is used in the treatment of GVB + GRVS[9-13].

Recent years, some studies have shown that TIPS combined with variceal embolization prevented recurrent variceal bleeding and improved liver function[14,15]. However, there are no similar studies to evaluate the effectiveness of a combination of these two methods for patients with GVB + GRVS. The aim of this study was therefore to evaluate the efficacy and safety of TIPS + SEVE for patients with GVB + GRVS.

MATERIALS AND METHODS

Patients

Between October 2013 and December 2015, a total of 107 patients in whom TIPS + SEVE had been successfully performed in our hospital were recruited for this study. Inclusion criteria were as follow: (1) age > 18 years; (2) history of cirrhosis and GVB (based on findings of histological or typical cross-sectional imaging, such as ultrasound, endoscopy, computed tomography, or magnetic resonance imaging); and (3) patients was diagnosed as having GRVS by computed tomography angiography (CTA). Exclusion criteria were: (1) hepatocellular carcinoma or other malignancies; (2) chronic renal failure; (3) portal vein thrombosis; (4) infection; and (5) coagulation disorder. Of the 107 patients, 26 with EVB or GVB without GRVS were excluded from this study. Thus, the final population for study consisted of 81 patients. The main clinical and biochemical characteristics of these 81 patients are presented in Table 1. All patients provided their informed written consent. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Anhui Medical University.

Procedural protocol

Procedures were performed with general anesthesia in the angiography suite. The procedure of TIPS + SEVE has been described previously[14-16]. Briefly, before cathe-

Table 1 Characteristics of the 81 patients treated with transjugular intrahepatic portosystemic shunt + stomach and esophageal variceal embolization

No. of patients	81 (%)
Men	63 (77.8)
Female	9 (22.2)
Age (yr)	
Mean ± SD	50.9 ± 10.9
Range	25-76
Cause of liver disease	*n* (%)
Viral	61 (75.4)
Alcoholic	7 (8.7)
Viral and alcoholic	1 (1.2)
Primary biliary cirrhosis	4 (4.9)
Autoimmune hepatitis	1 (1.2)
Cryptogenic	7 (8.6)
Child-Pugh class	*n* (%)
A	15 (18.5)
B	47 (58.0)
C	19 (23.5)
Endoscopic findings	
IGV1	25 (30.9)
GOV1	10 (12.3)
GOV2	46 (56.8)
Pre-PPG (mmHg)	
Mean ± SD	38.0 ± 6.4
Range	26.0-48.0
Follow-up (mo)	
Mean ± SD	7.87 ± 5.57
Range	1-18

PPG: Portosystemic pressure gradient.

terization of the hepatic vein was performed through the right internal jugular vein, inferior vena cava pressure was measured when the tip of the catheter floated in the inferior vena cava at the junction with the hepatic vein. A needle and guide-wire were advanced through the liver parenchyma into a branch of the portal vein with fluoroscopic guidance, which was then followed by direct portography and measurement of portal vein pressure. A catheter was passed into the gastroesophageal collateral vessels and embolization of the collateral vessels was initiated, which formed coils of varying diameters and resulted in the disappearance of varices at post-embolization angiography. The catheter was then exited *via* the liver parenchyma. After the parenchymal tract between the hepatic vein and portal vein was dilated with an angioplasty balloon catheter, the patency of the TIPS was facilitated by deployment of a covered stent (8 mm in diameter, BARD E LUMINEXX Vascular Stent, France). The PPG was determined *via* the difference between the portal vein pressure and inferior vena cava pressure. The mid-chest was used as the external zero reference. Pressure tracings must remain stable for at least 30 s to be considered satisfactory. The mean value of two PPG measurements was used for analysis.

All patients received intravenous antibiotic prophylaxis 1 d before the procedure. Intravenous heparin was given as an anti-coagulate during the procedure and for 1 wk post-procedure, which then changed to oral aspirin and warfarin for 1 year. Oral lactulose was used to prevent hepatic encephalopathy (HE).

Follow-up

All patients were asked to enroll in the follow-up protocol. PPG, biochemical examination, TIPS-related complications, post-HE, primary patency, rebleeding, and death were recorded respectively. Patients were examined during follow-up with Doppler ultrasound, endoscopy, and CTA at 1, 3, 6, and 12 mo after TIPS placement and then every 6 mo thereafter. Patients suffering from HE, rebleeding, or any other severe complications were invited to our TIPS unit at any time. Liver functions were assessed by testing albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, prothrombin time (PT) levels, and Child-Pugh score at 1 wk before and 1 mo after TIPS. TIPS patency could be assessed by Doppler ultrasonography. Endoscopy confirmed sources of bleeding and variceal disappearance. CTA was used to define the GRVS. Patients were followed until death or liver transplantation, while first rebleeding, first HE, and first shunt insufficiency were followed-up on to a maximum of 2 years after the procedure (closure date: December 31, 2015).

Definitions

The following definitions were used: (1) rebleeding: Any subsequent hematemesis or melena confirmed endoscopically; (2) HE: Diagnosis of HE was made according to the final report of the 1998 Working Party at the 11th World Congress of Gastroenterology in Vienna[17], and patients with clinical evidence of HE were classified according to the West Haven criteria grades: HE ⩾ grade Ⅰ; (3) shunt dysfunction[18]: Doppler criteria for shunt insufficiency was that maximal flow velocity was less than 50 cm/s or that there was an absence of flow within the shunt. Suspected shunt dysfunction was confirmed by portography that showed shunt stenosis > 50%; (4) primary patency: The absence of shunt insufficiency without intervention during TIPS surveillance; and (5) endoscopic findings of esophagogastric varices were recorded as proposed by the Japanese Society for portal hypertension[19].

Statistical analysis

The data were expressed as means ± SD. Quantitative variables were compared using Student's *t* test. The rates of primary patency, HE, survival, and variceal rebleeding were analyzed using Kaplan-Meier analyses. A statistically significant difference was assessed for any of the analyses with results of $P < 0.05$. Analyses were performed using the SPSS 10.0 software package.

RESULTS

Basic data

Table 2 summarizes the basic clinical and biochemical characteristics of patients. As shown, the PPG before TIPS placement was greater than 12 mmHg in all patients. The mean PPG dropped from 37.97 ± 6.36 mmHg to

Table 2 Comparison of main biochemical data and portosystemic pressure gradient before and after the transjugular intrahepatic portosystemic shunt + stomach and esophageal variceal embolization

	Before TIPS	After TIPS	*P*
Albumin (mg/dL)	32.24 ± 5.88	33.90 ± 7.26	0.199
ALT (u/L)	30.00 ± 17.51	30.85 ± 20.60	0.806
AST (u/L)	38.00 ± 25.95	41.88 ± 24.03	0.318
Bilirubin (mg/dL)	1.41 ± 0.76	1.45 ± 0.65	0.561
PT (%)	52 ± 14	51 ± 15	0.903
Creatinine (mg/dL)	1 ± 0.3	1 ± 0.4	0.58
Child-Pugh score	6.91 ± 1.44	6.79 ± 1.34	0.563
PPG (mmHg)	38.0 ± 6.4	28.2 ± 6.5	< 0.001

PPG: Portosystemic pressure gradient; TIPS: Transjugular intrahepatic portosystemic shunt; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; PT: Prothrombin time.

28.15 ± 6.52 mmHg after TIPS (t = 19.22, P < 0.001), with reductions in PPG greater than 20% from baseline. There were no significant differences in albumin, ALT, AST, bilirubin, PT, or Child-Pugh score 1 wk before or 1 mo after operation.

Rebleeding

Rebleeding from the upper gastrointestinal tract occurred in ten patients (12.3%) after TIPS placement. One patient had 4 U of blood transfused within 24 h after the TIPS procedure, with no symptoms of rebleeding observed thereafter. The cumulative rates of rebleeding (Kaplan-Meier estimation) after 1, 3, 6, 12, and 18 mo were 3%, 6%, 12%, 18%, and 18%, respectively. The actual probability of rebleeding is presented in Figure 1. One patient underwent tissue adhesive administration 6 mo after TIPS implantation and is, at the time of writing, alive and free of rebleeding. One patient was found to have portal hypertensive gastropathy, which resulted in rebleeding. The other rebleeding patients were found to have shunt stenosis or obstruction.

Survival

Five patients died within the follow-up period because of procedure-related complications. In one patient, a shunt obstruction was observed 6 mo after TIPS placement; the patient refused intervention treatment and died seven months after TIPS due to recurrent bleeding. The other four patients died 5 to 12 mo after TIPS placement. The cumulative rates of survival (Kaplan-Meier estimation) after 1, 3, 6, 12, and 18 mo were 100%, 100%, 95%, 90%, and 90%, respectively. Survival curves are shown in Figure 2.

HE

Five patients experienced HE sometimes before the operation and were also diagnosed as having HE after TIPS placement. A protein-restricted diet and/or lactulose treatment were given to prevent the recurrence of HE. The cumulative rates of HE (Kaplan-Meier estimation) after 1, 3, 6, 12, and 18 mo were 9%, 13%, 18%, 18%, and 18%, respectively (Figure 3).

Primary shunt patency

The cumulative rates of primary shunt patency (Kaplan-Meier estimation) after 1, 3, 6, 12, and 18 mo were 100%, 96%, 91%, 74%, and 74%, respectively (Figure 4). During the follow-up period, 10 (12.3%) patients were diagnosed as having shunt stenosis or obstruction, of which 8 patients successfully underwent shunt recanalization with balloon angioplasty. Although one patient with shunt obstruction died 7 mo after TIPS (as previously mentioned), at the time of writing, the remaining patients are alive and well, albeit with one patient who had to receive anticoagulant therapy.

Other complications

During the follow-up period, the rare complication of hepatic myelopathy (HM) occurred in two patients 6 to 8 mo after the TIPS procedure, which exerted a significant impact on their mobility and quality of life. Due to economic factors, the patients received conservative medical treatment and are, at the time of writing, alive.

DISCUSSION

The rate of GVB is significantly lower than that of EVB[1,2], but is usually more severe, requires more transfusions, and is associated with higher mortality than EVB[1-3]. Currently, the optimal treatment of GVB remains a difficult issue for clinicians.

Variceal embolotherapy has been recognized as an efficient method for preventing bleeding caused by portal hypertension[19,20], while TIPS is used worldwide for the prevention of variceal bleeding[4,5,8]. Previous studies have advocated TIPS combined with variceal embolization in the prevention of recurrent variceal bleeding and improvement of liver function[14,15]. However, there are no similar studies evaluating the combination of these two methods in patients with GVB + GRVS.

In the current study, we found that the PPG before TIPS placement was greater than 12 mmHg in patients with GVB + GRVS. All included patients had previously experienced at least one instance of bleeding. Ou *et al*[21] found that 35% (14/40) of patients with GVB had a PPG ⩽ 12 mmHg at the time of TIPS[22]. The differing results may be related to the number of cases and the size of spontaneous GRVS in our study, despite previous studies illustrating that PPG appears to correlate inversely with the presence and size of spontaneous GRVS[6,21]; to date, there have been no attempts to measure the size of GRVS and the definition of GRVS size remains as yet undetermined.

It has been reported that patients with strong GVB have a lower PPG than those with EV, which may be a result of GRVS development[6,23]. Several studies have found that decompressive methods such as TIPS do not seem to confer much of a benefit for GVB + GRVS[9-13]. Our results suggest that the rebleeding rate after TIPS was 12% after 1 year, which was similar to the typically

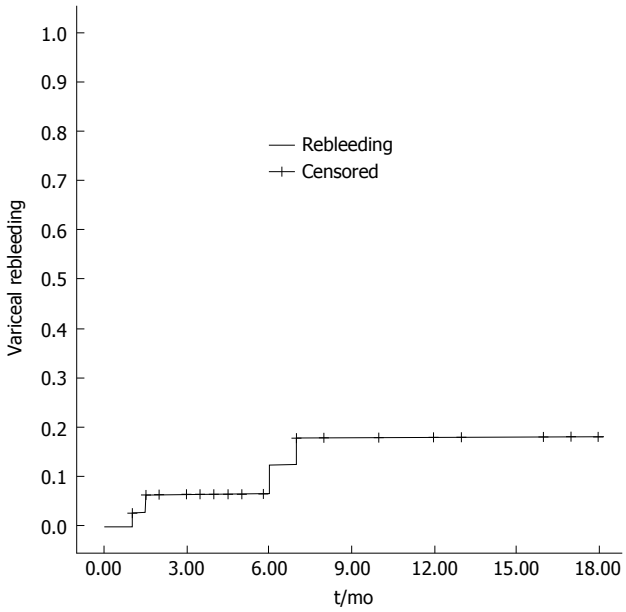

Figure 1 Graph of Kaplan-Meier estimation of cumulative percentages of rebleeding.

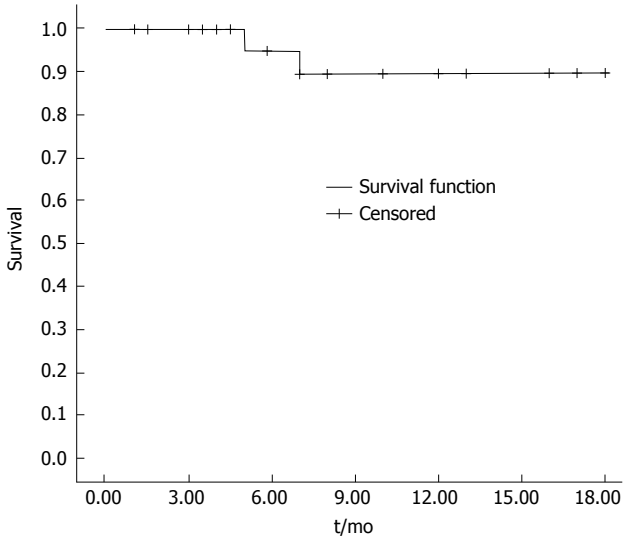

Figure 2 Kaplan-Meier plot shows the rates of survival after transjugular intrahepatic portosystemic shunt placement.

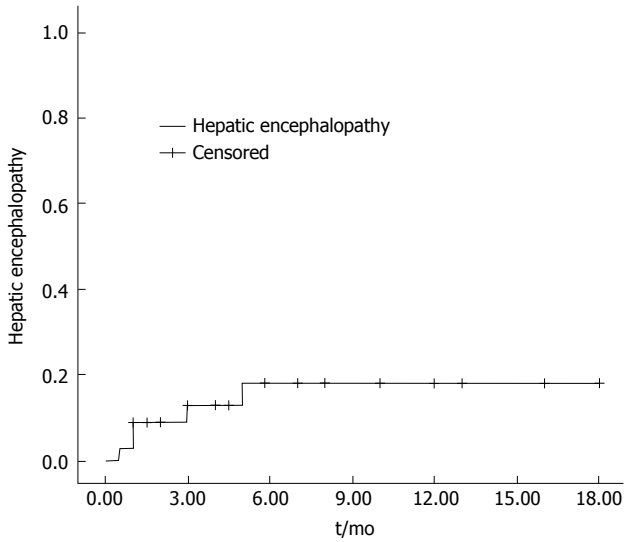

Figure 3 Actuarial probability of hepatic encephalopathy in 81 patients treated with transjugular intrahepatic portosystemic shunt + stomach and esophageal variceal embolization.

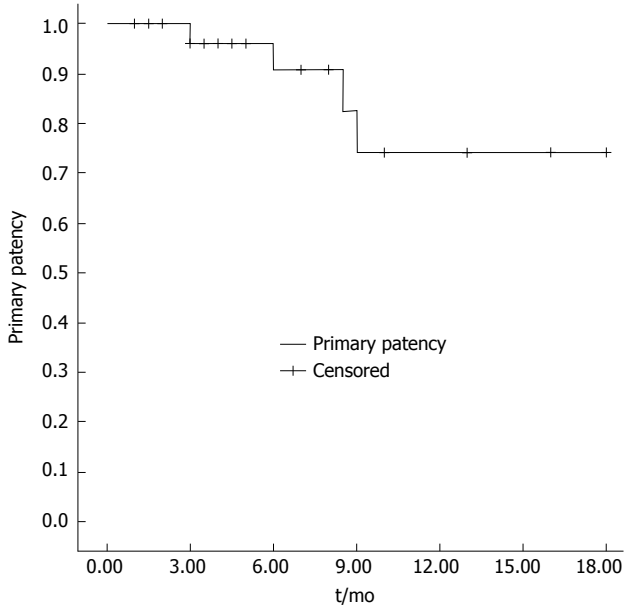

Figure 4 Graph of Kaplan-Meier estimation of cumulative percentages of patients with primary shunt patency in all patients undergoing transjugular intrahepatic portosystemic shunt + stomach and esophageal variceal embolization.

reported result of between 10% to 40%[24,25], while the reduction in PPG was greater than 20% from the baseline. Moreover, we noticed that TIPS + SEVE may reduce the risk of rebleeding. It should be noted that previous studies of TIPS differed from our own in that they used bare stents with TIPS alone placement or did not limit the stent diameter. In our study, all patients underwent decompressive operation and embolotherapy *via* coil, as well as the embolization of extensive collateral circulation (such as that of the short or posterior gastric vein), which may contribute to the occlusion of GRVS. All covered stents were dilated to 8 mm, which may be regarded as limited shunts that accord with natural hemodynamic features.

Survival is usually regarded as the strongest evidence for evaluating the effectiveness of a therapy. In previous studies, total survival 1-year post-TIPS ranged from 58% to 80% and depended mainly on the severity of the underlying liver disease[25,26]. The survival rate was 94% at 1 year in our study; such a high rate may be related to the patients' liver function (76.5% patients with Child-Pugh class A or B). Although our results support patients with Child-Pugh class C as well, TIPS placement should be used with extreme caution. Taken together, improving liver function before TIPS may increase the survival rates.

TIPS has been extensively used within the last 20 years. Previous studies showed that TIPS increases the

incidence of HE without improving survival[27-29], which may be the reason why it is currently only recommended as a rescue therapy. HE has been reported to occur in 16%-31% of patients who receive a TIPS in the presence of GVB + GRS[30]. Our results indicated that 15% of our patients were diagnosed as having HE after TIPS placement, which is very similar to the reports of other studies, and that only one patient required admission. Importantly, our results were attributed to three effective improvements. First, oral lactulose was used to prevent HE after operation. Second, the left portal vein could be successfully punctured in 58% patients. As we know, the left portal vein receives blood from the splenic vein and inferior mesenteric vein, which have fewer digestive products but more electrolytes. Most recent studies have illustrated that introducing TIPS to the left portal vein instead of the right portal vein could decrease the risk of HE[31-33]. Third, 8 mm stents were used in patients. It has been previously reported in the literature that the incidence of portosystemic HE increased with increasing diameter of the stent[31].

It has been shown that occlusion and stenosis are the main disadvantages of TIPS. Studies have demonstrated that stent insufficiency occurs in 14% to 82% of patients by 1 year post-TIPS[25,33]. Our findings suggest that 12% of patients in our study were diagnosed as having stenosis or obstruction one year after TIPS; our results therefore showed higher patency rates when compared to historical data. It was reported that the routine administration of anticoagulants and the use of covered stents play important roles in the improved patency rate[34-36]. Thus, the higher patency rate of our patients was partially attributed to the use of covered stents and anticoagulant therapy. Other possible reasons for our results are that patients were regularly followed-up on and that TIPS was placed in the left portal vein.

During the follow-up period, two patients were diagnosed with HM, in which the spontaneous shunt found by CTA was not completely closed. Embolization only with coils may be an insufficient embolization factor that was thought to be secondary to the increased systemic circulation of shunting portal venous toxins from the hypoperfusion and ischemia of the hepatocytes. Studies showed that a liver transplant could fully reverse the effects of HM in patients with early stage disease[37,38], however, due to economic factors, patients only received conservative medical treatment. Despite previous studies advocating TIPS combined with variceal embolization to improve liver function[15,39], there were no significant differences in liver functions before and after TIPS placement in our study.

In spite of these results, we may conclude that PPG before TIPS placement may be greater than 12 mmHg in patients with GVB + GRVS, and that the efficacy and safety of TIPS + SEVE were satisfactory in these patients.

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COMMENTS

Background

The optimal treatment for gastric variceal bleeding (GVB) + gastrorenal vessel shunt (GRVS) is still controversial. Transjugular intrahepatic portosystemic shunt (TIPS) alone cannot be widely used in the treatment for GVB + GRVS. Previous studies have advocated TIPS combined with variceal embolization in order to prevent recurrent variceal bleeding and improve liver function. However, the efficacy and safety of TIPS + stomach and esophageal variceal embolization (SEVE) in patients with GVB + GRVS was unclear.

Research frontiers

In recent years, more and more patients have undergone TIPS procedure to prevent variceal bleeding. For the use of the TIPS procedure, the research hot spot is how to increase the patient survival rate and reduce complications by bettering the patient selection and improving techniques. Interestingly, TIPS + SEVE may decrease portal pressure and embolize extensive collateral circulation, thereby potentially reducing the risk of rebleeding.

Innovations and breakthroughs

Most GVB is associated with a GRVS. The efficacy of tissue adhesives in patients with GVB + GRVS is controversial, due to the potential for systemic embolism secondary to migration of cyanoacrylate into the systemic circulation through a GRVS. TIPS alone cannot be widely used in the treatment of GVB + GRVS. In the study, all patients underwent TIPS + SEVE with *via* coil, with extensive collateral circulation, such as short or posterior gastric vein, potentially contributing to the occlusion of GRVS. In this study, the authors found that the efficacy and safety of TIPS + SEVE were satisfactory in patients with GVB + GRVS.

Applications

The results suggest that the efficacy and safety of TIPS + SEVE were satisfactory in patients with GVB + GRVS. Additional studies with long-term follow-up are needed to confirm the results.

Peer-review

The authors have provided a well-designed study that shows the satisfactory efficacy and safety of combination TIPS + SEVE in cirrhotic patients with gastric variceal bleeding associated with a gastrorenal vessel shunt.

REFERENCES

1 **Sarin SK**, Lahoti D, Saxena SP, Murthy NS, Makwana UK. Prevalence, classification and natural history of gastric varices: a long-term follow-up study in 568 portal hypertension patients. *Hepatology* 1992; **16**: 1343-1349 [PMID: 1446890 DOI: 10.1002/hep.1840160607]

2 **Kim T**, Shijo H, Kokawa H, Tokumitsu H, Kubara K, Ota K, Akiyoshi N, Iida T, Yokoyama M, Okumura M. Risk factors for hemorrhage from gastric fundal varices. *Hepatology* 1997; **25**: 307-312 [PMID: 9021939 DOI: 10.1002/hep.510250209]

3 **Thakeb F**, Salem SA, Abdallah M, el Batanouny M. Endoscopic diagnosis of gastric varices. *Endoscopy* 1994; **26**: 287-291 [PMID: 8076547 DOI: 10.1055/s-2007-1008969]

4 **Boyer TD**, Haskal ZJ. The Role of Transjugular Intrahepatic Portosystemic Shunt (TIPS) in the Management of Portal Hypertension: update 2009. *Hepatology* 2010; **51**: 306 [PMID: 19902484 DOI: 10.1002/hep.23383]

5 **de Franchis R**. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2010; **53**: 762-768 [PMID: 20638742 DOI: 10.1016/j.jhep.2010.06.004]

6 **Watanabe K**, Kimura K, Matsutani S, Ohto M, Okuda K. Portal hemodynamics in patients with gastric varices. A study in 230

patients with esophageal and/or gastric varices using portal vein catheterization. *Gastroenterology* 1988; **95**: 434-440 [PMID: 3391371]

7 **Irisawa A**, Obara K, Sato Y, Saito A, Orikasa H, Ohira H, Sakamoto H, Sasajima T, Rai T, Odajima H, Abe M, Kasukawa R. Adherence of cyanoacrylate which leaked from gastric varices to the left renal vein during endoscopic injection sclerotherapy: a histopathologic study. *Endoscopy* 2000; **32**: 804-806 [PMID: 11068842 DOI: 10.1055/s-2000-7702]

8 **García-Pagán JC**, Caca K, Bureau C, Laleman W, Appenrodt B, Luca A, Abraldes JG, Nevens F, Vinel JP, Mössner J, Bosch J. Early use of TIPS in patients with cirrhosis and variceal bleeding. *N Engl J Med* 2010; **362**: 2370-2379 [PMID: 20573925 DOI: 10.1056/NEJMoa0910102]

9 **Caldwell S**. Gastric varices: is there a role for endoscopic cyanoacrylates, or are we entering the BRTO era? *Am J Gastroenterol* 2012; **107**: 1784-1790 [PMID: 23211846 DOI: 10.1038/ajg.2012.160]

10 **Matsumoto A**, Matsushita M, Sugano Y, Takimoto K, Yasuda M, Inokuchi H. Limitations of transjugular intrahepatic portosystemic shunt for management of gastric varices. *Gastroenterology* 2004; **126**: 380-381 [PMID: 14753222 DOI: 10.1053/j.gastro.2003.07.021]

11 **Ryan BM**, Stockbrugger RW, Ryan JM. TIPS for gastric varices. *Gut* 2003; **52**: 772; author reply 772 [PMID: 12692074 DOI: 10.1136/gut.52.5.772]

12 **Sanyal AJ**, Freedman AM, Luketic VA, Purdum PP, Shiffman ML, DeMeo J, Cole PE, Tisnado J. The natural history of portal hypertension after transjugular intrahepatic portosystemic shunts. *Gastroenterology* 1997; **112**: 889-898 [PMID: 9041251 DOI: 10.1053/gast.1997.v112.pm9041251]

13 **Choi YH**, Yoon CJ, Park JH, Chung JW, Kwon JW, Choi GM. Balloon-occluded retrograde transvenous obliteration for gastric variceal bleeding: its feasibility compared with transjugular intrahepatic portosystemic shunt. *Korean J Radiol* 2003; **4**: 109-116 [PMID: 12845306 DOI: 10.3348/kjr.2003.4.2.109]

14 **Tesdal IK**, Filser T, Weiss C, Holm E, Dueber C, Jaschke W. Transjugular intrahepatic portosystemic shunts: adjunctive embolotherapy of gastroesophageal collateral vessels in the prevention of variceal rebleeding. *Radiology* 2005; **236**: 360-367 [PMID: 15955858 DOI: 10.1148/radiol.2361040530]

15 **Chen S**, Li X, Wei B, Tong H, Zhang MG, Huang ZY, Cao JW, Tang CW. Recurrent variceal bleeding and shunt patency: prospective randomized controlled trial of transjugular intrahepatic portosystemic shunt alone or combined with coronary vein embolization. *Radiology* 2013; **268**: 900-906 [PMID: 23657891 DOI: 10.1148/radiol.13120800]

16 **Rössle M**, Haag K, Ochs A, Sellinger M, Nöldge G, Perarnau JM, Berger E, Blum U, Gabelmann A, Hauenstein K. The transjugular intrahepatic portosystemic stent-shunt procedure for variceal bleeding. *N Engl J Med* 1994; **330**: 165-171 [PMID: 8264738 DOI: 10.1056/NEJM199401203300303]

17 **Ferenci P**, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy--definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002; **35**: 716-721 [PMID: 11870389 DOI: 10.1053/jhep.2002.31250]

18 **Han G**, Qi X, He C, Yin Z, Wang J, Xia J, Yang Z, Bai M, Meng X, Niu J, Wu K, Fan D. Transjugular intrahepatic portosystemic shunt for portal vein thrombosis with symptomatic portal hypertension in liver cirrhosis. *J Hepatol* 2011; **54**: 78-88 [PMID: 20932597 DOI: 10.1016/j.jhep.2010.06.029]

19 **Tajiri T**, Yoshida H, Obara K, Onji M, Kage M, Kitano S, Kokudo N, Kokubu S, Sakaida I, Sata M, Tajiri H, Tsukada K, Nonami T, Hashizume M, Hirota S, Murashima N, Moriyasu F, Saigenji K, Makuuchi H, Oho K, Yoshida T, Suzuki H, Hasumi A, Okita K, Futagawa S, Idezuki Y. General rules for recording endoscopic findings of esophagogastric varices (2nd edition). *Dig Endosc* 2010; **22**: 1-9 [PMID: 20078657 DOI: 10.1111/j.1443-1661]

20 **Kwok AC**, Wang F, Maher R, Harrington T, Gananadha S, Hugh TJ, Samra JS. The role of minimally invasive percutaneous embolisation technique in the management of bleeding stomal varices. *J Gastrointest Surg* 2013; **17**: 1327-1330 [PMID: 23546560 DOI: 10.1007/s11605-013-2180-y]

21 **Ou HY**, Huang TL, Chen TY, Tsang LL, Concejero AM, Chen CL, Cheng YF. Emergency splenic arterial embolization for massive variceal bleeding in liver recipient with left-sided portal hypertension. *Liver Transpl* 2005; **11**: 1136-1139 [PMID: 16123955 DOI: 10.1002/lt.20543]

22 **Tripathi D**, Therapondos G, Jackson E, Redhead DN, Hayes PC. The role of the transjugular intrahepatic portosystemic stent shunt (TIPSS) in the management of bleeding gastric varices: clinical and haemodynamic correlations. *Gut* 2002; **51**: 270-274 [PMID: 12117893 DOI: 10.1136/gut.51.2.270]

23 **Ohnishi K**, Nakayama T, Koen H, Saito M, Saito M, Chin N, Terabayashi H, Iida S, Nomura F, Okuda K. Interrelationship between type of spontaneous portal systemic shunt and portal vein pressure in patients with liver disease. *Am J Gastroenterol* 1985; **80**: 561-564 [PMID: 4014107]

24 **Chao Y**, Lin HC, Lee FY, Wang SS, Tsai YT, Hsia HC, Lin WJ, Lee SD, Lo KJ. Hepatic hemodynamic features in patients with esophageal or gastric varices. *J Hepatol* 1993; **19**: 85-89 [PMID: 8301048 DOI: 10.1016/S0168-8278(05)80180-1]

25 **Garcia-Pagán JC**, Barrufet M, Cardenas A, Escorsell A. Management of gastric varices. *Clin Gastroenterol Hepatol* 2014; **12**: 919-928.e1; quiz e51-e52 [PMID: 23899955 DOI: 10.1016/j.cgh.2013.07.015]

26 **Ryan BM**, Stockbrugger RW, Ryan JM. A pathophysiologic, gastroenterologic, and radiologic approach to the management of gastric varices. *Gastroenterology* 2004; **126**: 1175-1189 [PMID: 15057756 DOI: 10.1053/j.gastro.2004.01.058]

27 **Berry K**, Lerrigo R, Liou IW, Ioannou GN. Association between Transjugular Intrahepatic Portosystemic Shunt and Survival in Patients with Cirrhosis. *Clin Gastroenterol Hepatol* 2016; **14**: 118-123 [PMID: 26192147 DOI: 10.1016/j.cgh.2015.06.042]

28 **Papatheodoridis GV**, Goulis J, Leandro G, Patch D, Burroughs AK. Transjugular intrahepatic portosystemic shunt compared with endoscopic treatment for prevention of variceal rebleeding: A meta-analysis. *Hepatology* 1999; **30**: 612-622 [PMID: 10462365 DOI: 10.1002/hep.510300316]

29 **Khan S**, Tudur Smith C, Williamson P, Sutton R. Portosystemic shunts versus endoscopic therapy for variceal rebleeding in patients with cirrhosis. *Cochrane Database Syst Rev* 2006; (**4**): CD000553 [PMID: 17054131]

30 **Escorsell A**, Bañares R, García-Pagán JC, Gilabert R, Moitinho E, Piqueras B, Bru C, Echenagusia A, Granados A, Bosch J. TIPS versus drug therapy in preventing variceal rebleeding in advanced cirrhosis: a randomized controlled trial. *Hepatology* 2002; **35**: 385-392 [PMID: 11826413 DOI: 10.1053/jhep.2002.30418]

31 **Sabri SS**, Abi-Jaoudeh N, Swee W, Saad WE, Turba UC, Caldwell SH, Angle JF, Matsumoto AH. Short-term rebleeding rates for isolated gastric varices managed by transjugular intrahepatic portosystemic shunt versus balloon-occluded retrograde transvenous obliteration. *J Vasc Interv Radiol* 2014; **25**: 355-361 [PMID: 24468043 DOI: 10.1016/j.jvir.2013.12.001]

32 **Xue H**, Yuan J, Chao-Li Y, Palikhe M, Wang J, Shan-Lv L, Qiao W. Follow-up study of transjugular intrahepatic portosystemic shunt in the treatment of portal hypertension. *Dig Dis Sci* 2011; **56**: 3350-3356 [PMID: 21643741 DOI: 10.1007/s10620-011-1744-5]

33 **Chen L**, Xiao T, Chen W, Long Q, Li R, Fang D, Wang R. Outcomes of transjugular intrahepatic portosystemic shunt through the left branch vs. the right branch of the portal vein in advanced cirrhosis: a randomized trial. *Liver Int* 2009; **29**: 1101-1109 [PMID: 19386025 DOI: 10.1111/j.1478-3231.2009.02016.x]

34 **Bai M**, He CY, Qi XS, Yin ZX, Wang JH, Guo WG, Niu J, Xia JL, Zhang ZL, Larson AC, Wu KC, Fan DM, Han GH. Shunting branch of portal vein and stent position predict survival after transjugular intrahepatic portosystemic shunt. *World J Gastroenterol* 2014; **20**: 774-785 [PMID: 24574750 DOI: 10.3748/wjg.v20.i3.774]

35 **Bureau C**, Garcia-Pagan JC, Otal P, Pomier-Layrargues G, Chabbert V, Cortez C, Perreault P, Péron JM, Abraldes JG, Bouchard L, Bilbao JI, Bosch J, Rousseau H, Vinel JP. Improved clinical outcome using polytetrafluoroethylene-coated stents for TIPS: results of a randomized study. *Gastroenterology* 2004; **126**: 469-475 [PMID: 14762784 DOI: 10.1053/j.gastro.2003.11.016]

36 **Yang Z**, Han G, Wu Q, Ye X, Jin Z, Yin Z, Qi X, Bai M, Wu K, Fan D. Patency and clinical outcomes of transjugular intrahepatic portosystemic shunt with polytetrafluoroethylene-covered stents versus bare stents: a meta-analysis. *J Gastroenterol Hepatol* 2010; **25**: 1718-1725 [PMID: 21039832 DOI: 10.1111/j.1440-1746.2010.06400.x]

37 **Sauer P**, Theilmann L, Herrmann S, Bruckner T, Roeren T, Richter G, Stremmel W, Stiehl A. Phenprocoumon for prevention of shunt occlusion after transjugular intrahepatic portosystemic stent shunt: a randomized trial. *Hepatology* 1996; **24**: 1433-1436 [PMID: 8938176 DOI: 10.1002/hep.510240622]

38 **Baccarani U**, Zola E, Adani GL, Cavalletti M, Schiff S, Cagnin A, Poci C, Merkel C, Amodio P, Montagnese S. Reversal of hepatic myelopathy after liver transplantation: fifteen plus one. *Liver Transpl* 2010; **16**: 1336-1337 [PMID: 21031552 DOI: 10.1002/lt.22149]

39 **Weissenborn K**, Tietge UJ, Bokemeyer M, Mohammadi B, Bode U, Manns MP, Caselitz M. Liver transplantation improves hepatic myelopathy: evidence by three cases. *Gastroenterology* 2003; **124**: 346-351 [PMID: 12557140 DOI: 10.1053/gast.2003.50062]

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CASE REPORT

Hepatitis C virus cures after direct acting antiviral-related drug-induced liver injury: Case report

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Abstract

The United States Food and Drug Administration recently warned that the direct acting antiviral (DAA) combination hepatitis C virus (HCV) treatment of Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir, and Ribavirin (PODr + R) can cause severe liver injury in patients with advanced liver disease. Drug induced liver injury was observed in a small number of patients with decompensated cirrhosis treated with other DAAs, but has not been reported in patients with compensated cirrhosis. We report a case of a 74-year-old woman with chronic HCV and Child-Pugh class A cirrhosis (compensated cirrhosis) treated with PODr + R. The patient presented on day 14 of PODr + R therapy with jaundice and new-onset ascites. Her total bilirubin level increased to 23 mg/dL and international normalized ratio rose to 1.65, while aminotransferase levels remained relatively stable. Hepatitis C treatment was discontinued on day 24 and she gradually recovered. Follow-up testing showed that she achieved a sustained virologic response. In conclusion, hepatic decompensation developed within two weeks of starting treatment with

PODr + R in a patient with Child-Pugh class A cirrhosis and was characterized by jaundice and ascites with stable aminotransferase levels. Careful monitoring is warranted in patients with HCV-related cirrhosis treated with PODr + R.

Key words: Direct antiviral agent; Drug-induced liver injury; Hepatitis C; Mathematical modeling; Sustained virological response; Viral kinetics

Core tip: To the best of our knowledge, this is the first report of hepatic decompensation in a hepatitis C patient with Child Pugh class A cirrhosis due to treatment with Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir and Ribavirin (PODr + R). Liver aminotransferase levels did not increase prior to decompensation, depriving us of our usual alarm signs heralding hepatic decompensation. The patient achieved sustained virologic response despite very early discontinuation of therapy (day 24).

Hasin Y, Shteingart S, Dahari H, Gafanovich I, Floru S, Braun M, Shlomai A, Verstandig A, Dery I, Uprichard SL, Cotler SJ, Lurie Y. Hepatitis C virus cures after direct acting antiviral-related drug-induced liver injury: Case report. *World J Hepatol* 2016; 8(20): 858-862 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i20/858.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i20.858

INTRODUCTION

Globally, it was estimated that in 2005, more than 185 million people were anti-hepatitis C virus (HCV) seropositive (prevalence 2.8%)[1]. End-stage liver disease due to HCV is a leading indication for liver transplantation and accounts for almost 500000 deaths annually[2]. The growing proportion of patients with chronic HCV infection and cirrhosis has the highest priority for treatment, but are at risk for complications of liver disease[3].

The interferon-free, 12-wk Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir and Ribavirin (PODr + R) regimen is approved for the treatment of patients with Child's-Pugh class A cirrhosis and was shown to achieve a sustained virologic response (SVR) rate exceeding 90% in such patients[4]. However, on 10/22/15, an Food and Drug Administration (FDA) Drug Safety Communication warned that PODr + R can cause severe drug-induced liver injury (DILI) in patients with advanced liver disease[5]. Since December 2014, 26 cases of severe liver injury in patients with advanced liver disease were reported to the FDA Adverse Event Reporting System database that were considered probably or possibly related to PODr + R. Ten patients developed liver failure resulting in death or liver transplantation. The pattern of liver injury consisted of an acute increase in bilirubin level without alanine aminotransferase (ALT) elevations. The FDA Drug Safety Communication emphasized that PODr + R is contraindicated in patients with Child's class B and C cirrhosis. More recently, four cases of severe DILI were identified in patients who had decompensated cirrhosis at pre-treatment baseline who were treated with sofosbuvir and a NS5A inhibitor, with or without Ribavirin[6-8]. Three of the patients were human immunodeficiency virus-co-infected and were receiving antiretroviral therapy. To date, severe DILI has not been reported in patients with compensated cirrhosis who receive direct acting antiviral (DAA).

CASE REPORT

A 74-year-old woman presented to Shaare Zedek Medical Center in Jerusalem on 12/30/14 for management of HCV genotype 1b infection with compensated cirrhosis (Child's Pugh class A-score 6, MELD score 7), and a 5.6 cm × 6.8 cm right lobe liver lesion with diagnostic features of hepatocellular carcinoma (HCC) on ultrasonography and contrast-enhanced computed tomography. Her baseline laboratory data are shown in Figure 1.

On 1/1/15 she underwent transcatheter arterial chemoembolization (TACE) as treatment for HCC. Her ALT and aspartate transaminase levels increased transiently and then declined to baseline (Figure 1B). She showed no signs of hepatic decompensation before or after the procedure. Treatment with Ombitasvir, Paritaprevir, Ritonavir (2 tablets daily), Dasabuvir (250 mg twice daily, and Ribavirin (400 mg, twice daily) was initiated on 3/1/15, two months after TACE. The HCV RNA level declined from a pre-treatment baseline of 2000000 IU/mL to 474 IU/mL in 7 d (Figure 2). However, on day 14 of treatment, she presented with nausea and increased abdominal girth. Her medications were limited to PODr + R and Ramipril for hypertension, which she had taken for four months. She reported taking no other prescription, over the counter, or alternative medications during the four months prior to the onset of liver injury. She did not drink alcohol. On physical examination, she had icteric sclera and moderate ascites. She developed vomiting and anorexia. Serial laboratories showed a rising total bilirubin (maximum 23 mg/dL) and international normalized ratio (maximum 1.65) levels and her albumin level decreased to 2.5 g/dL (Figure 1A). Of note, her aminotransferase levels remained stable (Figure 1B). An abdominal ultrasound with Doppler study showed flow in the portal and hepatic veins and no new mass lesions in the liver.

Antiviral therapy was discontinued on treatment day 24 (3/24/15) for suspected DILI. The patient was hospitalized on 4/1/15 for management of ascites. After hospital discharge, she was treated with weekly intravenous albumin and Furosemide until November 2015. Her ascites slowly improved and her liver biochemistries normalized (Figure 1). Seven months after discontinuation of antiviral therapy, she has recovered and her HCV RNA remains undetectable (Figure 2). She

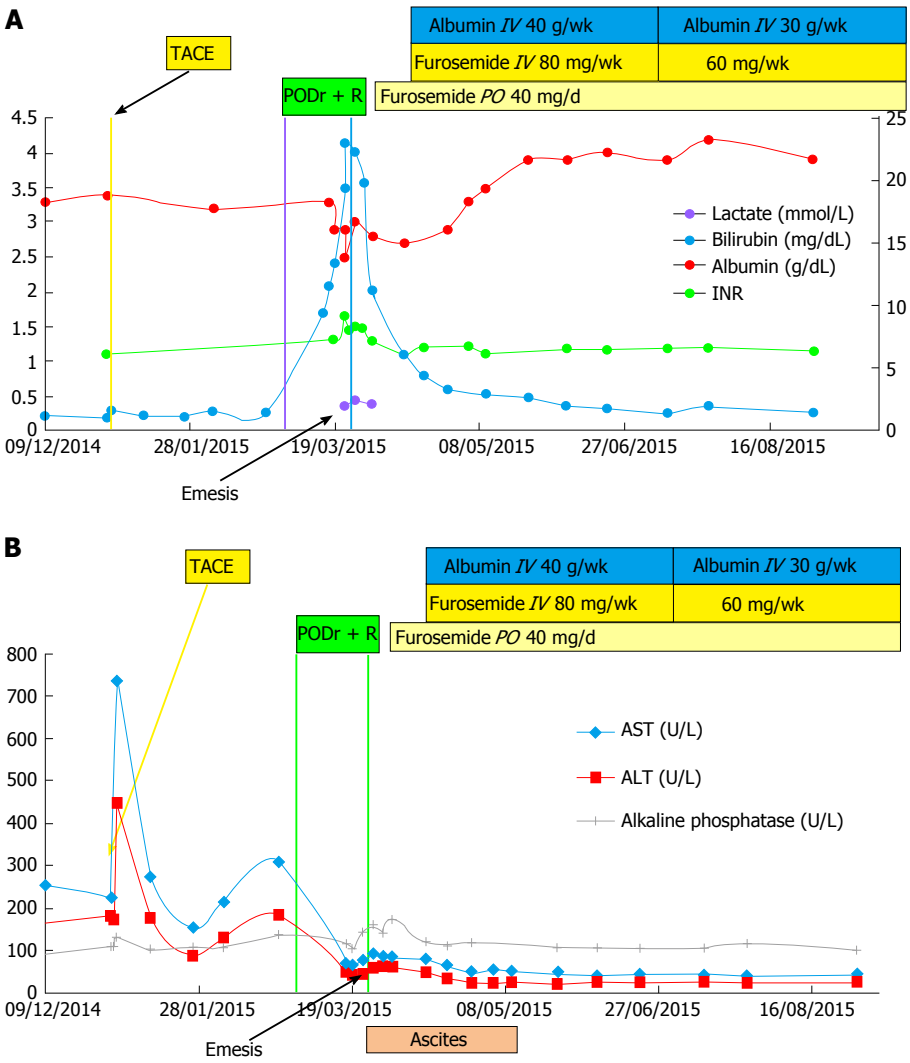

Figure 1 Change in laboratories in relation to antiviral therapy (A) and in aminotransferase and Alkaline Phosphate levels over time (B). A: Change in laboratories in relation to antiviral therapy. Shortly after initiation of hepatitis C treatment, and after 4 mo of stable levels, laboratory values deteriorated indicating a decline in hepatic synthetic function; peak bilirubin - 23 mg/dL, PT/INR - 1.65, lactate-2.44 mg/dL, lowest albumin - 2.5 g/L. Shortly after treatment discontinuation, laboratory values slowly returned to baseline; B: Change in aminotransferase and alkaline phosphate levels over time. There was a spike in aminotransferase levels after the TACE procedure. Following HCV treatment initiation, aminotransferase levels declined over time, reaching plateau levels of AST = 40 U/L and ALT = 25 U/L. Aminotransferase levels remained stable when total bilirubin and PT/INR levels increased (shown in Figure 1A above). AST: Aspartate transaminase; ALT: Alanine aminotransferase; TACE: Transcatheter arterial chemoembolization; INR: International normalized ratio; PODr + R: Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir, and Ribavirin; *IV*: Intravenous; *PO*: By mouth.

has no evidence of viable HCC by magnetic resonance imaging.

The time to reach cure, or SVR, was previously defined as the time to reach less than one hepatitis C virion in the extracellular fluid volume (approximately 13.5-15 L)[9-12]. Thus a value of approximately 3×10^{-5} IU/mL is the threshold for viral clearance (termed here cure boundary). The fact that the patient achieved SVR despite a very short course of therapy (24 d) was striking since her viral load level 10 d before DAA therapy stopped (3/15/15) was 97 IU/mL, which is several log IU/mL higher than the cure boundary. To estimate, retrospectively, when the patient reached cure we used the standard biphasic HCV treatment model[11,12] and the multiscale HCV treatment model[13,14]. Both these models predicted that cure occurred 3 to 6 wk after therapy was stopped (not shown).

DISCUSSION

Herein we detail hepatic decompensation in a patient treated with PODr + R. The patient had Child's-Pugh class A cirrhosis prior to initiation of treatment and presented with jaundice and ascites 14 d after DAA therapy was started. Treatment was discontinued on day 24. She had a prolonged recovery and gradually returned to her baseline condition. She achieved SVR despite a truncated course of therapy. No causes of liver injury other than DAA were identified in the present case and the available data are consistent with the minimal elements for reporting DILI[15].

The patient's presentation is consistent with the pattern of PODr + R-related liver injury reported by the FDA with an early onset of hyperbilirubinemia without a rise in ALT level[5]. Similarly, two cases of severe liver

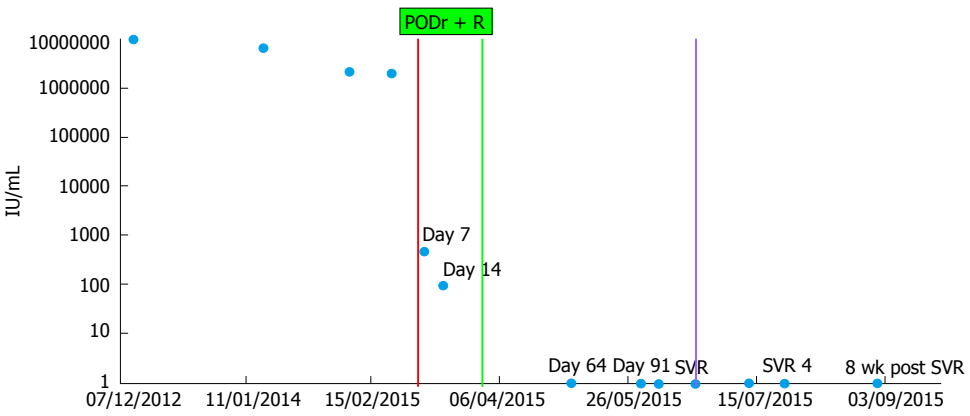

Figure 2 Hepatitis C virus RNA measurements over time. HCV RNA levels (circles) declined rapidly during treatment reaching not detected levels by the first measurement after treatment discontinuation and the patient achieved a sustained virologic response (SVR) defined as no detectable viral RNA 12 wk post treatment (purple line). HCV: Hepatitis C virus; PODr + R: Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir, and Ribavirin.

injury in patients with decompensated cirrhosis treated with sofosbuvir and a NS5A inhibitor were characterized by a rising bilirubin level with stable ALT levels[6]. Aminotransferase levels were not reported in the other two sofosbuvir/NS5A inhibitor DILI cases[7,8]. It is critical that clinicians be aware that aminotransferase levels do not tend to rise prior to, or in parallel with bilirubin levels in patients with DAA-related DILI, as failure to recognize this pattern could delay recognition of severe liver injury and discontinuation of therapy.

The most novel and important feature of the present case is that the patient had well compensated liver disease before starting treatment with a Child Pugh score of 6 and a MELD score of 7. In contrast, the FDA Drug Safety Communication emphasized that PODr + R is contraindicated in patients with Child's Pugh class B and C cirrhosis. The current case provides evidence that patients with Child's Pugh class A cirrhosis are at risk for severe DILI with PODr + R.

Of interest, HCV was eradicated with only 24 d of treatment. Both the standard biphasic and the multiscale models of HCV kinetics during therapy suggest that cure might have occurred between 3 to 6 wk after therapy was stopped. While it is not feasible that the drugs had a prolonged direct antiviral effect, in theory the ongoing liver injury (*i.e.*, cell loss) and/or resulting inflammation might have exerted an immune mediated antiviral effect. Alternatively, cure might have occurred by the end of DAA therapy if the DAAs affected the ratio between non-infectious and infectious viral particles (*i.e.*, viral infectivity) as previously observed during HCV DAA treatment in cell culture[16]. That is, DAAs may reduce the infectivity of the virus particles produced such that only a small fraction of the viral RNA detected 10 d before drug cessation was infectious. This alternative explanation is also consistent with reports in which some patients treated with DAAs were documented to achieve SVR despite having detectable HCV RNA at end of treatment[17,18]. Further experimental and modeling efforts are needed to provide insights into the biological and/or immunological aspects that gave rise to HCV cure after such short durations of DAA therapy[19].

Our study has several limitations. Blood samples during therapy are not available for further measurements to test for superimposed acute viral infections such as hepatitis A or B or to measure paritaprevir concentration at the time of the rise in bilirubin. However, the temporal relation between PODr + R therapy and hepatic decompensation and recovery following discontinuation of therapy provides strong evidence for DILI.

Careful laboratory and clinical monitoring, beginning early in the course of therapy, is prudent in patients with compensated cirrhosis treated with PODr + R.

COMMENTS

Case characteristics

Patient suffered from jaundice, nausea and vomiting 14 d following treatment with Paritaprevir, Ombitasvir, Dasabuvir, Ritonavir, and Ribavirin (PODr + R).

Clinical diagnosis

Aminotransferase levels did not increase. Despite that, Nausea, Vomiting, Ascites, Jaundice, hypoalbuminemia and coagulopathy occurred.

Differential diagnosis

Ribavirin induced hemolysis, possible drug induced liver injury, recurrence of hepatocellular carcinoma (HCC), or vascular causes for hepatic failure. The authors performed liver ultrasonography to exclude Recurrence of HCC and vascular causes. Blood tests to exclude hemolysis as cause for jaundice were performed.

Laboratory diagnosis

The authors witnessed increase in bilirubin levels, decrease in albumin levels and impaired coagulation tests with no increase in aminotransferase levels. Despite the above, eradication of hepatitis C virus was achieved (Figures 1 and 2).

Imaging diagnosis

The authors performed liver ultrasonography to rule out recurrence of HCC or vascular causes as the cause of decompensation. Eventually magnetic resonance imaging scan was also performed as follow-up on HCC.

Treatment

The main treatment in this case was discontinuation of PODr + R treatment. Albumin infusions and Ⅳ furosemide were administered.

Term explanation

All acronyms are explained in the main text.

Experiences and lessons

The new direct acting antiviral have an excellent safety record. Still, careful clinical and laboratory monitoring, beginning early in the course of therapy, is warranted especially in compensated cirrhotic patients treated with PODr + R because decompensation can occur without aminotransferase flareup.

Peer-review

The author presents an interesting and valuable case report.

REFERENCES

1 **Mohd Hanafiah K**, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]

2 **Holtzman D**. Hepatitis C 2016. CDC Health Information for International Travel. [updated 2015 Jul 10]. Available from: URL: http://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/hepatitis-c#4627

3 **Panel AIHG**. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015; **62**: 932-954 [PMID: 26111063 DOI: 10.1002/hep.27950]

4 **Poordad F**, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM, Forns X, Lovell SS, Da Silva-Tillmann B, Collins CA, Campbell AL, Podsadecki T, Bernstein B. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med* 2014; **370**: 1973-1982 [PMID: 24725237 DOI: 10.1056/NEJMoa1402869]

5 **FDA Drug Safety Communication**. FDA warns of serious liver injury risk with hepatitis C treatments Viekira Pak and Technivie. 2015. Available from: URL: http://www.fda.gov/Drugs/DrugSafety/ucm468634.htm

6 **Dyson JK**, Hutchinson J, Harrison L, Rotimi O, Tiniakos D, Foster GR, Aldersley MA, McPherson S. Liver toxicity associated with sofosbuvir, an NS5A inhibitor and ribavirin use. *J Hepatol* 2016; **64**: 234-238 [PMID: 26325535 DOI: 10.1016/j.jhep.2015.07.041]

7 **Dyson JK**, McPherson S. Reply to "Liver failure in human immunodeficiency virus - Hepatitis C virus coinfection treated with sofosbuvir, ledipasvir and antiretroviral therapy". *J Hepatol* 2016; **64**: 753-754 [PMID: 26682725 DOI: 10.1016/j.jhep.2015.11.038]

8 **Marchan-Lopez A**, Dominguez-Dominguez L, Kessler-Saiz P, Jarrin-Estupiñan ME. Liver failure in human immunodeficiency virus - Hepatitis C virus coinfection treated with sofosbuvir, ledipasvir and antiretroviral therapy. *J Hepatol* 2016; **64**: 752-753 [PMID: 26682727 DOI: 10.1016/j.jhep.2015.10.033]

9 **Snoeck E**, Chanu P, Lavielle M, Jacqmin P, Jonsson EN, Jorga K, Goggin T, Grippo J, Jumbe NL, Frey N. A comprehensive hepatitis C viral kinetic model explaining cure. *Clin Pharmacol Ther* 2010; **87**: 706-713 [PMID: 20463660 DOI: 10.1038/clpt.2010.35]

10 **Guedj J**, Perelson AS. Second-phase hepatitis C virus RNA decline during telaprevir-based therapy increases with drug effectiveness: implications for treatment duration. *Hepatology* 2011; **53**: 1801-1808 [PMID: 21384401 DOI: 10.1002/hep.24272]

11 **Dahari H**, Shteingart S, Gafanovich I, Cotler SJ, D'Amato M, Pohl RT, Weiss G, Ashkenazi YJ, Tichler T, Goldin E, Lurie Y. Sustained virological response with intravenous silibinin: individualized IFN-free therapy via real-time modelling of HCV kinetics. *Liver Int* 2015; **35**: 289-294 [PMID: 25251042 DOI: 10.1111/liv.12692]

12 **Dahari H**, Canini L, Graw F, Uprichard SL, Araújo ES, Penaranda G, Coquet E, Chiche L, Riso A, Renou C, Bourliere M, Cotler SJ, Halfon P. HCV kinetic and modeling analyses indicate similar time to cure among sofosbuvir combination regimens with daclatasvir, simeprevir or ledipasvir. *J Hepatol* 2016; **64**: 1232-1239 [PMID: 26907973 DOI: 10.1016/j.jhep.2016.02.022]

13 **Rong L**, Guedj J, Dahari H, Coffield DJ, Levi M, Smith P, Perelson AS. Analysis of hepatitis C virus decline during treatment with the protease inhibitor danoprevir using a multiscale model. *PLoS Comput Biol* 2013; **9**: e1002959 [PMID: 23516348 DOI: 10.1371/journal.pcbi.1002959]

14 **Guedj J**, Dahari H, Rong L, Sansone ND, Nettles RE, Cotler SJ, Layden TJ, Uprichard SL, Perelson AS. Modeling shows that the NS5A inhibitor daclatasvir has two modes of action and yields a shorter estimate of the hepatitis C virus half-life. *Proc Natl Acad Sci USA* 2013; **110**: 3991-3996 [PMID: 23431163 DOI: 10.1073/pnas.1203110110]

15 **Barritt AS**, Lee J, Hayashi PH. Detective work in drug-induced liver injury: sometimes it is all about interviewing the right witness. *Clin Gastroenterol Hepatol* 2010; **8**: 635-637 [PMID: 20363371 DOI: 10.1016/j.cgh.2010.03.020]

16 **Sansone N**, Dahari H, Subramanya G, Perelson AS, Uprichard SL. Modeling HCVcc infection reveals new insights into the dynamics that maintain the in vitro HCV steady state and the mechanisms of action of the NS5A inhibitor daclatasvir. *Hepatology* 2014; 60: **4** (Suppl): 1165A

17 **Kohli A**, Osinusi A, Sims Z, Nelson A, Meissner EG, Barrett LL, Bon D, Marti MM, Silk R, Kotb C, Gross C, Jolley TA, Sidharthan S, Petersen T, Townsend K, Egerson D, Kapoor R, Spurlin E, Sneller M, Proschan M, Herrmann E, Kwan R, Teferi G, Talwani R, Diaz G, Kleiner DE, Wood BJ, Chavez J, Abbott S, Symonds WT, Subramanian GM, Pang PS, McHutchison J, Polis MA, Fauci AS, Masur H, Kottilil S. Virological response after 6 week triple-drug regimens for hepatitis C: a proof-of-concept phase 2A cohort study. *Lancet* 2015; **385**: 1107-1113 [PMID: 25591505 DOI: 10.1016/S0140-6736(14)61228-9]

18 **Harrington PR**, Deming DJ, Komatsu TE, Naeger LK. Hepatitis C Virus RNA Levels During Interferon-Free Combination Direct-Acting Antiviral Treatment in Registrational Trials. *Clin Infect Dis* 2015; **61**: 666-667 [PMID: 26002846 DOI: 10.1093/cid/civ402]

19 **Meissner EG**, Nelson A, Marti M, Masur H, Osinusi A, Kottilil S. Sustained Virologic Response for Chronic Hepatitis C Infection after 27 Days of Treatment with Sofosbuvir and Ribavirin. *Open Forum Infect Dis* 2014; **1**: 013 [PMID: 25411655 DOI: 10.1093/ofid/ofu013]

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