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REVIEW

Biliary atresia: Where do we stand now?

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Abstract

The pathway from clinical suspicion to establishing the diagnosis of biliary atresia in a child with jaundice is a daunting task. However, investigations available help to point towards the correct diagnosis in reasonable time frame. Imaging by Sonography has identified several parameters which can be of utility in the diagnostic work up. Comparison of Sonography with imaging by Nuclear medicine can bring out the significant differences and also help in appropriate imaging. The battery of Biochemical tests, available currently, enable better understanding of the line-up of investigations in a given child with neonatal cholestasis. Management protocols enable standardized care with optimal outcome. The place of surgical management in biliary atresia is undisputed, although Kasai procedure and primary liver transplantation have been pitted against each other. This article functions as a platform to bring forth the various dimensions of biliary atresia.

Key words: Biliary atresia; Neonatal cholestasis; Kasai procedure; Neonatal jaundice; Hyperbilirubinemia

Core tip: The etiology of biliary atresia is intriguing with a myriad of diagnostics available to work up a child with neonatal jaundice. This article attempts to review the pathogenesis, evaluation, management and outcome for current update of biliary atresia.

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INTRODUCTION

Biliary atresia is the commonest surgical cause for neonatal cholestasis, although the diagnosis is one of exclusion of the various causes of neonatal cholestasis which require non-surgical management. Incidence of neonatal cholestasis is noted to be 1 in 2500 newborn children[1]. Among the group of children with neonatal cholestasis, about 34%-42% have been noted to have Biliary atresia[2,3]. The actual incidence is around 1 in

8000-18000 live births[4]. The etio-pathogenesis is not entirely convincing to point towards a particular offending agent, inspite of several studies describing the causal association of infective or autoimmune origin. The investigations for the establishment of the diagnosis are elaborate, and require extensive workup. As timely surgical intervention is essential, an appropriate and prompt work up is required.

ETIOLOGY

For the purpose of better understanding, biliary atresia is categorized into 2 forms, the perinatal or the acquired form and the embryonic or the congenital form. The embryonic form is the less common variant of the two (20%), with a link to syndromic association such as Biliary atresia Splenic Malformation (BASM - polysplenia, intestinal malrotation, preduodenal portal vein, absent inferior vena cava, aberrant hepatic artery, abdominal heterotaxia), known to be due to gene mutations controlling the bile duct development. The commoner perinatal form (80%) is supposed to be the end result of viral trigger and complex interactions between innate and adaptive immune responses[5].

The complete deletion of inversin gene in mice was shown to produce laterality defects in the abdominal organs along with malformations of the hepatobiliary system, similar to that of the fetal form[6]. However, the role of the inversin gene in humans is unlikely in the fetal form of Biliary atresia, as established by Schön *et al*[7].

Several viral agents such as human papilloma virus, cytomegalo virus, respiratory syncytial virus, reovirus, rotavirus[8-12], epstein barr virus, herpes virus, hepatitis B virus[13-15] have been implicated in the past, but none have been consistently and convincingly shown to be associated with the pathogenesis of Biliary atresia in humans.

The cystic biliary atresia is believed to be an exclusive subtype, based on the following observations: (1) jaundice noticed at birth; (2) diagnosed antenatally by the identification of a cystic lesion at the porta on sonography; and (3) intra-operatively a cystic lesion seen, not communicating with intrahepatic ductal system or duodenum.

Reports on cystic type biliary atresia declare that the entity has a better outcome[16].

PATHOGENESIS

To explain the pathogenesis of Biliary atresia, the concept of an initial viral infection damaging the biliary duct, followed by exaggerated autoimmune directed inflammation of biliary ducts and secondary biliary cirrhosis as a result of progressive ductal injury and obstruction has been mooted[17,18].

Interestingly, studies have noted the ability of Rota virus to target cholangiocytes and cause tissue specific inflammation and pathogenic effects in mouse models. The theory of viral mediated damage and progressive obliterative inflammation of bile ductules has been put forward, on the basis of this murine model. The virus is found to be tropic to cholangiocytes, leaving behind gamma interferon producing CD4 and 8 lymphocytes which target the hepatobiliary system, culminating in fibrosis of the injured ductal elements, bearing the striking resemblance to Biliary atresia[19].

Furthermore, it was concluded that the gamma interferon triggered the inflammatory changes responsible for progressive bile duct obstruction and obliteration[19]. It is believed that DNA hypomethylation changes in CD4 lymphocytes leads onto uncontrolled gamma interferon expression[20]. Gamma interferon through release from T lymphocytes, has been projected as the pivotal player, orchestrating the sequence of events, specifically the later occurrence of intraductal inflammation, ductal fibrosis and loss of epithelial integrity. However, the initial response of neutrophilic inflammation to the provoking viral agent was not altered, lending to the surmise that the gamma interferon is responsible for the ultimate damage and loss of extrahepatic bile ducts[21]. It is noteworthy that, in their attempt to achieve viral clearance, the CD8 lymphocytes secondarily cause ductular damage resulting in the experimental type of Biliary atresia[19]. Alpha2 beta1 integrin has been identified to be the medium of interaction responsible for predisposition of the cholangiocytes to Rhesus Rota virus infection[22].

Regulatory T lymphocyte defects in the presence of viral infection, has also found to be contributory to the unchecked bile ductal inflammation and destruction[23].

ANIMAL MODELS

The use of intrahepatic injection of chemicals like carbon tetrachloride, ethanol, formalin have been found to simulate inflammation similar to biliary atresia in adult rat[24,25]. Other animals like lamb fetus has also been studied[26]. Attempted *in vivo* replication of biliary atresia includes bile duct excision or ligation. Sea Lamprey as a model has been propagated with the advantage of seamless progress into biliary atresia without the need for intervention with injection of chemicals or surgical bile duct ligation[27].

CLINICAL PRESENTATION

The consistent passage of clay coloured stools, dark coloured urine, icterus at about 2 wk of age in a neonate should prompt the complete work up for cholestasis, especially biliary atresia.

INVESTIGATIONS

Simple macroscopic examination showing clay coloured acholic stool raises a strong suspicion of biliary atresia. When the stools are not acholic, additional features such as fecal fat and consistency can provide more information. Soil like consistency of stool with massive fat droplets on Sudan Ⅲ stain is a finding which has

high sensitivity, although not specific for biliary atresia detection[28].

Blood biochemistry

Gamma glutamyl transpeptidase (GGT) has been found to be an important parameter in the differential diagnosis of neonatal cholestasis. Children with Biliary atresia consistently had higher GGT levels than those without Biliary atresia (902.7 mmol/L *vs* 263.2 mmol/L)[29]. Tang *et al*[30] demonstrated that an elevated GGT more than 300 IU/L had a specificity of 98% and sensitivity of 38% to differentiate biliary atresia from Neonatal Hepatitis. In addition, the association between GGT and Alanine transferase ALT (GGT/ALT ratio more than 2) was put forth as a useful adjunct in the differential diagnosis of biliary atresia[30].

It is to be noted that, more relevance is placed on the correlation of GGT with age, than an absolute GGT value. To elaborate further, GGT is best diagnostic when evaluating cholestasis in children aged less than 120 d. Among infants aged 31-60 d, GGT levels more than 268 IU/L had a sensitivity of 80.5% and specificity of 75.6%, respectively, with an accuracy of 79.1% in the diagnostic evaluation of Biliary atresia. Recommended cut-off values of GGT for various age groups include 303 IU/L for age 61-90 d, 298 IU/L for age 91-120 d, 252 IU/L for age more than 121 d[31]. Another study brought out the optimal threshold for GGT for various ages, 150 IU/L for age less than 4 wk, 250 IU/L for age between 4-8 wk and 300 IU/L for age more than 8 wk[32].

On the contrary, alkaline phosphatase levels were noted to be higher in those children without biliary atresia[31].

The Apolipoprotein E has been found to be useful in the diagnostic workup as the serum levels have been consistently elevated in biliary atresia[33]. Rafeey *et al*[34] in a recent study showed Apolipoprotein E to have positive predictive value of 71% and negative predictive value of 67% in differentiating biliary atresia from other neonatal cholestatic disorders, indicating that its utility as a stand-alone diagnostic test is limited. Similar results have been seen with procalcitonin, which is an inflammatory marker, synthesized in the liver. Hence it could be used possibly in combination with other tests to improve the diagnostic accuracy[34].

Recently, microRNA assay has been pointed to be a novel method of quick diagnosis of biliary atresia. Injury to liver tissue in biliary atresia is supposed to release certain microRNAs, which are non-coding RNAs regulating target genes. High levels of these micro RNAs are found in the intrahepatic bile ducts confirming the source of release and their specificity. The study by Zahm *et al*[35] has established the high levels of serum miR220b/429 in Biliary atresia patients in comparison to other cholestatic disorders, implying the potential and promising utility of these in aiding in the early diagnosis.

Imaging

Sonography has distinct advantages of being non-invasive, repeatable, less expensive, readily available bedside and non-ionising, although limited by operator dependancy. Hence, this is used as the initial screening modality in the work up of neonatal cholestasis.

The usefulness of sonography, as an initial diagnostic tool is well brought out in several studies. Presence of a triangular cord sign which is the visualization of the fibrotic cord in the portal hilum is one of the hallmarks of sonographic imaging with a positive predictive value of 95%[36]. Triangular or tubular structure with echogenic density cranial to portal vein bifurcation at the liver hilum is indicative of triangular cord sign[37]. Gall bladder (GB) morphology is looked into as the primary diagnostic factor on sonography. If the GB morphology is normal on sonography, the next step of measuring the triangular cord thickness is undertaken, which if more than 3.4 mm, the sonographic diagnosis of Biliary atresia is very likely[38].

In addition, the GB contractility, size and dimension, regular mucosal contour all go together in the diagnostic imaging. Findings pertaining to GB on sonography can be absent/non visualized GB, irregular contour of GB, small shrunken GB, non contractile GB despite 4 h of fasting, cystic structure replacing GB and absent echogenic mucosal lining of GB. The liver echotexture signifying the presence of cirrhosis is another finding useful on sonography for prognostication[39]. At a cut off GB length of 1.5 cm, high index of suspicion for biliary atresia to be kept while evaluating a baby with neonatal cholestasis[40]. In the early stage of the disease, the triangular cord sign may be not prominent, leading to missed diagnosis. Triangular cord sign combined with GB length can act as twin hallmarks in the sonographic diagnosis of biliary atresia. In the setting of periportal inflammation or cirrhosis, sonographic diagnosis may be difficult as triangular cord sign may not be apparent. Utility of the GB ghost triad, including GB length less than 1.9 cm, irregular contour of GB and lack of smooth, regular mucosal echogenicity of GB may be helpful in the above scenario. With an accuracy of 97%, it appears to be an invaluable diagnostic feature on sonography[41].

As adjuncts to the above sonographic parameters, the right hepatic artery diameter more than 1.5 mm and ratio of the right hepatic artery to that of the portal vein more than 0.45 were of use in the sonographic evaluation[42].

The visualisation of hepatic subcapsular flow due to hepatic arteriopathy and fibrosis in biliary atresia is another sonographic feature on colour Doppler study[43]. El-Guindi *et al*[44] in a recent study reported the superiority of demonstration of hepatic subcapsular flow over the other sonographic parameters such as triangular cord sign, GB contractility, GB size and dimensions of hepatic artery. Even when the sonographic hallmark of triangular cord sign could not be satisfactorily demonstrated, presence of hepatic sub capsular flow can be of significant value in sonographic examination[45].

The measurement of liver span below the costal margin by sonography can help in the workup, as consistently "small" livers are seen in non-biliary atresia

children[29].

Using a special transducer sonography probe, it is now possible to measure liver fibrosis, based on the technique of transient elastography. Consequently, prognostication of the state of the advanced liver disease can be predicted in a non-invasive manner. It is predicted to be useful as a follow up tool, without the need to resort to performing a liver biopsy. However, the sensitivity of this test in identifying early stages of liver fibrosis is limited[46].

It is recommended that a confident demonstration of the triangular cord sign can route the algorithm towards operative cholangiography, rather than subjecting to liver biopsy, in view of the accuracy of the sonographic sign[29].

Antenatal diagnosis

The presence of a cystic structure at the porta hepatis without intrahepatic biliary ductular dilatation goes towards the diagnosis of biliary atresia, in the antenatal period. This is also known to be associated with additional anomalies[47,48].

Sonography vs scintigraphy

Compared to nuclear scintigraphy, sonography has better discriminatory value in the differential diagnosis. This is evidenced by the higher specificity of the triangular cord sign (95.8%) against scintigraphy (72.9%). Also, the positive predictive value of the triangular cord sign scoring twice higher than scintigraphy (77.8% *vs* 38.1%) puts sonography ahead, in the correct detection of biliary atresia[49].

Magnetic resonance cholangiopancreatography

The utility of Magnetic resonance cholangiopancreatography (MRCP) has not been encouraging in view of the cost, varying results and the need for immobilisation. Negative and positive predictive value have been reported as 91%-100%, 75%-96% respectively[50,51]. The requirement of sedation, preferably general anesthesia is a significant concern in addition to long image acquisition time. In a recent study, the image acquisition time using three dimensional MRCP has been reported to be around 180 s. The sensitivity 99.08% and negative predictive value 96.88% were high but the specificity 36.05% and positive predictive value 65.19% were low[52].

Nuclear scintigraphy

Nuclear scintigraphy is non-invasive, simple and is supposed to have practical utility in view of the logical assumption of the functional ability of liver to take up the tagged agent and subsequent excretion in the intestine, enabling visualization of gut activity. Studies caution regarding excessive reliance of scintigraphy, as it may contribute to misdiagnosis in infants with jaundice[29]. However, in the background of elevated bilirubin levels and likely deranged liver function, ability to take up the agent may be compromised. To overcome this, cholegogues such as Ursodeoxy cholic acid, Phenobarbitone, Phenytoin are used as pre-treatment agents, to ensure adequate "priming"[53].

The value of delayed or 24 h imaging has been pointed out to decrease the false positive results as nearly 50% of the bowel visualization was seen in the delayed image[54]. As an adjunct, SPECT has been put forth in dealing with poor bowel visualization. More studies are required before concluding in favor of its usage[55]. However, arguments against, have discouraged the same citing the poor image resolution with consequent difficult interpretation. Excretion is expected to be less due to reduced uptake primarily, given the background of deranged liver function and high bilirubin levels, competing with the tagged agent effectively to decrease the uptake. Furthermore, it has been proposed that this would be time consuming and lead to more delay in the work up[56].

A recent meta-analysis places the scintigraphy in the correct perspective, at a low specificity of 70.4%, although pooled sensitivity was high at 98.7%. This would mean that almost every case of biliary atresia gets detected, but when the scintigraphy shows no excretion, it does not necessarily diagnose biliary atresia amongst the other causes of neonatal cholestasis[53].

Histology

Liver biopsy is considered as gold standard in the diagnosis of biliary atresia, with an accuracy of 88.2%-96.9%[57,58]. To cope with the delayed referrals and the negative laparotomy rate, histology of liver biopsy is proposed as the best alternative. Also, histology has a definitive role, where the various imaging modalities may not be able to suggest the suitable diagnosis, especially in younger neonatal cholestatic children. Among the several findings in histology, ductular proliferation, bile plugs in the ducts and the ductules and portal fibrosis were found to be statistically significant in the diagnostic workup of biliary atresia. On further multivariate analysis, the ductular proliferation emerged as the sole parameter of paramount importance. Of note, age was not found to be a factor in altering the diagnostic histological features in biliary atresia. Multinucleate giant cell formation and myeloid metaplasia were noted to be seen more commonly in neonatal hepatitis[58]. Utility of the liver biopsy in the work up of neonatal cholestasis has been recommended as a guideline[59].

Histology can also prognosticate in addition to providing a diagnosis, by cirrhosis assessment and ductal plate malformation. Ductal plate malformation which refers to presence of fetal type intrahepatic duct, is identified to be a poor prognostic factor as it is known to be associated with poor bile flow after Portoenterostomy[60].

Ductal diameter less than 100 microns was a feature identified with children requiring liver transplantation[61]. Whereas, when ductal size was more than 150 microns, in combination with a columnar lined epithelium, it was predictive of good prognosis after surgical mana-

gement[62].

Fibrosis as an independent prognostic marker in histological evaluation is established by various studies[63-65]. Also, it has been utilized to predict the long term outcome in post-operative biliary atresia patients[66].

Ductopenia and secondary biliary cirrhosis were consistently found to be late histological features[67].

Endoscopy

Use of endoscopy in biliary atresia is mainly for dealing with the sequelae of portal hypertension and varices. However, endoscopy can be of use in aiding diagnostic workup, in addition to duodenal intubation for bile detection. Sampling of Duodenal contents to improve the accuracy of scintigraphy, as gamma camera may not pick up minimal activity, is a step towards improvisation by means of non-imaging method[68].

Scoring system

Based on the variable nature of the diagnostic tests and their overlapping tendency, it would be best to rely on a combination of investigations with correlation to the clinical condition, to reach a prompt and confident diagnosis in the individual child with neonatal cholestasis. Most investigations by themselves do not point to a clear cut differentiation between biliary atresia and other causes of neonatal cholestasis. Hence this has led to a strategy of mix and match of modalities to evolve a meaningful scoring system to attempt to objectively categorize the children with biliary atresia from the group of Neonatal cholestasis. The proposal of El-Guindi *et al*[69] consists of a twelve-point scoring system, according to clinical, laboratory, ultrasonographic, and histopathological parameters, with a reported accuracy of 98.3% in pin pointing biliary atresia. Strikingly, scintigraphy was not included in their scoring, referring to its low specificity and time lost to prime the patient. Confining to histology, Chen *et al*[70] have evolved a 8-feature (liver fibrosis, portal ductal proliferation, bile plugs in portal ductules, cholestasis, hepatocellular changes inflammatory cells infiltration in portal region, extramedullary hematopoiesis, and ductal plate malformation), 21-point (0 to 21) scoring system declaring an accuracy of 91.9% in correctly identifying biliary atresia[70].

OPERATIVE MANAGEMENT

Surgery is the main stay of treatment in biliary atresia to effectively establish bile drainage and jaundice clearance. Left untreated, there is an incessant progression towards Biliary cirrhosis, end stage liver failure and death by 3 years of age[4]. The hallmark of biliary atresia is the difficulty in prediction of the natural course and outcome, given that it should not be considered a single disease entity with a predictable natural history and stereotypical response to surgery[71].

Kasai portoenterostomy relies on the realization that the microscopic structures in the porta hepatis will act as micro-conduits of bile as an internal biliary fistula is created with a segment of bowel. Use of gall bladder, appendix has been tried earlier as conduits instead of the bowel segment, but none were successful like the bowel. In view of higher revision rates, other conduits except bowel have been abandoned[72].

The extended Kasai procedure attempts at utilising more anastomotic area for achieving effective bile drainage by extending the dissection into the Rex recess (the space between segments Ⅲ and Ⅳ under the liver bridge) and around the bifurcation of the right vascular pedicle of portal hilum[73,74].

Laparoscopic portoenterostomy has not been shown to have better outcome than the open portoenterostomy[75]. Although proponents have defended the minimal access approach with the claim that the risk for damage to small bile ductules around the porta hepatis is minimal, due to avoidance of deep suturing and extensive dissection[76]. The advantage of minimal adhesions after laparoscopic intervention, enabling future liver transplantation has also been negated[77]. Hence, the open portoenterostomy continues to be the gold standard for biliary atresia[78].

The recommendation to perform per op cholangiography directly without a liver biopsy where clinical suspicion is high, reflects the equivocal state of the liver biopsy[3].

Post-operative management

The role of corticosteroids is hotly debated and controversial, as there is no conclusive evidence in terms of long term improved outcome[79]. However, there does seem to be a positive impact of improved clearance of jaundice when steroids are used for a short course in the post-operative period. Thus the lack of translation of beneficial effect with usage of steroids has generally discouraged its prescription in the long term management, although there is a strong link between continuing inflammation, altered immunity and ongoing fibrosis in biliary atresia after Kasai procedure[80]. Unlike steroids, Urso deoxycholic acid does play a positive and significant role in the bile flow and finds a place in the post-operative protocol of Biliary atresia management[81].

OUTCOME

Lower degree of biliary fibrosis, bile ductular proliferation, absence of ductal plate malformation, large ducts more than 150 μm and younger age were found to be associated with better long term outcome[66].

The cystic dilatation of the intrahepatic biliary system on sonography following Kasai during long term follow-up, is considered as a poor prognostic feature lowering the survival rate with native liver[82].

The children with BASM tend to have a poorer prognosis[83,84]. Younger age at Kasai was linked with better outcome in those with the cystic type biliary atresia and BASM. Whereas younger age at surgery was not a determining factor in isolated biliary atresia[83].

The long term survival with native liver is significantly lower, establishing the dictum that liver transplant is the

ultimate recipe for biliary atresia management. Adult outcome studies in Biliary atresia patients quote the survival with native liver at 20% in the adults 20 years post Kasai and 10% among those who are 30 years post Kasai[85,86].

Centralisation of services, such that biliary atresia surgery is managed at select centres, has been shown to remarkably increase surgical outcome and overall survival. Standardisation of protocolised management with uniform pre operative work up, surgical technique, post operative management and follow-up seem to be the cohesive factors towards achieving a better outcome. To quote the Finnish study, jaundice clearance rate improved from 27% to 75% and overall survival from 64% to 92% with all the above measures[87].

Kasai portoenterostomy effectively acts as a bridging procedure, enabling retention of native liver in about a quarter of patients and maintaining the rest till an organ is available for transplant in the long term[88]. The importance of surveillance is underlined by the fact that majority of the patients (58.3%) after Kasai procedure develop features of chronic liver disease such as Cirrhosis and Portal hypertension[89].

Early neonatal screening with stool charts has a beneficial effect as evidenced by the fact that 5 year survival with native liver increased from 27.3% to 64.3%[90].

Nutritional management for optimal outcome would include feeding regime with a medium chain triglyceride formula. Also, the follow-up of these children should monitor the regular vitamin supplementation of fat soluble vitamins. However, the question of nutritional resuscitation is relevant from the point of view of those awaiting liver transplant[4].

SCREENING

Various screening methods other than stool charts have been studied, but none are effective as a simple, cost effective and useful tool in screening general population. Serum bile acid, direct bilirubin, Apo CⅡ/CⅢ proteins, urine sulfated bile acid, fecal bilirubin and fat[91-95] were some of the biomarkers used in the literature for screening of Biliary atresia.

CONCLUSION

Biliary atresia is a multifactorial disorder with varied outcome depending upon the time of surgical treatment and histology. Strict adherence to protocols in the form of investigations would lead to seamless progression from diagnosis to management. Post operative management with appropriate medications is required to ensure an optimal outcome. Long term follow-up is essential as the native liver can fail over a period of time requiring the need for liver transplantation. Although advances regarding understanding of progressive inflammation after portoenterostomy have been made, translation into significant treatment has not evolved yet.

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MINIREVIEWS

Restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension

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Abstract

In recent years, defined progress has been made in understanding the mechanisms of hemodynamic disturbances occurring in liver cirrhosis, which are based on portal hypertension. In addition to pathophysiological disorders related to endothelial dysfunction, it was revealed: There is the restructuring of the vasculature, which includes vascular remodeling and angiogenesis. In spite of the fact that these changes are the compensatory-adaptive response to the deteriorating conditions of blood circulation, taken together, they contribute to the development and progression of portal hypertension causing severe complications such as bleeding from esophageal varices. Disruption of systemic and organ hemodynamics and the formation of portosystemic collaterals in portal hypertension commence with neovascularization and splanchnic vasodilation due to the hypoxia of the small intestine mucosa. In this regard, the goal of comprehensive treatment may be to influence on the chemokines, proinflammatory cytokines, and angiogenic factors (vascular endothelial growth factor, placental growth factor, platelet-derived growth factor and others) that lead to the development of these disorders. This review is to describe the mechanisms of restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension. Development of pathogenetic methods, which allow correcting portal hypertension, will improve the efficiency of conservative therapy aimed at prevention and treatment of its inherent complications.

Key words: Portal hypertension; Vascular remodeling; Angiogenesis; Pathogenesis; Liver cirrhosis

Core tip: The purpose of the review is to describe the mechanisms of restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension. In addition to pathophysiological disorders related to endothelial dysfunction, it was revealed: There is the restructuring of the vasculature, which includes vascular remodeling and angiogenesis. In spite of the fact that these changes are the compensatory-

adaptive response to the deteriorating conditions of blood circulation, taken together, they contribute to the development and progression of portal hypertension causing severe complications such as bleeding from esophageal varices.

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INTRODUCTION

The majority of severe complications of liver cirrhosis are directly related to its characteristic hemodynamic disturbances, and portal hypertension is at their core[1]. Due to various anatomical and functional factors, the increase in hepatic vascular resistance causes the development of portal hypertension. The synthesis of extracellular matrix components leads to serious changes in the liver cytoarchitectonics. Accompanying this process, hypoxia with the participation of vascular endothelial growth factor (VEGF) induce pathological angiogenesis. In addition, sinusoidal endothelial dysfunction in liver cirrhosis disturbs the balance between vasoconstrictors and vasodilators produced by the endothelium. There are endothelin-1 (ET-1) and nitrogen oxide (NO), the most studied at present[2].

In spite of the formation of portosystemic shunts, the subsequent development of hyperdynamic circulatory syndrome contributes to the progression of portal hypertension. Appearing in this case circulatory disorders are caused by endothelial dysfunction and restructuring of the vascular bed that includes vascular remodeling and angiogenesis[3] (Figure 1).

The purpose of the review is to describe the mechanisms of restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension.

CURRENT CONCEPT OF VASCULAR REMODELING

The term "remodeling" started to be used in the 1980s of the last century, mainly in cardiology. In the strict interpretation, it means the process of reorganization of the existing structure, during which it joins a new material or it is entirely changed. Vascular remodeling is an adaptive response to long-term hemodynamic disturbances. This process includes several stages[4]: (1) perception of signals about modified conditions of blood circulation and circulating humoral factors; (2) signal transmission within a cell and between adjacent cells; (3) synthesis, activation, or release of substances affecting cell growth, death, migration, or extracellular matrix construction; and (4) structural changes of the vascular wall (both cellular and extracellular components), its mechanics, and function.

Endothelial cells are the main sensors perceiving changes in blood flow and the impact of various humoral factors. They are constantly activated by mechanical stimuli, such as shear stress and intravascular pressure, which are transformed into intracellular and extracellular chemical signals within endothelial cells. These changes occur in the early stage of the mechanotransduction process[5]. It involves a variety of physiological elements including ion channels, molecules of cell-matrix and cell-cell interactions [integrins, platelet-endothelial cell adhesion molecule-1 (PECAM-1) or CD31, adherent compounds], tyrosine kinase receptors, caveolae, G protein-coupled receptors and G-proteins, glycocalyx, endothelial cell cytoskeletal components, and others. In response to mechanotransduction, there are formation and secretion of biologically active substances produced by the endothelium. They regulate the development of extracellular matrix, proliferation, migration, and organization of endothelial and smooth muscle cells, as well as sensitivity to growth factors - the key events in the vascular remodeling. Currently, the most studied ones are the vasodilators NO and prostacyclin (PGI2), and the vasoconstrictor ET-1[6].

Structural changes of blood vessels consist in eutrophic, hypertrophic, and hypotrophic remodeling. In inward eutrophic remodeling, outer and lumen diameters are reduced, and media cross-sectional area is unaltered. Inward hypertrophic remodeling is characterized by an increase in media/lumen ratio owing to media cross-sectional area increasing. Outward hypotrophic remodeling refers to an increase in lumen diameter and a decrease in cross-sectional area[7].

Distensibility is an important mechanical characteristic of the blood vessels defined as the percentage of change of their volume for each 1 mmHg change in intraluminal pressure. It depends primarily on the stiffness of the vascular wall, which mainly consists of collagen, elastin, and smooth muscles. It has been shown on the experimental model of spontaneously hypertensive rats that reduced distensibility of major arteries is related to increased quantity and changing structure of elastin, whereas its reduction in mesenteric resistance vessels mainly results from the modification of the elastin structure and possibly collagen accumulation[8].

MOLECULAR MECHANISMS OF THE ANGIOGENIC PROCESS

Angiogenesis is the complicated physiological process of creating new blood vessels. It occurs *via* activation of endothelial cells, expression of proteases in them, extracellular matrix destruction, proliferation, migration of endothelial cells, and creation of the highly permeable primary vascular structures, which is reconstructed into the three-dimensional vascular network after stabilization

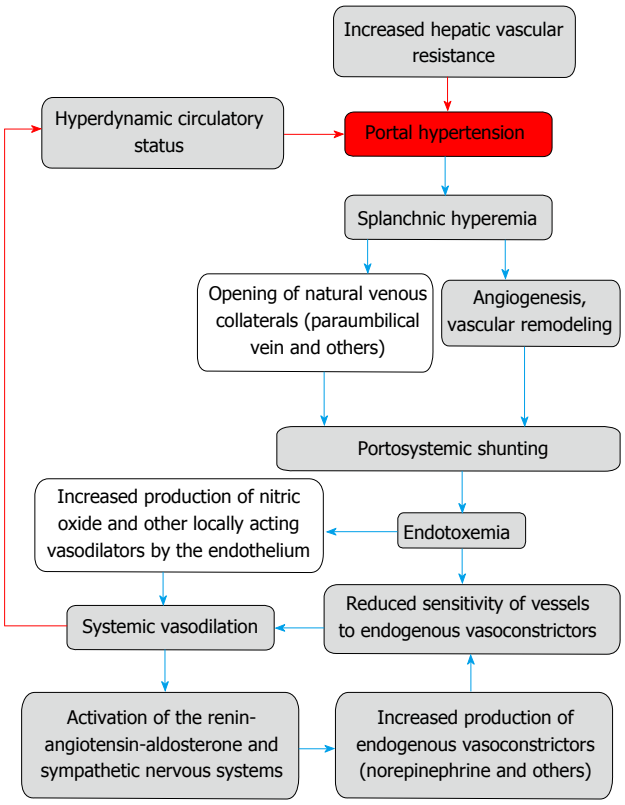

Figure 1 Potential mechanisms of the pathogenesis of portal hypertension in liver cirrhosis.

and "maturation" by attracting pericytes and smooth muscle cells[9].

The major inductor of angiogenesis in both pathological and physiological conditions is hypoxia. The cells react to lack of oxygen by several mechanisms including accumulation of hypoxia-inducible factors (HIFs). They are activated in the physiologically important sites of regulation of the oxygen pathways to provide quick and adequate responses to hypoxic stress and activate genes that regulate the angiogenesis process, vasomotor control, energy metabolism, erythropoiesis, and apoptosis[10].

The HIFs family include three α-subunits, which are connected with a β-subunit (HIF-1β). HIF-1α is ubiquitously expressed, whereas HIF-2α is found in a more limited set of cell types, particularly in vascular ECs, type Ⅱ pneumocytes, hepatocytes, and macrophages. The role of HIF-3α is less well understood in hypoxic responses[11].

HIFs are considered to be the major transcriptional regulators of genes involved in response to the lack of oxygen, whereas miRNAs regulate gene expression post-transcriptionally[12].

The most investigated angiogenic growth factors are the family of VEGF. It consists of five homologs: VEGF-A, B, C, D and placental growth factor (PlGF). All VEGFs binds to various related receptors: VEGFR-1, VEGFR-2, VEGFR-3. Only the first two of them are responsible for transmission of angiogenic signals. What is more, the binding of VEGF-A to VEGFR-2 and the increase of vascular permeability under the influence of nitric oxide are the mechanisms triggering vasculogenesis and angiogenesis[13].

Members of the fibroblast growth factor (FGF) family are also capable of stimulating angiogenesis. Cellular response to the impact of FGFs develops *via* specific binding to FGF-receptors (FGFR), which have internal tyrosine kinase activity. Dimerization of FGFR is a precondition for activation and phosphorylation of signaling molecules occurring with the participation of heparin-binding proteins. The process stimulates cell differentiation, proliferation, migration, and destruction of the extracellular matrix. It is important to note that while members of the VEGF family are involved mostly in the capillary formation, the FGF mainly involved in arteriogenesis[14].

Although the platelet-derived growth factor (PDGF) influence on angiogenesis is not so explicit in contrast with VEGF and FGF, studies *in vivo* have shown that it is capable of causing the formation of blood vessels and regulating their tone[15].

Tie-2 (Tek), a tyrosine kinase receptor expressed by endothelial cells, and its ligands, the angiopoietins (Ang), play an important role in the coordination of the angiogenesis process. Angiopoetin-1 (Ang-1) promotes stabilization of vessels *via* inhibiting endothelial cells apoptosis and stimulating its formation. In contrast, Ang-2, an antagonist of Ang-1, results in destabilization of vessels by converting the endothelial cells to the proliferative phenotype from the stable state. However, Ang-2 is also able to stimulate angiogenesis with the participation of VEGF[16].

Integrin alpha v beta 3 (αvβ3) and alpha v beta 5 (αvβ5) were regarded as positive regulators of angiogenesis for a long time. Nevertheless, the recent studies have shown their inhibitory role in this process[17].

Vascular endothelial (VE)-cadherin, an endothelial-specific adhesion molecule, promotes cell-cell junctions during neovascularization and manages the transport of molecules through the endothelial lining[18].

Thrombospondin-1, an antiangiogenic protein, has a direct impact on migration and apoptosis of endothelial cells. Furthermore, it prevents the release of VEGF from the extracellular matrix by suppressing the activation of matrix metalloproteinases (MMPs)[19]. Endostatin, a fragment of the C-terminal part of the collagen XⅧ α1-chain, and angiostatin, a plasminogen degradation product, are also inhibitors of angiogenesis[20].

The first step in the formation of new blood vessels is vasodilatation. Ang-2 and VEGF affect the formation of endothelial fenestrae. Increased vascular permeability causes extravasation of plasma proteins, which will further serve as a scaffold for migrating endothelial cells. Meanwhile, integrins provide them information about the presence of the angiogenic sites. The next step is the destruction of the basement membrane and the extracellular matrix by activated MMPs. This induces the subsequent migration and proliferation of endothelial cells with the participation of angiogenic growth factors such

as VEGF, FGF, and epidermal growth factor (EGF). One of the regulators of this process is a transmembrane protein ESDN - endothelial and smooth muscle cell-derived neuropilin-like protein[21].

Endothelial progenitor cells differentiate into endothelial cells. VE-cadherin and integrins coordinate their binding, and tumor necrosis factor α (TNF-α), FGF, and PDGF create conditions for the formation of new capillaries. Endothelial cells form the new basement membrane and the extracellular matrix with the participation of surrounding pericytes. Ang-1 provides final vascular stabilization[9].

MECHANISM OF PORTAL-SYSTEMIC COLLATERALS FORMATION

In the early stages of the development of portal hypertension, a moderate increase in the portal pressure leads to a redistribution of blood flow toward the muscle layer of the small intestine. The appearance of mucosal hypoxia causes a significant increase in NAD(P)H oxidase activity, the main source of reactive oxygen species (ROS) in the mucous membrane, and also leads to increased production of VEGF and NO by arterioles, contributing splanchnic vasodilation[22]. In addition, multiple signaling pathways are stimulated, such as mitogen-activated protein kinases, tyrosine kinases, and transcription factors that are involved in VEGF-induced neovascularization[23]. It was shown that overexpression of Kruppel-like factor 2 in duodenal tissue with the assistance of microRNAs causes hemodynamic stimuli integration and VEGF-driven angiogenesis in patients with liver cirrhosis[24]. Besides the wall of the small intestine[25], the elevated levels of VEGF, VEGFR-2, and CD31 (PECAM-1) is observed in the mesentery[26].

These pathophysiological disturbances may be the initial step in the development of portosystemic collateral circulation in portal hypertension[27]. Monocytes adhere to the surface of activated endothelial cells and produce growth factors and proteases, such as urokinase plasminogen activator and MMPs, promoting the division and migration of smooth muscle cells. Proinflammatory cytokines [macrophage chemotactic protein-1, granulocyte-macrophage colony-stimulating factor, transforming growth factor β1 (TGF-β1), TNF-alpha] also promote the growth of blood vessels, as well as growth factors such PlGF, which stimulates the growth of endothelial and smooth muscle cells, FGF - through upregulated expression of PDGF receptor, and VEGF by reaction with Ang-1. At the same time, anti-inflammatory cytokines (*e.g.*, interleukin-10) inhibit the process[28].

It was shown in animal model of prehepatic portal hypertension induced by partial portal vein ligation, that the blockade of VEGFR-2 with anti-VEGFR-2 monoclonal antibody for 5-7 d and inhibition of VEGF/VEGFR-2 signalization using autophosphorylation inhibitor VEGFR-2 for 5 d after the operation resulted in 50% reduction of portosystemic collateral vessel formation[29,30]. Blockade of NAD(P)H also contributed to this owing to the reduced splanchnic expression of VEGF, VEGFR-2 and CD31[31].

It should be noted that the emerging shunts are very dynamic vascular bed because of the expression of various receptor types on the surface of the endothelial lining, for example, α and β adrenoreceptors, 5-HT2 receptors. Furthermore, vasoactive substances such as NO, ET-1, prostaglandins can affect their tonus[32]. In particular, it was noted that excessive discharge of blood through portosystemic collaterals because of postprandial splanchnic hyperemia promotes their dilation due to shear stress, which in turn induces the overproduction of NO by endothelial cells[33]. Although natural portosystemic anastomoses are found in all patients with portal hypertension, they acquire the highest clinical significance in the development of gastroesophageal varices, because their rupture leads to life-threatening bleeding. The determining factor in their formation is the type of hepatofugal blood flow, and a gastroesophageal drainage path is the most important in this situation. The left gastric vein plays the main role in this path. It drains blood from both surfaces of the stomach, ascends from right to left along the lesser curvature into the lesser omentum, to the esophageal opening of the diaphragm, where it receives esophageal veins. It then turns backward and passes from left to right behind the omental bursa and drains into the portal vein. Anastomoses between the left and right gastric veins and the left and short gastric veins, respectively indicated by terms "coronary vein" and "posterior gastric vein", have clinical significance only in portal hypertension, because they are involved in the formation of esophageal and related with them paraesophageal varices[34].

Immunohistochemical studies, which was conducted in patients with portal hypertension, revealed the existence of the pronounced expression of PDGF, basic FGF-2, EGF and TGF-α in the wall of the coronary vein of the stomach. This fact shows that the increase in pressure in this vein activates smooth muscle cells and induces the release of growth factors that stimulate their proliferation, differentiation, and migration, as well as contribute to the disruption of the metabolism of collagen and elastin fibers. Phenotypic changes of smooth muscle cells is a response to chronic mechanical stimuli. They are lead to thickening of the venous wall and reduce its elasticity[35].

VASCULAR STRUCTURE OF THE LOWER ESOPHAGUS IN CLINICAL PORTAL HYPERTENSION

The venous system of the distal portion of the esophagus includes intraepithelial, subepithelial superficial, deep submucosal and adventitial veins. The largest varices are generally localized 2-3 cm above and 2 cm below the cardia, mainly in the lamina propria of the mucous membrane. They have two types of vascular structure: Palisading type and bar type. The palisading type has

dilated intraepithelial channels and numerous small superficial collateral veins. The bar type has triply dilated subepithelial superficial veins and deep submucosal veins which erode the epithelium[36]. Structural changes in the veins of the distal portion of the esophagus in portal hypertension are characterized by thickening of the medial layer because of hyperplasia of elastic and collagen fibers. Elastic fibers become fragmented and sharply tortuous directly in the varicose veins of the esophagus in the background of increasing sclerosis of the vascular wall[37].

Four distinct intramural vascular zones of the gastroesophageal junction were defined as follows: Gastric zone, palisade zone, perforating zone, and truncal zone. Portacaval shunts in this area are formed because of increased pressure in the portal venous system[38].

Gastric zone

The longitudinal veins of the gastric area are located in the submucosa and the lamina propria of the proximal portion of the stomach. They are more abundant near the esophagus, have a small diameter, and form a group of several longitudinal vessels. The veins merge in the submucosa of the distal part of the gastric zone and form large tortuous trunks draining blood into the portal vein system.

Palisade zone

The palisade zone is an extension of the gastric zone. It begins in the projection of the gastroesophageal junction and ends 2-3 cm above it. Veins in that zone are located randomly, close to each other, and are arranged longitudinally and in parallel as a palisade.

Numerous anastomoses are identified between vessels of both gastric and palisade zones. They are localized in the submucosa of the gastroesophageal junction, penetrate the muscularis mucosa, and pass into the lamina propria mainly in a longitudinal direction.

Veins of a proximal portion of the palisade zone simultaneously converge at one point and, perforating the muscularis mucosa, pass into the submucosa again as four or five big trunks. There are arched transverse anastomoses between them. Veins perforating the muscular layer of the esophagus were not detected in this zone.

Perforating zone

Veins of the perforating zone, which is located 3-5 cm above the gastroesophageal junction, are not so homogeneous and constant. Vessels form five polygonal networks in the lamina propria of the esophageal mucosa (as a continuation of the veins of the palisade zone) and perforate the muscular layer, communicating with adventitial veins located on the outer esophageal surface. They were referred to as (treble clef) veins because of their similarity with music symbols.

The perforating zone is the "critical area" for variceal rupture in portal hypertension. This is due to increased resistance to blood flow in this anatomical area, as well as increased fragility and superficial location of perforating veins[39].

Truncal zone

The truncal zone is a region from 8 to 10 cm in length with the bottom edge 5 cm above the gastroesophageal junction. Large longitudinal venous trunks, discovered here in the lamina propria, constitute a continuation of the polygon vascular networks of the perforating zone. They have a small diameter in the proximal portion. Between them, there are several transversely oriented anastomoses. Perforating veins, locating randomly along the zone, pass from the submucosa of the esophagus to its outer surface and communicate with adventitial veins.

In physiological terms, palisade zone is the most important part of the vascular structure of the gastroesophageal junction. Veins are located there mainly in the lamina propria. Their superficial location decreases venous blood flow resistance to a minimum, which would otherwise arise in the high-pressure zone in the area of the lower esophageal sphincter.

A large number of small caliber vessels in the palisade zone with a longitudinal stroke and parallel to each other perfectly adapted to the physiological pressure variations that leads to a bi-directional flow during breathing. When the venous outflow is carried out in the caudal direction, the gastric zone collects and drains the blood into the portal vein system.

Deep submucosal veins are enlarged because of the blood outflow in the cranial direction in portal hypertension. They drain the blood into the enlarged adventitial veins (periesophageal collateral veins) through the numerous veins perforating the esophageal smooth muscle layer in the perforating zone. Adventitial veins, in turn, communicate with paraesophageal collateral veins, which are located in the posterior mediastinum. The blood flows from them usually into the azygos vein[40], which structural changes in response to increased blood flow are characterized by focal destruction, hyperplasia and chaotic arrangement of elastic fibers[37].

THE SYSTEMIC AND SPLANCHNIC ADAPTIVE RESPONSE OF VASCULAR BED TO HEMODYNAMIC DISTURBANCES IN PORTAL HYPERTENSION

The development of portosystemic collateral circulation is a compensatory mechanism, which purpose is decompression of increased portal pressure. However, this does not happen. Conversely, there is a hyperdynamic circulatory state accompanied by increased cardiac output, decreased peripheral vascular resistance, and the opening of arteriovenous communications, which exacerbates portal hypertension. The cause of these disorders may be the flow of vasodilator substances (*e.g.*, glucagon, endocannabinoid, atrial natriuretic

peptide, bacterial endotoxin) through the network of portosystemic shunts, as well as increased production of topical vasodilators by endothelium, such as NO, carbon monoxide, PGI2, endothelium-derived hyperpolarizing factor, adrenomedullin, hydrogen sulfide. Furthermore, in spite of increased circulating levels of endogenous vasoconstrictors (noradrenaline, ET-1, angiotensin Ⅱ), vascular sensitivity to them is significantly reduced[41].

Abdominal aorta

Adaptive response of the abdominal aorta to shear stress, induced by the blood flow in the conditions of the hyperdynamic circulation, may be associated with oxidative stress. Production of ROS, such as superoxide and hydrogen peroxide, which are toxic metabolic products of the cells, leads to non-specific damage of nucleic acids, proteins, lipids, and its other components. ROS regulate vascular tone, endothelial cells sensitivity to oxygen, their growth, proliferation, and apoptosis. Furthermore, they promote the expression of inducible genes by transcription factors, including NF-κB. These genes contribute to the synthesis of chemokines, chemokine receptors, proinflammatory cytokines, and adhesion molecules, inducing an inflammatory response. Potential sources of ROS are various enzyme systems: NAD(P)H oxidase, xanthine oxidase, enzymes of arachidonic acid metabolism (cyclooxygenase and lipoxygenase), and the mitochondrial respiratory chain[42].

Increased levels of TNF-α, IL-1β, and IL-6 in the aorta, as a result of oxidative stress, plays an important role in the induction of immune-mediated systemic vascular process in portal hypertension. Particularly, TNF-α induces activation and translocation of NF-κB to the nucleus with activation of NF-κB-dependent target genes. The subsequent increase in expression of connective tissue growth factor may enhance the synthesis of extracellular matrix proteins, particularly, collagen Ⅰ type, whereas the decrease of the level of MMP-2/TIMP-2 complex (tissue inhibitor of metalloproteinase-2) will contribute to reducing the degradation of extracellular matrix proteins. These processes lead to significant histological changes in the aorta. Its wall thickness decreases, as well as the ratio of medial layer thickness to lumen diameter. Elastic fibers lose their ordered arrangement, and well-marked collagen fibers become more narrow and separated because of the increase of the extracellular matrix in the interstitium of media with a significant decrease in the number of smooth muscle cells[43,44].

The left gastric artery is the first branch of the celiac artery. It is assumed that the hemodynamics in the left gastric artery in portal hypertension may act as the initiator of variceal formation, showing close linkage with variceal recurrence[45].

Mesenteric resistance arteries

Similar infringements also occur in mesenteric resistance arteries. The mechanical stimuli, generated by shear stress, activate endothelial cells and induce hyperproduction of NO and prostaglandins, causing vasodilation[46]. The significantly reduced isometric stiffness of blood vessels and their increased elongation may cause structural changes in the internal elastic membrane and increase fenestrations in it[47]. This contributes to excessive NO-mediated vascular permeability and angiogenic processes in the mesentery of the small intestine because of the high VEGF and eNOS expression in microvessels located there[48].

Portal vein and hepatic artery

Splanchnic hyperemia leads to increased portal venous inflow. The portal vein becomes dilated under the influence of shear stress. Its intima and media are thickened due to the high content of collagen fibers here, hypertrophy, and hyperplasia of smooth muscle cells, which significantly reduce the vascular wall elasticity[49]. At the same time, portal blood flow, supplying the liver, decreases because of collateral circulation, and so-called hepatic arterial buffer response maintains hepatic perfusion constancy. This phenomenon, first described by Lautt[50], was identified in physiological conditions and in various pathological conditions. In liver cirrhosis, it is caused by intrahepatic hypoxia. Oxidative stress contributes to hepatic artery remodeling, which is accompanied by its dilation, decreased elasticity and thinning of the wall, as well as increased expression of adenosine and NO[51]. This reduces hepatic arterial vascular resistance and allows to maintain oxygen supply to the liver, providing protection of the organ structure and function[52].

Splenic artery and vein

Significant histopathological changes also occur in the blood vessels of the spleen. Damaged splenic artery intima becomes thicker, and smooth muscle cells grow into it. The internal elastic lamina is stratified, that is accompanied by the destruction of both included in its structure and localized in media elastic fibers.

Smooth muscle cells, randomly located in media, have a different size and morphology, and the content of separating them collagen fibers, as well as the extracellular matrix, increases significantly, causing the "collagenization" of the vascular wall, thickening, and rigidity[53]. The splenic vein expanding and its intima and media thickening is due to high content of collagen fibers, hypertrophy, and hyperplasia of smooth muscle cells[54]. These pathologic changes in the blood vessels of the spleen lead to a significant reduction of their flexibility.

CONCLUSION

In recent years, defined progress has been made in understanding the mechanisms of hemodynamic disturbances occurring in liver cirrhosis, which are based on portal hypertension. In addition to pathophysiological disorders related to endothelial dysfunction, it was revealed: There is the restructuring of the vasculature, which includes vascular remodeling and angiogenesis. In spite of the fact that these changes are the compensatory-adaptive response to the violated conditions

of blood circulation, taken together, they promote the development and progression of portal hypertension causing severe complications such as bleeding from esophageal varices. Disruption of systemic and organ hemodynamics and the formation of portosystemic collaterals in portal hypertension commence with neovascularization and splanchnic vasodilation due to the hypoxia of the small intestine mucosa. In this regard, the goal of comprehensive treatment may be to influence on the chemokines, proinflammatory cytokines, and angiogenic factors (VEGF, PIGF, PDGF and others) that lead to the development of these disorders. Although pathogenetically reasonable methods of correction of portal hypertension are studied mainly at the molecular, cellular level, and in animal experiments, it can be expected that their clinical implementation will improve the efficiency of conservative therapy aimed at prevention and treatment of its inherent complications.

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ORIGINAL ARTICLE

Basic Study

Fractionation of gamma-glutamyltransferase in patients with nonalcoholic fatty liver disease and alcoholic liver disease

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Author contributions: Sueyoshi S, Sawai S, Satoh M, Seimiya M, Sogawa K, Fukumura A, Tsutsumi M and Nomura F designed research; Sueyoshi S and Satoh M performed research, contributed new analytic tools and analyzed data; Nomura F wrote the paper.

Institutional review board statement: All routine liver biopsy specimens and blood samples from the patients were taken after informed consent and ethical permission was obtained for participation in the study.

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Abstract

AIM

To assess how serum gamma-glutamyltransferase (GGT) fractions vary in patients with alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD).

METHODS

Serum samples were obtained from 14 patients with biopsy-proven alcoholic liver diseases and 9 patients with biopsy proven non-alcoholic fatty liver disease. In addition to these biopsy-proven cases, 16 obese (body mass index > 25) patients without any history of alcohol consumption but with a fatty liver on ultrasound examination and with elevated GGT were included for an additional analysis. Serum GGT fractionation was conducted by high-performance gel filtration liquid chromatography and was separated into the four fractions, big-GGT, medium-GGT, small-GGT (s-GGT), and free-GGT (f-GGT).

RESULTS

The results were expressed as a ratio of each fraction including the total GGT (t-GGT). The s-GGT/t-GGT ratios

were lowest for the control group and highest for the ALD group. The differences between the control and NAFLD groups and also between the NAFLD and ALD groups were statistically significant. In contrast, the f-GGT/t-GGT ratios were highest in the control group and lowest in the ALD group, with the differences being statistically significant. As a result, the s-GGT/f-GGT ratios were markedly increased in the NAFLD group as compared with the control group. The increase of the s-GGT/t-GGT ratios, the decrease of the f-GGT/t-GGT ratios, and the increase of s-GGT/F-GGT ratios as compared with the control group subjects were also found in obese patients with clinically diagnosed fatty change of the liver.

CONCLUSION

Serum GGT fractionation by high-performance gel filtration liquid chromatography is potentially useful for the differential diagnosis of ALD and NAFLD.

Key words: Gamma-glutamyltransferase; f-GGT/t-GGT ratios; Alcoholic liver disease; Non-alcoholic fatty liver disease; Gel filtration liquid chromatography

Core tip: The aim of this study was to assess whether fractionation of serum gamma-glutamyltransferase (GGT) into four fractions by high-performance gel filtration chromatography is useful for the differential diagnosis of alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD). In patients with ALD, small-GGT (s-GGT)/total GGT (t-GGT) ratios were significantly higher and free-GGT (f-GGT)/t-GGT ratios were lower than in those in NAFLD. Consequently, there were marked differences in the s-GGT/f-GGT ratio between ALD and NAFLD. These preliminary results indicate that a large-scale study to clarify the diagnostic values of serum GGT fractionation in the differential diagnosis of ALD and NAFLD is warranted.

Sueyoshi S, Sawai S, Satoh M, Seimiya M, Sogawa K, Fukumura A, Tsutsumi M, Nomura F. Fractionation of gamma-glutamyltransferase in patients with nonalcoholic fatty liver disease and alcoholic liver disease. *World J Hepatol* 2016; 8(36): 1610-1616 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i36/1610.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i36.1610

INTRODUCTION

Gamma-glutamyltransferase (GGT) [(5-glutamyl)-peptide: Amino acid 5-glutamyltransferase, EC 2.3.2.2] is present in many tissues, including the kidneys, pancreas, and liver[1]. GGT in serum is mainly derived from the liver and this enzyme is often used as a marker of hepatobiliary diseases. Although sensitive, GGT elevation is not specific enough for the differential diagnosis of hepatobiliary disorders. GGT is present in serum in multiple forms in molecular complexes that vary in size, charge, and density[2]. These forms were evaluated in the past by electrophoretic methods to enhance the diagnostic value of GGT measurements[3]. These methods, however, were not sensitive enough to facilitate the differential diagnosis of liver diseases. To overcome this limitation, Franzini *et al*[4] developed a high-performance liquid chromatography method to quantify four plasma GGT fractions on the basis of molecular size exclusion chromatography, followed by a GGT-specific post-column reaction.

GGT is widely used as a marker of excessive alcohol intake in patients with alcoholic liver disease (ALD)[5]. Induction of hepatic microsomal GGT by chronic alcohol consumption may account, at least in part, for GGT elevation in alcoholics[6]. In addition, serum GGT levels are often increased in patients with non-alcoholic fatty liver disease (NAFLD)[7].

Distinguishing ALD from NAFLD is difficult because self-reported history of alcohol consumption is unreliable. Detection of patients with high alcohol intake by general practitioners is not necessarily easy[8,9]. Accurate diagnosis of NAFLD relies on a liver biopsy; hence, a less-invasive evaluation strategy is desirable[10].

The aim of this preliminary study was to assess how serum GGT fraction patterns, obtained by a high-performance liquid chromatography method, vary in patients with ALD and NAFLD.

MATERIALS AND METHODS

Patients and blood sample preparation

Serum samples were obtained from 23 patients with biopsy-proven NAFLD or ALD at the Department of Hepatology, Kanazawa Medical University. Fourteen patients (11 males and 3 females, age 53.0 ± 10.6 years) with biopsy-proven ALD (3 patients with fatty liver, 2 alcoholic fibrosis, 5 alcoholic hepatitis, 3 alcoholic hepatitis, and 3 liver cirrhosis) and 9 patients (6 males and 3 females, age 57.2 ± 9.86 years) with biopsy-proven NAFLD (6 patients with non-alcoholic steatohepatitis and 3 with simple steatosis) were included in the study. In addition to these biopsy-proven cases, 16 obese (body mass index > 25) patients (16 males, age 48.3 ± 6.97 years) without any history of alcohol consumption but with a fatty liver on ultrasound examination and with elevated GGT were included for an additional analysis. Subjects suspected to have autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson's disease and alpha 1 anti-trypsin deficiency were excluded from this study. Serum samples were also obtained from 10 apparently healthy and age-matched subjects for a control group. The clinical data for these 49 patients are presented in Table 1. All samples were frozen by liquid nitrogen and were stored at -80 ℃ until analysis. Written informed consent was obtained from all the patients. The ethics committees of each institute approved the protocol.

GGT fractionation by high-performance gel filtration chromatography

Serum GGT fractionation by high-performance liquid chromatography was conducted on the basis of the

Table 1 Comparison of the characteristics of the controls and study patients with biopsy proven

	Biopsy-proven ALD (n = 14)	Biopsy-proven NAFLD (n = 9)	Clinically diagnosed NAFLD (n = 16)	Controls (n = 10)	P value Biopsy-proven ALD *vs* NAFLD
Age (yr)	53 (45-60)	54 (49-66)	49 (43-54)	51 (39-60)	NS
Gender (male:female)	11:3	6:3	16:0	10:0	NS
AST (U/L)	74 (41-105)	45 (38-95)	32 (24-42)	19 (15-21)	NS
ALT (U/L)	19 (13-22)	35 (27-67)	48 (35-74.5)	18 (14-20)	0.0166
Albumin (g/dL)	4.1 (3.2-4.4)	4.6 (4.1-4.7)	4.4 (4.3-4.6)	4.6 (4.3-4.8)	NS
Total bilirubin (mg/dL)	1 (0.6-3.1)	0.7 (0.6-0.8)	0.8 (0.6-0.9)	0.9 (0.6-1.2)	NS
Triglyceride (mg/dL)	186 (99-284)	184 (101-298)	187 (102-212)	130 (111-139)	NS
HDL-cholesterol (mg/dL)	40 (30-56)	43 (29-52)	42 (39-54)	50 (42-56)	NS
LDL -cholesterol (mg/dL)	75 (43-105)	116 (97-153)	122 (98-157)	113 (101-136)	0.0181
GGT (U/L)	368 (296-421)	94 (62-170)	74 (57-112)	24 (19-42)	0.0018
b/t-GGT ratio	0.1 (0.07-0.13)	0.16 (0.13-0.26)	0.18 (0.14-0.24)	0.12 (0.08-0.15)	NS
m/t-GGT ratio	0.04 (0.02-0.05)	0.04 (0.02-0.05)	0.04 (0.03-0.05)	0.02 (0.02-0.03)	NS
s/t-GGT ratio	0.78 (0.65-0.80)	0.55 (0.49-0.64)	0.54 (0.49-0.60)	0.4 (0.31-0.44)	0.0158
f/t-GGT ratio	0.12 (0.08-0.15)	0.18 (0.15-0.26)	0.23 (0.15-0.29)	0.45 (0.37-0.63)	0.0055
b/s-GGT ratio	0.14 (0.09-0.20)	0.33 (0.21-0.53)	0.35 (0.24-0.51)	0.29 (0.19-0.52)	0.0456
s/f-GGT ratio	6.68 (3.67-10.58)	3.1 (2.18-4.32)	2.09 (1.69-3.98)	0.96 (0.48-1.11)	0.0086

Data are presented as median (25^{th}-75^{th} percentile). Statistical analysis: Wilcoxon-Mann-Whitney U test. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyltransferase; ALD: Alcoholic liver disease; NAFLD: Non-alcoholic fatty liver disease; NS: Not significant; HDL: High density lipoprotein; LDL: Low density lipoprotein; b-GGT: Big-GGT; m-GGT: Medium-GGT; s-GGT: Small-GGT; f-GGT: Free-GGT.

methods described by Franzini *et al*[4]. A 100-μL aliquot of serum was injected into a Superose 6 HR 10/300 GL column (diameter 10 mm, length 300-310 mm; GE Healthcare, Parsippany, NJ, United States) attached to a LC-10AD high-performance liquid chromatography system (Shimadzu Co., Kyoto, Japan). Gel filtration chromatography was performed using the isocratic mode with a binary mobile phase composed of 0.1 mol/L sodium phosphate buffer (pH 7.4), containing 0.2 mol/L NaCl, 0.1 mmol/L EDTA, and 5.4 mmol/L Gly-Gly to support the GGT reaction[11,12]. The flow rate of the mobile phase was 0.5 mL/min. Total run time was 60 min, and fractions were collected every 30 s. Serum containing high GGT (> 150 U/L) levels was difficult to separate into small-GGT (s-GGT) and free-GGT (f-GGT) fractions. To make an appropriate comparison of elution profiles of GGT fractions, serum samples with high-GGT-level sera were diluted to approximate 30-50 U/L with the mobile phase solvent prior to analysis. All results were expressed as compared with total GGT activity subjected to the high-performance liquid chromatography analysis.

Measurement of serum total and fractionated GGT activities

Serum total GGT (t-GGT) activities were determined using an enzymatic assay (Serotec Co. Ltd., Sapporo, Japan) with an autoanalyzer (JCA-2250; JEOL Ltd., Tokyo, Japan). This measurement conformed to the International Federation of Clinical Chemistry reference mode for GGT, implemented at a serum volume of 1.2 μL and a reagent volume of 75 μL[4]. Moreover, GGT activity in each fraction improved in the sensitometer mode, which was implemented at a serum volume of 25 μL and a reagent volume of 40 μL. The limit of quantitation at a 10% coefficient of variation was 0.102 U/L.

Statistical analysis

Total GGT activity and those in each high-performance liquid chromatography fraction in the NAFLD and ALD groups were analyzed using the non-parametric Wilcoxon-Mann-Whitney U test. Between-group comparisons of the laboratory data were made with Spearman's rank correlation coefficient. P values of < 0.05 were considered significant.

RESULTS

Elution pattern of the GGT fractions

Figure 1A-E shows the GGT-specific elution profiles of representative serum samples obtained from the control group (Figure 1A and B), and patients with ALD (Figure 1C and D) and NAFLD (Figure 1E and F). Three distinct peaks and a low one were found by fractionation every 30 s. The area of the peaks was calculated using a blank for the average GGT eluted with elution volumes of 5.00-6.25 mL. Each GGT fraction was calculated by dividing the area of each single peak. As indicated in Figure 2, it was confirmed that the area under the chromatogram curve was proportional to the GGT enzyme activities.

On the basis of the molecular weight calibration curve (data not shown), these four peaks are equivalent to big-GGT (b-GGT) (MW > 2000 kDa, eluted between 6.25-9.50 mL), medium GGT (m-GGT) (MW 940 Da, eluted between 9.5-12.25 mL), s-GGT (MW 140 kDa, eluted between 12.25-15.5 mL) and f-GGT (MW 70 kDa, eluted between 15.5-20 mL), respectively.

GGT fraction profiles in four patient groups and the control group

The s-GGT/t-GGT ratios were lowest for the control group

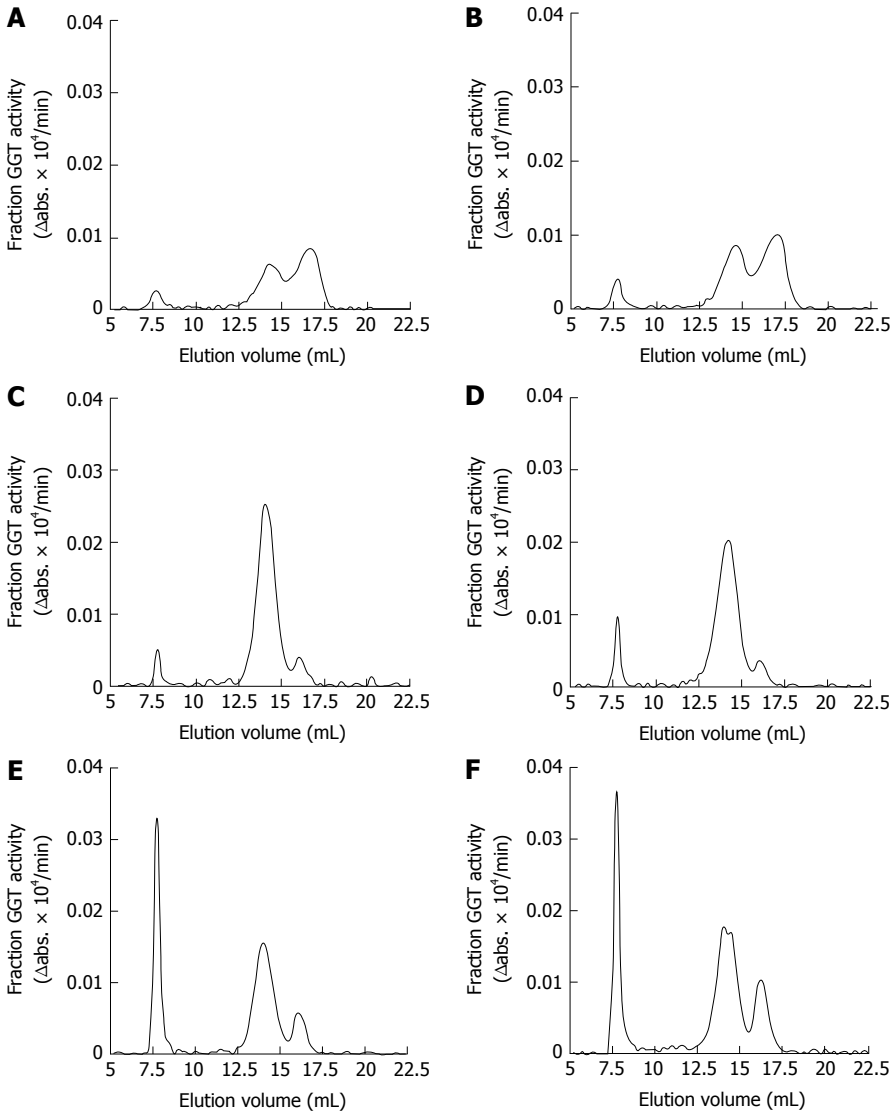

Figure 1 Gamma-glutamyltransferase-specific elution profiles of representative serum samples (two of each) obtained from the control group subjects (A and B), patients with alcoholic liver disease (C and D), and patients with non-alcoholic fatty liver disease (E and F). On the basis of the molecular weight calibration curve (data not shown), these four peaks are equivalent to big-GGT (MW > 2000 kDa, eluted between 6.25-9.50 mL), medium GGT (MW 940 kDa, eluted between 9.5-12.25 mL), small GGT (MW 140 kDa, eluted between 12.25–15.5 mL) and free-GGT (MW 70 kDa, eluted between 15.5-20 mL), respectively. GGT: Gamma-glutamyltransferase.

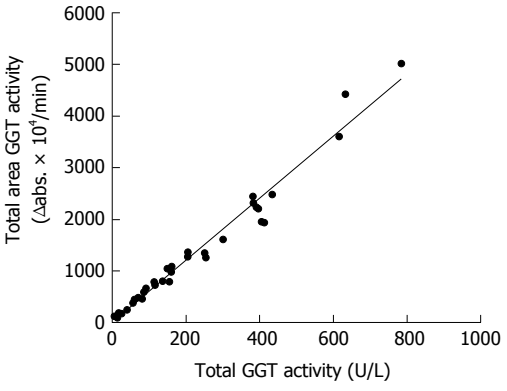

Figure 2 Linear correlation between the total area under the chromatograph curve multiplied by serum dilution factors and total serum gamma-glutamyltransferase activity. Elution volume (between 12-38 mL). $y = 5.9667x + 15.303$; $r = 0.988$; $P < 0.001$; $n = 49$. GGT: Gamma-glutamyltransferase.

and highest for the ALD group. The differences between the control and NAFLD groups and also between the NAFLD and ALD groups were statistically significant, as indicated in Figure 3B. In contrast, the f-GGT/t-GGT ratios were highest in the control group and lowest in the ALD group, with the differences being statistically significant (Figure 3C). As a result, the s-GGT/f-GGT ratios were markedly increased in the NAFLD group as compared with the control group (Figure 3E). The increase of the s-GGT/t-GGT ratios, the decrease of the f-GGT/t-GGT ratios, and the increase of s-GGT/f-GGT ratios as compared with the control group subjects were also found in obese patients with clinically diagnosed fatty change of the liver (Figure 4C-E). There was also a positive correlation between b-GGT activity and levels of low-density lipoprotein and apolipoprotein B in the NAFLD group, but not in the ALD

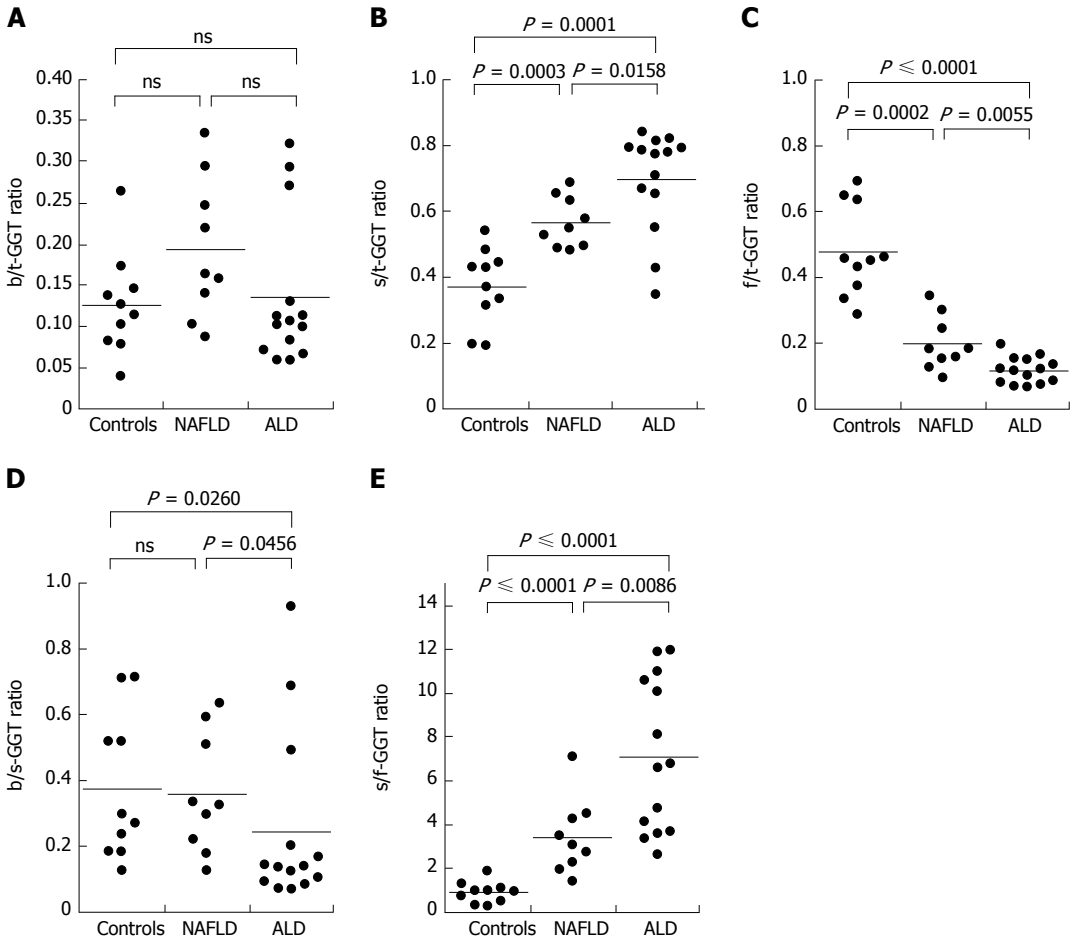

Figure 3 Serum b-GGT/t-GGT, s-GGT/t-GGT, f-GGT/t-GGT, b-GGT/s-GGT and s-GGT/f-GGT ratios in patients with biopsy-proven non-alcoholic fatty liver disease, biopsy-proven alcoholic liver disease and the controls group. ALD: Alcoholic liver disease; NAFLD: Non-alcoholic fatty liver disease; GGT: Gamma-glutamyltransferase; b-GGT: Big-GGT; t-GGT: Total-GGT; s-GGT: Small-GGT; f-GGT: Free-GGT; ns: No significant differences.

group, in the present study (data not shown).

DISCUSSION

Franzini *et al*[4] described a high-performance gel filtration chromatography method for plasma GGT fraction analysis. This method permitted the quantification of four GGT fractions; b-GGT, m-GGT, s-GGT (likely lipoprotein-bound, molecular masses > 2000, 940 and 140 kDa, respectively) and a f-GGT fraction.

It is common for serum GGT levels to be elevated in patients with ALD[5] or obesity-related NAFLD[7]. Patients with elevated serum GGT levels who are obese and are also excessive, habitual alcohol drinkers are frequently encountered in clinical practice. It is necessary to have non-invasive measures to assess the relative contribution of overweight and excessive alcohol consumption on GGT elevations. We wondered how serum GGT fraction patterns obtained by the high-performance liquid chromatography method vary in patients with ALD and NAFLD.

The results of this preliminary study indicate that in patients with ALD, s-GGT/t-GGT ratios were significantly increased and f-GGT/t-GGT ratios were lower, compared with those in NAFLD patients. As a result, there was a marked difference in the s-GGT/f-GGT ratios between patients with ALD and NAFLD. These results indicate that a large-scale study to clarify the diagnostic value of serum GGT fractionation in the differential diagnosis of ALD and NAFLD is warranted.

High-sensitivity GGT fraction patterns of various liver diseases were evaluated by Franzini *et al*[13-17], Elawdi *et al*[18], Fornaciari *et al*[19] and Corti *et al*[20,21]. They reported that the b-GGT/s-GGT ratio was significantly lower in both alcoholics and abstainers than in the control group, consistent with our study[13]. Patients with NAFLD and chronic hepatitis C have different GGT fraction patterns: b-GGT is increased in NAFLD, but not in chronic hepatitis C[14]. More recently, GGT fractions were measured in cirrhosis patients, revealing that, irrespective of etiology, s-GGT showed the greatest increase in cirrhotic patients and the b-GGT/s-GGT ratio was even lower than that in patients with chronic hepatitis C[18].

To the best of our knowledge, the present study is the first direct comparison of serum GGT fraction profiles between patients with ALD and NAFLD. However, there are several limitations to the present study. The numbers of the biopsy-proven cases was small. In addition, how serum GGT profiles change with disease progression

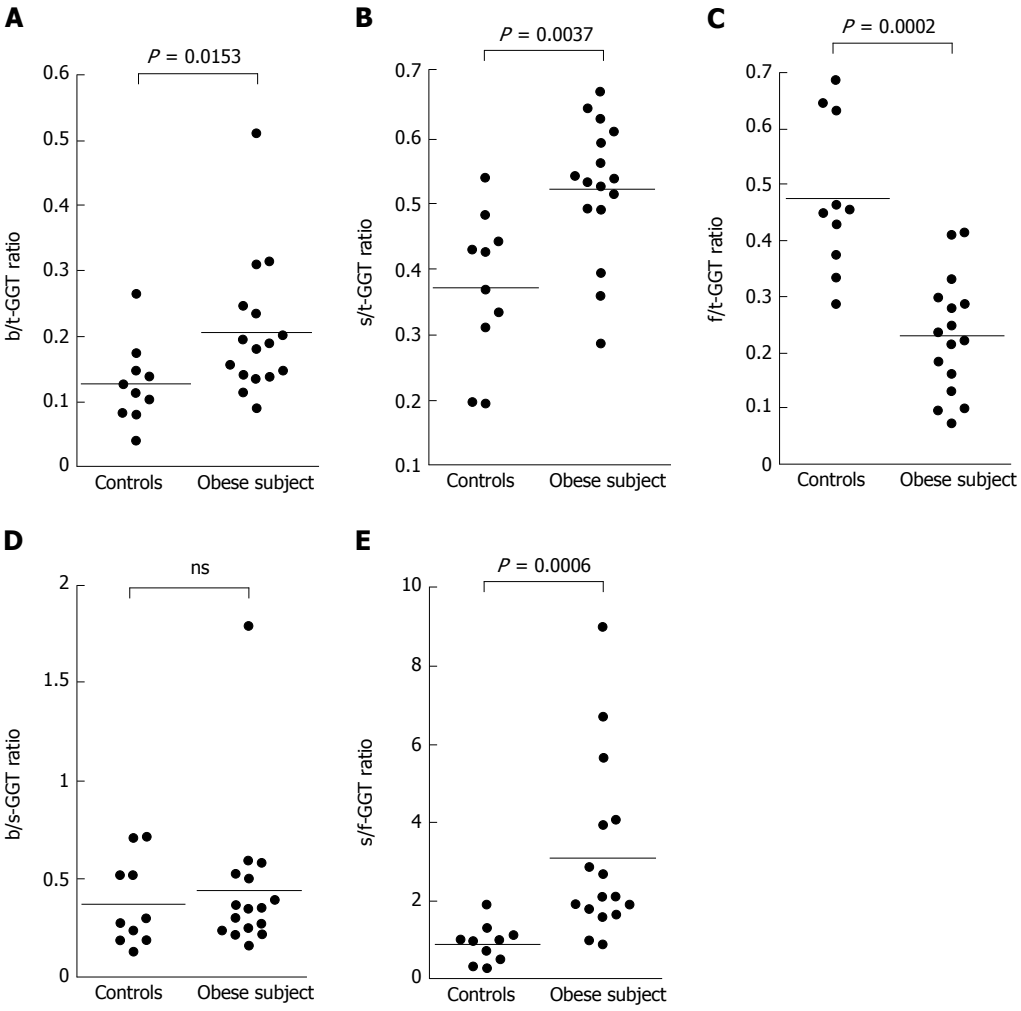

Figure 4 Serum b-GGT/t-GGT, s-GGT/t-GGT, f-GGT/t-GGT, b-GGT/s-GGT, and s-GGT/f-GGT ratios in patients with non-alcoholic fatty liver disease clinically diagnosed by ultrasonography. GGT: Gamma-glutamyltransferase; b-GGT: Big-GGT; t-GGT: Total-GGT; s-GGT: Small-GGT; f-GGT: Free-GGT; ns: No significant differences.

from fatty liver to liver cirrhosis in patients with NAFLD and ALD remains unclear. Also, the diagnostic value of GGT profiles remains to be compared with other markers including cytochrome C[22].

In addition to the well-known alterations in hepatobiliary disorders, GGT is associated with cardiovascular disease (CVD)[23]. In a recent review article, the predictive value of GGT for assessing CVD and cancer mortality was described, including assessment at the physiological level of the enzyme activity[24]. Taking advantage of the high-performance gel filtration chromatography method for plasma GGT fraction analysis, Franzini *et al*[15] demonstrated that CVD risk factors were associated with b-GGT.

In conclusion, the serum GGT fraction patterns in patients with NAFLD are significantly different from those in patients with ALD. In patients with ALD, s-GGT/t-GGT ratios were significantly higher and f-GGT/t-GGT ratios were lower than in those in NAFLD. Consequently, there were marked differences in the s-GGT/f-GGT ratio between ALD and NAFLD. A large-scale study is needed to further evaluate the diagnostic value of serum GGT fractionation in the differential diagnosis of ALD and NAFLD.

COMMENTS

Background

Distinguishing alcoholic liver disease (ALD) from non-alcoholic fatty liver disease (NAFLD) is difficult because self-reported history of alcohol consumption is unreliable. Detection of patients with high alcohol intake by general practitioners is not necessarily easy. Accurate diagnosis of NAFLD relies on a liver biopsy; hence, a less-invasive evaluation strategy is desirable.

Research frontiers

Gamma-glutamyltransferase (GGT) in serum is mainly derived from the liver and this enzyme is often used as a marker of hepatobiliary diseases. Although sensitive, GGT elevation is not specific enough for the differential diagnosis of hepatobiliary disorders. GGT is present in serum in multiple forms in molecular complexes that vary in size, charge, and density. These methods, however, were not sensitive enough to facilitate the differential diagnosis of liver diseases. To overcome this limitation, Franzini *et al* developed a high-performance liquid chromatography method to quantify four plasma GGT fractions on the basis of molecular size exclusion chromatography, followed by a GGT-specific post-column reaction.

Innovations and breakthroughs

To the best of our knowledge, the present study is the first direct comparison of

serum GGT fraction profiles between patients with ALD and NAFLD.

Applications

Although preliminary, determination of serum GGT profiles may serve for differential diagnosis of ALD and NAFLD.

Terminology

High-performance liquid chromatography (previously called high-pressure liquid chromatography), is a useful analytical tool which is able to separate various compounds based on their size, electrical charge and biochemical affinity.

Peer-review

The research presents a screening for future investigations about AFLD and NAFLD diagnosis differentiation. It is an interesting approach of ALD and AFLD diagnosis which was not executed in the best possible way.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Spontaneous bacterial peritonitis prevalence in pre-transplant patients and its effect on survival and graft loss post-transplant

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Abstract

AIM

To investigate the incidence of spontaneous bacterial peritonitis (SBP) in pre-transplant patients and its effect on post transplant mortality and graft failure.

METHODS

We conducted a retrospective cohort study of patient records from the organ procurement and transplant network data set. Patients were identified by the presence of SBP pre-transplant. Univariate post-transplant survival models were constructed using the Kaplan-Meier technique and multivariate models were constructed using the Cox proportional hazards model. Variables that affected post-transplant graft survival were identified in the SBP population.

RESULTS

Forty-seven thousand eight hundred and eighty patient records were included in the analysis for both groups, and 1966 (4.11%) patients were identified in the data set as having pre-transplant SBP. Patients that had pre-transplant SBP had higher rates of graft loss from recurrent hepatitis C virus (HCV) (3.6% *vs* 2.0%, $P < 0.0001$), infections leading to graft loss (1.9% *vs* 1.3%, $P = 0.02$), primary non-function (4.3% *vs* 3.0%, $P < 0.0001$) and chronic rejection (1.1% *vs* 0.7%, $P = 0.04$). Kaplan-Meier survival analysis showed a statistically significant difference in all-cause survival in patients with a history of SBP *vs* those without ($P < 0.0001$). Pre-transplant history of SBP was independently predictive

of mortality due to recurrent HCV (HR = 1.11, 95%CI: 1.02-1.21, $P < 0.017$) after liver transplantation.

CONCLUSION

HCV patients prior to the advent of directing acting anti-viral agents had a higher incidence of pre-transplant SBP than other patients on the liver transplant wait list. SBP history pre-transplant resulted in a higher rate of graft loss due to recurrent HCV infection and chronic rejection.

Key words: Spontaneous bacterial peritonitis; Liver transplant; Graft failure; Hepatitis C

Core tip: Prevention of spontaneous bacterial peritonitis (SBP) pre-transplant may affect graft outcomes and ultimately patient survival post-transplant. Patients with hepatitis C virus (HCV) in whom therapy is deferred until the time of transplant due to hepatic decompensation, may benefit from expedited treatment if they possess a history of SBP to avoid complications related to HCV recurrence.

Shah NL, Intagliata NM, Henry ZH, Argo CK, Northup PG. Spontaneous bacterial peritonitis prevalence in pre-transplant patients and its effect on survival and graft loss post-transplant. *World J Hepatol* 2016; 8(36): 1617-1622 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i36/1617.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i36.1617

INTRODUCTION

According to the Scientific Registry of Transplant Recipients database, since 2005 over 6000 liver transplants have been performed on an annual basis. Hepatitis C virus (HCV) has consistently been the most common indication for liver transplantation and challenging due to the possibility of viral recurrence post-transplant. Recurrent HCV had been a major problem for patients post liver transplantation, but with the new direct acting anti-viral (DAA) therapies, this problem is steadily declining. Even with resolution of this challenge, identifying factors that may accelerate graft loss or damage is still essential.

Originally established for mortality post transjugular intrahepatic portosystemic shunts, the laboratory based Model for End Stage Liver Disease (MELD and MELD-Na) is now the primary measure for liver organ allocation in the United States[1]. Since the use of MELD began in 2002, wait list mortality has significantly decreased as patients are prioritized effectively, however patients on the wait list still suffer from complications of end stage liver disease and portal hypertension such as gastrointestinal bleeding, encephalopathy, ascites, renal failure, and infection[2].

Liver disease patients, with decreased levels of complement proteins and decreased opsonization, live in a relatively immunocompromised state and are at high risk of developing bacterial, viral, or fungal infections[3]. Ascites reportedly occurs in 7% to 23% of hospitalized end stage liver disease patients[4]. An initial episode of spontaneous bacterial peritonitis (SBP) occurs in about 10% of these patients[5]. Infection related to SBP can have severe ramifications in the development of renal failure and mortality of patients while on the transplant wait list.

The utility of MELD in organ allocation is well established, but its extrapolation to post-transplant survival or graft outcomes is still unclear. Further, in the post MELD era, limited studies have investigated the role of SBP on post-transplant outcomes[6]. Therefore, in order to understand the role of infections on post-transplant outcomes, we aimed to investigate the incidence of SBP in pre-transplant patients and its effect on post transplant mortality and graft failure in the era prior to DAA therapy.

MATERIALS AND METHODS

We investigated the United States organ procurement and transplant network (OPTN) dataset for liver transplants from February 2002 until November 2009 for liver graft recipients with a reported history of pre-transplant SBP. All patients who eventually underwent liver transplantation were included in the analysis. If the patient did not have a history of SBP or if the question was left blank (or "unknown" was selected) on the Adult Liver Transplant Recipient Registration Worksheet submitted to the United Network for Organ Sharing, the recipient was assumed to not have pre-transplant SBP. The population with a history of SBP was compared to those without the history of SBP for multiple pre- and post-transplant characteristics. Recipient etiology of disease was categorized as HCV, hepatitis B, non-alcoholic steatohepatitis or cryptogenic, alcohol alone, cholestatic liver disease, autoimmune, liver malignancy, or other. Etiologies of graft failure included biliary, *de novo* autoimmune hepatitis, recurrent (non-viral) disease, infection, primary non-function, recurrent viral hepatitis, acute rejection, chronic rejection, and vascular thrombosis. Laboratory (non-exception) MELD scores were used for all recipients.

Demographics, recipient, donor, and surgical characteristics were compared between groups using the χ^2 test for categorical variables and the Student *t*-test for continuous variables. Univariate post-transplant survival models were constructed using the Kaplan-Meier technique and multivariate models were constructed using the Cox proportional hazards model. Variables known to influence post-transplant survival from previous studies or those variables found to be significant in the univariate analysis to a level of less than 0.20 were included in multivariate models using a whole model (non-stepwise) analysis. Because of the finding of a relationship between SBP and graft failure due to recurrent HCV, a multivariate logistic regression model was developed to determine

Table 1 Population characteristics

Population characteristic	History of pre-transplant SBP (n = 1966)	No history of SBP (n = 45914)	P-value
Recipient age, yr	50.51 (49.96-51.07)	47.97 (47.81-48.13)	< 0.0001
Donor age, yr	38.08 (38.84-39.61)	38.29 (38.12-38.46)	0.165
MELD score at transplant	25.28 (24.83-25.74)	20.34 (20.25-20.44)	< 0.0001
Male	1429 (72.69)	29950 (65.23)	< 0.0001
Ethnicity African American	149 (7.58)	4591 (10.00)	0.027
Etiology of recipient liver disease			< 0.0001
Alcohol alone	318 (16.17)	4621 (10.06)	
Autoimmune	64 (3.26)	1149 (2.50)	
Cholestatic disease	113 (5.75)	3337 (7.27)	
Hepatitis B	69 (3.51)	1014 (2.21)	
Hepatitis C	809 (41.15)	13557 (29.53)	
Liver malignancy	126 (6.41)	6435 (14.02)	
NASH/cryptogenic	175 (8.90)	4214 (9.18)	
Other	292 (14.85)	11587 (25.24)	
Liver retransplantation	145 (7.38)	3711 (8.08)	0.259

Categorical variables are expressed as number and column percent. Continuous variables are expressed as mean and 95%CIs unless otherwise specified. SBP: Spontaneous bacterial peritonitis; MELD: Model for End Stage Liver Disease; NASH: Nonalcoholic steatohepatitis.

those variables independently predictive of recurrent HCV. No data imputation was used. All statistical testing was two sided and the level of type one error deemed to be statistically significant was assumed to be less than or equal to 0.05. All dataset manipulation and analysis was performed using SAS (version 9.2, Cary, NC, United States). Local institutional review board approval was not required for analysis of this de-identified dataset.

RESULTS

The OPTN data set contained information on 47880 patients transplanted during the study period. The characteristics of the study population are outlined in Table 1. Of this population, 1966 (4.11%) patients were reported to have a history of pre-transplant SBP. Patients with a history of SBP tended to be older (50.5 mean years in the SBP population *vs* 48.0 in the non-SBP population, $P < 0.0001$), male (72.7% *vs* 65.2%, $P < 0.0001$), and have a higher MELD score at the time of liver transplantation (25.3 *vs* 20.3, $P < 0.0001$). The etiology of liver disease was sigificantly different between those recipients who had pretransplant SBP compared to those that did not. HCV was signficantly more prevelant in the SBP population (41.1% *vs* 29.5%, $P < 0.0001$).

Table 2 shows the distribution and causes of graft failure in the post-transplant time period. While overall graft loss was uncommon, compared to those without a history of SBP, patients that had pre-transplant SBP had higher rates of graft loss from recurrent HCV (3.6% *vs* 2.0%, $P < 0.0001$), infections leading to graft loss (1.9% *vs* 1.3%, $P = 0.02$), primary non-function (4.3% *vs* 3.0%, $P < 0.0001$) and chronic rejection (1.1% *vs* 0.7%, $P = 0.04$). In regards to all-cause survival, patients having pre-transplant SBP had worse unadjusted one year post-transplant survival (82.8% *vs* 86.5%, $P < 0.0001$) and this difference widened at two years (76.5% *vs* 81.6%, $P < 0.0001$). Figure 1 shows the Kaplan-Meier survival analysis. There was a statistically significant difference in all-cause survival in patients with a history of SBP *vs* those without ($P < 0.0001$).

In order to account for multiple factors influencing graft loss in this population, we developed a multivariable logistic regression model including factors known to affect survival rates: Age of recipient, age of donor, MELD score, history of previous transplant, and history of HCV. Table 3 shows the results of this analysis. A pre-transplant history of SBP was found to be an independent risk factor for post-transplant graft failure imparting a 57% increased risk of graft failure (OR = 1.57, 95%CI: 1.22-2.02, $P < 0.001$). Table 4 shows the results of a mutivariate proportional hazards survival model predicting death due to recurrent HCV. Once again, a pre-transplant history of SBP was independently predictive of mortality due to recurrent HCV (HR = 1.11, 95%CI: 1.02-1.21, $P < 0.017$) after liver transplantation.

DISCUSSION

We have shown that HCV patients prior to the advent of DAA agents had a higher incidence of pre-transplant SBP than other patients on the liver transplant wait list. Further, SBP history resulted in a higher rate of graft loss due to recurrent HCV infection and chronic rejection. This group also had an 11.2% greater risk of post-transplant mortality. In a multivariate Cox regression model, SBP was found to be an independent risk factor for post-transplant mortality.

Patients with a diagnosis of active HCV infection at the time of transplant have a known predisposition to graft failure due to HCV recurrence. However, the rate of progression to cirrhosis and graft failure is unpredictable. Certain known risk factors such as the donor's age, post-transplant CMV infection, HIV co-infection, the use of T cell depleting therapies, pulsed steroids, and other donor risk factors have all been associated with poorer outcomes[7]. SBP has not been studied in this regard. Our results show that a history of pre-transplant SBP may

Table 2 Cause of graft failure in patients with and without a history of spontaneous bacterial peritonitis

Cause of graft failure	History of pre-transplant SBP (n = 1966)	No history of SBP (n = 45914)	P-value
Biliary	24 (1.22)	423 (0.92)	0.176
De novo autoimmune hepatitis	2 (0.10)	17 (0.04)	0.182
Recurrent viral hepatitis	71 (3.61)	936 (2.04)	< 0.0001
Infection	37 (1.88)	585 (1.27)	0.020
Primary non-function	84 (4.27)	1352 (2.94)	< 0.0001
Recurrent non-viral disease	26 (1.32)	547 (1.19)	0.600
Acute rejection	18 (0.92)	289 (0.63)	0.120
Chronic rejection	21 (1.07)	312 (0.68)	0.042
Vascular thrombosis	44 (2.24)	811 (1.77)	0.122

SBP: Spontaneous bacterial peritonitis.

Table 3 Mutlivariate analysis of independent predictors of graft failure due to recurrent viral hepatitis

	Odds ratio	95%CI	P-value
History of pre-transplant SBP	1.567	1.218-2.017	< 0.001
Hepatitis C (*vs* hepatitis B)	6.777	5.864-7.832	< 0.0001
Previous transplant	2.349	1.923-2.868	< 0.0001
MELD at transplant	0.989	0.982-0.996	0.0014
Age of recipient	0.992	0.986-0.997	0.0041
Age of donor	1.023	1.019-1.027	< 0.0001

SBP: Spontaneous bacterial peritonitis; MELD: Model for End Stage Liver Disease.

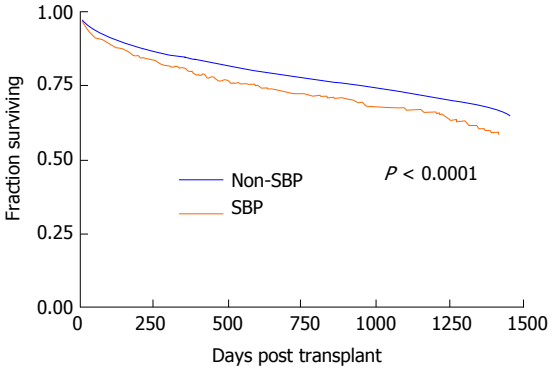

Figure 1 Kaplan-Meier post-transplant survival analysis of patients with a history of pre-transplant spontaneous bacterial peritonitis. SBP: Spontaneous bacterial peritonitis.

contribute to worse survival and an increased risk of graft failure. As our study shows, 40% of pre-transplant SBP patients in this cohort suffered from HCV, whereas only 30% of a control, non-SBP pre-transplant group suffered from HCV.

Prior treatment regimens, which included interferon, to decrease HCV levels in pre-transplant candidates possessed their own risks and had shown an increase the rate of bacterial infections in these patients[8]. The rate of infection, especially the incidence of SBP in patients not receiving prophylaxis therapy, in Child Pugh B/C patients was higher than matched controls[8]. Several etiologies had been proposed, including the neutropenic and granulo-toxic effect of interferon causing an increase susceptibility to bacterial infections. These studies show

Table 4 Mutlivariate analysis of independent predictors of all cause survival due to recurrent hepatitis C

	Hazard ratio	95%CI	P-value
History of pre-transplant SBP	1.112	1.019-1.212	0.017
Hepatitis C	1.176	1.126-1.228	< 0.0001
Previous transplant	1.979	1.860-2.107	< 0.0001
Gender male	0.983	0.941-1.027	0.443
Ethnicity non-African American	0.921	0.866-0.980	0.010
MELD at transplant	1.016	1.014-1.018	< 0.0001
Age of recipient	1.009	1.007-1.010	< 0.0001
Age of donor	1.009	1.008-1.010	< 0.0001

SBP: Spontaneous bacterial peritonitis; MELD: Model for End Stage Liver Disease.

an increased risk of infection, including SBP, in HCV patients with poor liver function. With our data showing the deleterious effect of SBP on transplant outcomes, it supports the careful choice of HCV patients that were selected in the past to undergo interferon based therapies pre-transplant and the importance of SBP prophylaxis in this group with proper indications[8,9].

Limited studies from the pre-MELD era did not show that pre-transplant SBP influenced post-transplant outcomes. A study following 100 patients, showed that 32% of patients developed a pre-transplant infection. The infections ranged from SBP (35.6%), blood stream infections (28.9%), cellulitis (13.3%), pneumonia (8.9%), urinary tract infections (6.7%), and other infections (6.7%)[10]. After following this group, the study found that patients with pre-transplant infections were less likely to be transplanted from home and required longer hospital stays, but the mortality at 90 and 180 d post-transplant did not differ significantly compared to individuals without pre-transplant infections. As shown by our Kaplan Meier survival curves in Figure 1, the survival of the two groups from our analysis of the OPTN database seems to be similar, but then diverges significantly starting at day 250. Therefore, even though the previously mentioned study did not find differences in survival, we feel that with longer follow-up this difference could have been statistically significant.

Another single center study, reported that patients with a history of SBP had more severe liver disease as measured by MELD and CTP score, but had similar

post-transplant mortality to those without a history of SBP[11]. The patients who developed SBP most commonly suffered from liver disease related to HCV or alcohol. The mean follow-up time period for this study was 3 years. While mortality or infection rates were not affected, these patients were more likely to require abdominal surgery 1 year post transplant for hernia repairs, bleeding, and vascular complications[11]. This study reinforces our findings of the large burden of SBP on pre-transplant cirrhosis patients and the higher rate of SBP in HCV patients. However, the relatively small sample size and inclusion of patients transplanted in the pre-MELD era may not be as applicable to the general population. Our cohort studies the transplants occurring after 2002, and includes a multi-center analysis with almost 48000 patient records.

There are several limitations to our study. One of the major limitations is the incomplete reporting of SBP in the OPTN database. We believe it is safe to assume that reported instances of SBP are accurate and have a clear documented episode recognized by the listing health care provider, which prompted this designation. However, the low rates of patients with SBP pre-transplant as compared to other studies in the literature, raises the speculation of recall bias from reporting centers on patient's history of SBP. The exact determination of SBP may not be uniform across all centers, and without full accessibility to numbers of SBP episodes, exact cell counts, or ascites fluid analysis we are dependent solely on information from the database. Further, those patients with severe infection due to SBP were likely excluded from transplant listing. Finally, while we may assume that survival statistics and graft loss are accurate in the OPTN database, other post-transplant variables are often incomplete including immunosuppression data and other details regarding HCV recurrence[12]. By using a worst case scenario analysis, if we assume that all patients in the OPTN database without data entered for SBP are included in the control group. Any mortality registered in these patients would only reduce the differences between our groups.

It has been shown that HCV patients being treated with interferon based therapies were at an increased risk of developing SBP[8]. It is unclear if this association is due to the underlying viral hepatitis disease process or related to the treatment regimen that were used. Regardless, stronger surveillance measures and antibiotic prophylaxis pre-transplant SBP may be necessary to improve post-transplant outcomes. Also, the new issue as we transition fully to DAA regimens is the decision to treat cirrhosis patients pre *vs* post-transplant[13,14]. If patients live in a region with a relatively high average MELD at time of transplant, treating HCV infection pre-transplant may put these patients into MELD "purgatory" - stable MELD that will not increase transplant prioritization, but continued low quality of life[15]. Due to the low rates of drug interactions between the DAAs and immunosuppression, some patients at higher MELD are deferred for therapy until after transplant. From these results and our experience prior to the advent of DAAs, in those HCV patients we deem necessary to treat post-transplant, we should consider prioritizing therapy in those with a history of SBP. Further, we should strive to reduce donor risk factors, minimize pulsed steroids or T cell depleting immunosuppression to prevent post-transplant HCV recurrence and graft failure[16,17]. Over time, we predict the number of transplants performed in the United States stemming from HCV infection to decrease, but the decision to treat these patients around the time of transplant and avoiding SBP will continue to be a challenge.

COMMENTS

Background

This study examines the effect of spontaneous bacterial peritonitis in cirrhosis patients on the wait list for liver transplantation. Using the patients identified in the organ procurement and transplant network (OPTN)/United Network for Organ Sharing (UNOS) database, in the era prior to direct acting anti-viral agents, we investigated the effect of spontaneous bacterial peritonitis (SBP) on liver transplant graft loss and post-transplant mortality.

Research frontiers

Future studies focused on the prevention of pre-transplant infections, like SBP, and the effect of direct acting anti-viral (DAA) therapies on graft loss and mortality is a potential area to study. Further, as the treatment paradigm for pre *vs* post-transplant hepatitis C virus (HCV) therapy changes, it may be worthwhile to examine the change it produces in graft loss from HCV complications.

Innovations and breakthroughs

From our knowledge, this is the first article to assess the effect of SBP on post transplant outcomes from the OPTN dataset. Further it provides a collective experience of our outcomes from the pre-DAA therapy era which can be used as a basis for future comparison.

Applications

This article has shown the importance of avoiding SBP infections in the pre-transplant population. Aggressive prophylaxis and treatment of this infection may have long term implications with regards to graft survival and mortality.

Terminology

SBP: Spontaneous bacterial peritonitis - infection found in the ascites fluid in the peritoneum; DAA: Direct acting anti-viral therapies - the new class of HCV medications which directly inhibits replication; OPTN/UNOS: The national organization responsible for managing organ allocation, obtaining individual center's data on transplantation, and reporting collective outcomes.

Peer-review

This is a good paper.

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ORIGINAL ARTICLE

Observational Study

Prevalence of significant liver disease in human immunodeficiency virus-infected patients exposed to Didanosine: A cross sectional study

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Abstract

AIM

To identify significant liver disease [including nodular regenerative hyperplasia (NRH)] in asymptomatic Didanosine (DDI) exposed human immunodeficiency virus (HIV) positive patients.

METHODS

Patients without known liver disease and with > 6 mo previous DDI use had liver stiffness assessed by transient elastography (TE). Those with alanine transaminase (ALT) above upper limit normal and/or TE > 7.65 kPa underwent ultrasound scan (U/S). Patients with: (1) abnormal U/S; or (2) elevated ALT plus TE > 7.65 kPa;

or (3) TE > 9.4 kPa were offered trans-jugular liver biopsy (TJLB) with hepatic venous pressure gradient (HVPG) assessment.

RESULTS

Ninety-nine patients were recruited, median age 50 years (range 31-70), 81% male and 70% men who have sex with men. Ninety-five percent with VL < 50 copies on antiretroviral therapy with median CD4 count 639 IU/L. Median DDI exposure was 3.4 years (range 0.5-14.6). Eighty-one had a valid TE readings (interquartile range/score ratio < 0.3): 71 (88%) < 7.65 kPa, 6 (7%) 7.65-9.4 kPa and 4 (6%) > 9.4 kPa. Seventeen (17%) met criteria for TJLB, of whom 12 accepted. All had HVPG < 6 mmHg. Commonest histological findings were steatosis (n = 6), normal architecture (n = 4) and NRH (n = 2), giving a prevalence of previously undiagnosed NRH of 2% (95%CI: 0.55%, 7.0%).

CONCLUSION

A screening strategy based on TE, liver enzymes and U/S scan found a low prevalence of previously undiagnosed NRH in DDI exposed, asymptomatic HIV positive patients. Patients were more likely to have steatosis highlighting the increased risk of multifactorial liver disease in this population.

Key words: Nodular regenerative hyperplasia; Human immunodeficiency virus; Steatosis; Non-cirrhotic portal hypertension; Didanosine

Core tip: Human immunodeficiency virus positive patients are at increased risk of liver disease. The aetiology is often multifactorial and includes exposure to antiretroviral therapy. We used a simple screening strategy based on transient elastography, liver enzymes and ultrasound scan to identify that 2% of asymptomatic patients exposed to Didanosine in a clinical cohort had undiagnosed nodular regenerative hyperplasia. A further 6% had undiagnosed steatosis. Implementation of a screening strategy enables identification of liver disease and initiation of earlier targeted interventions in this high-risk group.

Logan S, Rodger A, Maynard-Smith L, O'Beirne J, Fernandez T, Ferro F, Smith C, Bhagani S. Prevalence of significant liver disease in human immunodeficiency virus-infected patients exposed to Didanosine: A cross sectional study. *World J Hepatol* 2016; 8(36): 1623-1628 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i36/1623.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i36.1623

INTRODUCTION

Nodular regenerative hyperplasia (NRH) - the development of micronodules in liver parenchyma without intervening fibrosis[1] - has been reported in human immunodeficiency virus (HIV) positive patients who often present late in the course of the condition with complications associated with non-cirrhotic portal hypertension (NCPH). A strong association with NRH is current or previous use of Didanosine (DDI)[1-6]. Although DDI is no longer used as first line antiretroviral therapy (ART), many HIV positive patients have significant previous exposure with a reported prevalence of NRH of between 0.5%-35%[1,2,6]. This wide range is indicative of the unreliability of screening strategies and the fact that studies largely included individuals diagnosed with NRH or NCPH as a result of liver biopsy for other conditions or as a result of late presentation with complications of portal hypertension[1-6]. However prior to this the disease is largely sub clinical and a screening strategy may be useful to identify patients with DDI associated liver disease earlier in the disease process.

Other studies have reported raised transaminases in NRH, but in many cases transaminases are only mildly elevated or not at all this indicating the unreliability of this alone as a screening tool[1]. Hepatic transient elastography (TE) is a validated non-invasive tool with good correlation for identifying hepatic and peri-portal fibrosis[2,7]. TE is also associated with hepatic venous pressure gradients (HVPG) in cirrhosis[8]. The co-relation with NRH is less well delineated, however increased liver stiffness readings have been reported in patients with both NRH and NCPH[9-14], although one study found that liver stiffness readings did not predict the presence of portal hypertension in NRH[10]. Many studies also report reduced platelet levels and the presence of splenomegaly in individuals with NRH[1].

Our aim was to develop and implement a screening strategy incorporating 3 separate measures; TE, platelet and alanine transaminase (ALT) levels, with subsequent ultrasound and trans-jugular liver biopsy (TJLB) in those who met criteria to identify DDI related liver disease in HIV positive patients with previous significant DDI exposure, but who were currently asymptomatic.

MATERIALS AND METHODS

This study is a cross-sectional study in HIV outpatients at The Royal Free London NHS Foundation Trust from 2010 to 2011. Ethical approval was obtained (REC 10/H0720/54). Study subjects were identified from the HIV clinical database. HIV positive patients currently under active follow-up and previously exposed to DDI therapy for longer than 6 mo were eligible to take part. Exclusions were viral hepatitis co-infection, age < 18 years, a body mass index (BMI) > 40, pregnancy or ascites. Patients were sequentially recruited as they attended for routine clinic follow-up. Statistical review of the study was performed by a biomedical statistician (Dr. Colette Smith).

Patients completed a study specific questionnaire on sociodemographic factors, medical history, lifestyle

including smoking, alcohol [Michigan Alcoholism Screening test (MAST)] and drug use. Clinical data (HIV viral load, CD4 count, whether on/off treatment, date of diagnosis, date of ART start, lipids, liver panel bloods, blood glucose, BMI) were also collected.

Liver TE was measured using FibroScan (FS) (Echosens, Paris). A median stiffness reading was measured using at least ten readings with a valid reading recording 60% accuracy and an interquartile range (IQR) of less than 30% of the median. Subjects were offered further evaluation with ultrasound of liver and spleen with doppler waveforms of the hepatic vasculature if they had either: (1) an ALT level above 19 IU/mL for women and 31 IU/mL for men; or (2) a platelet count (PLT) less than 120×10^9/L; or (3) TE reading of > 7.65 kPa (IQR < 0.3).

Individuals with evidence on ultrasound of splenomegaly or fatty liver or coarse echotexture or abnormalities of hepatic doppler waveforms in conjunction with a raised ALT or TE reading as above were offered a TJLB with HVPG measurements. Patients with a raised ALT or platelets < 120×10^9/L for whom an elastography score was unobtainable (centripetal obesity) or uninterpretable (less than 10 valid readings or IQR/LSM > 30%) were offered ultrasound and TJLB.

All TJLB procedures were performed in the interventional radiology suite by an experienced operator (O'Beirne J) after a 6-h fast, under local anaesthesia. Biopsies were taken using a 19G Tru-cut type biopsy needle (Quick core; Cook, William Cook Europe, Denmark). Three or 4 passes were performed through the same hepatic vein wall (right or middle) to ensure that sufficient liver tissue was obtained. Wedge hepatic vein pressures (WHVP) were measured using a 5-F Berenstein balloon catheter (Boston Scientific, Natick, MA) using the technique described by Groszmann and Wongcharatrawee[15]. Three sets of measurements were taken. WHVP was measured for at least 1 min each time. HVPG was calculated as the mean of the 3 gradients (the difference between WHVP minus free hepatic pressure). Groups were compared using the Mann Whitney *U* test for non-parametric continuous variables and using the χ^2 test for categorical variables.

RESULTS

Four hundred and fifty-nine patients exposed to DDI for longer than 6 mo were identified from the clinic database. Eighty-four patients known to have co-infection with hepatitis B or C were excluded. No patients were excluded due to BMI > 40 or presence of ascites. Of the remaining 376 patients, 99 patients were recruited sequentially as they attended HIV clinic during the study time period and response rates in those approached to take part in the study were > 95%.

Characteristics of patients recruited (n = 99) were compared to those not approached to take part (n = 274) to assess potential for recruitment bias. There were no differences in those recruited by sex: 80.8% (80/99 recruited) *vs* 78.5% (215/274) not recruited were male; P = 0.41, total length of DDI exposure (mean 4.1 years recruited *vs* 4.1 years not recruited; P = 0.89), most recent median ALT (34 μ/L recruited *vs* 35 μ/L not recruited; P = 0.85) or most recent median platelets (210 recruited *vs* 210 not recruited; P = 0.09). However a larger proportion of those of white ethnicity were recruited (77% *vs* 64% P = 0.02) and of those they had a slightly older mean age (50 years *vs* 48 years; P = 0.001).

Of the 99 who took part in the study, 75 (75%) were men who have sex with men (MSM). Mean age was 50 years (range: 30 to 70), 76% were White, 19% Black and 5% were of another ethnicity. The majority had well-controlled HIV infection with a median CD4 count of 637 mm^3 (IQR: 254 to 1378) and 92% had a suppressed HIV VL at < 40 copies. All were on ART and had been for a median of 15 years (IQR: 13 to 16 years). None were currently on a DDI containing ART regime. Median cumulative time previously on DDI was 43 mo (IQR: 22 mo to 68 mo). Overall, 43 (43%) patients reported never drinking alcohol or consuming less than 2 units monthly. Only 2 patients scored > 6 on the MAST score indicating hazardous drinking.

The screening algorithm is shown in Figure 1. ALT above the upper limit normal (ULN) (19 IU/mL in women and 31 IU/mL in men) was found in 37% (n = 7/19) of women and 50% of men (n = 40/80). Median ALT in men was 32 μ/L (IQR 23-44) and 18 μ/L in women (IQR 15-22). Eight-two (82%) had a valid TE reading (IQR/score ratio < 0.3 and success rate > 60%). Of these, 73 (73%) were < 7.65 kPa, 5 (6%) between 7.65-9.4 kPa and 4 (4%) > 9.4 kPa (Figure 1). Only one subject had platelets < 120 and they were known to have cirrhosis of the liver at study entry.

Ultrasound assessment was offered to 49 patients (49%) based on TE reading and/or ALT result. All those that met the criteria for ultrasound were screened for autoimmune liver disease and thrombophilia with a coagulation screen. Four patients did not attend for ultrasound. The most common abnormality was increased reflectivity indicating fatty filtration in 8 patients (18%). A further 4 patients had a normal liver ultrasound but were offered a TJLB on the basis of their ALT and TE score. In total 17 met criteria for TJLB of whom 12 accepted. The characteristics of these patients are described in Table 1. There were no complications observed from the TJB procedures. In the 5 who did not accept liver biopsy, ultrasound appearances were normal in 2 subjects, indicative of fatty infiltration of the liver in 2 and demonstrated splenomegaly in one. Two had abnormal FS readings > 7.76 kPa.

Overall, the commonest histological finding on liver biopsy was steatosis (n = 5) or normal architecture (n = 4). All subjects had HVPG < 6 mmHg (n = 11) including the 2 patients with previously undiagnosed NRH on biopsy in-keeping with a pre-sinusoidal component. This gives a prevalence of previously undiagnosed NRH in our cohort of 2% (95%CI: 0.55%, 6.8%).

As a sensitivity analyses we applied our study algorithm to two other patients attending the HIV clinic with previously identified DDI related liver disease. One

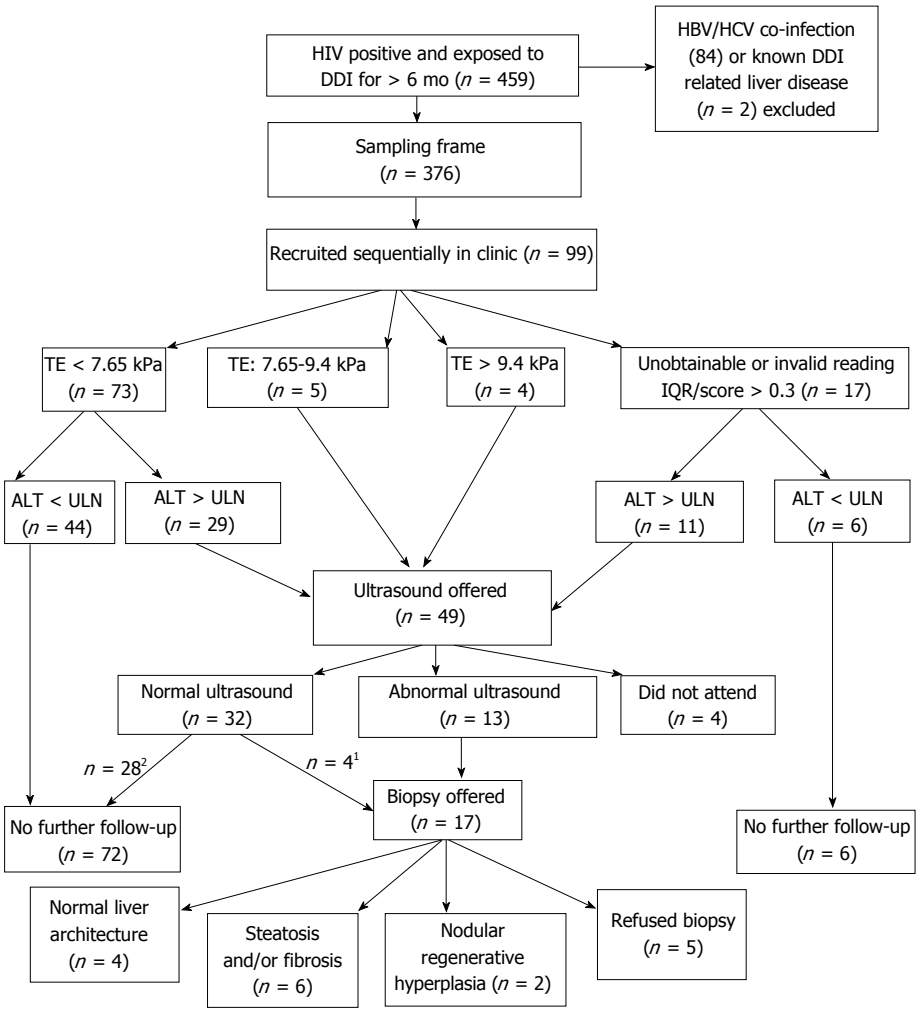

Figure 1 Screening algorithm to detect Didanosine related liver disease in human immunodeficiency virus positive patients previously exposed to Didanosine for > 6 mo. [1]ALT > upper limit normal (ULN) or platelets < lower limit normal (LLN) or TE > 7.65 kPa; [2]ALT < ULN and platelets > LLN and TE < 7.65 kPa. TE: Transient elastography; HIV: Human immunodeficiency virus; DDI: Didanosine; ALT: Alanine transaminase; HBV: Hepatitis B virus; HCV: Hepatitis C virus; IQR: Interquartile range.

case had been identified due to complications of portal hypertension and the other from liver biopsy undertaken due to abnormal ultrasound scan (U/S) appearances. Both cases met our study screening criteria to proceed to TJLB indicating they would have been detected using our screening process.

DISCUSSION

NRH largely presents with complications associated with NCPH after a prolonged asymptomatic period[1-6,9,12]. Although the aetiology may be multi-factorial, an over-riding association is use of DDI. A recent study also identified an association between single-nucleotide polymorphisms in the 5′-nucleotidase and xanthine oxidase genes and development of NCPH after DDI exposure[13] suggesting an element of genetic predisposition *via* the purine metabolism pathway.

Our study is the first to use a screening strategy to identify asymptomatic individuals with a previous DDI exposure but with no known liver disease at study entry. Such a strategy is important to identify liver disease at an earlier stage so that preventative measures and risk minimisation strategies may be instituted. Using our screening strategy we found 2 cases of previously undiagnosed NRH, but no cases of NCPH. Our strategy was based on TE, platelets and ALT levels, with ultrasound and subsequent TJLB in those who met criteria. We used a combination of methods to improve sensitivity of the screening strategy. Elevated transaminases, low platelets and moderate elevations in TE readings have all been described in known cases of NRH or NCPH. We chose a low TE cut off of 7.65 kPa as one study reported a median FS value of 7.9 kPa in subjects with biopsy confirmed NRH[10]. In addition in order to identify individuals with very low-level transaminase elevations we used a ULN cut off of 19 IU/mL in women and 31 IU/mL in men, contrasting with the ULN of 41 μL/L used in previous studies[2].

A recent multi-centre cohort of DDI-associated NCPH in HIV-infected adults identified thrombocytopenia, splenomegaly and elevated aminotransferases and alkaline phosphatase as significantly associated with NCPH[14]. The authors suggest a screening algorithm for NCPH consisting of DDI exposure or splenomegaly plus either

Table 1 Characteristics of those with liver disease identified as a result of study screening

Subject number	Gender	Age (yr)	Ethnicity	Prior DDI exposure (mo)	Time since DDI (yr)	BMI (kg/m^2)	MAST score	ALT (IU/mL)	PLTs (10^6/L)	PTT	Alk Phos (U/L)	FS (kPa)	Ultrasound results	Biopsy results	HVPG	Fib 4
Participants who accepted to undergo TJLB as a result of study screening																
1	Male	43	White British	81	7	25.58	0	77	175	14.0	83	10	Splenomegaly	NRH with mild steatosis	5	1.29
2	Male	59	White British	40	6	24.42	0	36	175	14.8	93	8	Normal	NRH	4	2.39
3	Male	48	White British	9	6	29.76	0	32	184	14.3	108	NR	Fatty liver, Splenomegaly	Moderate steatosis	3	1.08
4	Male	52	White British	70	7	24.69	0	50	242	11.7	44	5.6	Fatty liver	Mild steatosis	3	0.79
5	Male	49	White Other	34	4	28.54	1	60	215	14.0	83	12.6	Fatty liver	Moderate steatosis, moderate fibrosis	3	1.38
6	Male	52	White British	21	11	28.67	1	58	257	13.4	43	NR	Fatty liver	Moderate steatosis	Not done	1.02
7	Male	62	White Other	13	13	32.56	2	56	175	11.1	45	NR	Fatty liver	Moderate steatosis	2	2.25
8	Male	50	White British	117	3	31.18	0	125	204	10.7	58	8.7	Fatty liver	Mild fibrosis with mild steatosis	2	1.71
9	Female	56	Black African	24	11	36.96	0	27	208	12.3	67	4.4	Coarse echotexture	Normal Architecture	5	1.07
10	Male	57	White British	61	4		0	40	190	13.9	71	NR	Splenomegaly	Normal Architecture	3	1.74
11	Male	51	White Irish	103	4	20.30	0	56	228	10.8	154	4.8	Coarse echotexture	Normal Architecture	3	1.04
12	Male	52	White Other	44	5	21.07	6	24	224	11.4	62	9.1	Normal	Normal Architecture	2	1.16
Participants who refused TJLB offered as a result of study screening																
13	Male	46	White British	74	2	21.46	0	18	177		60	10	Normal	Declined		1.41
14	Male	41	White British	97	3	25.65	0	63	188		101	7.6	Splenomegaly	Declined		1.30
15	Male	47	White British	33	5	24.39	0	34	238		58	7.1	Fatty liver, dampened waveform	Declined		0.99
16	Male	61	White British	110	2	20.75	1	35	246		71	9.2	Normal	Declined		1.39
17	Male	54	White British	28	5	28.93	1	32	333		58	4.3	Fatty liver	Declined		0.67

DDI: Didanosine; BMI: Body mass index; MAST: Michigan Alcoholism Screening test; ALT: Alanine transaminase; PLT: Platelet count; FS: FibroScan; TJLB: Trans-jugular liver biopsy.

a raised serum aminotransferase or thrombocytopenia or raised alkaline phosphatase as a trigger for further assessment. Using this study's algorithm only one third of our cohort would have been offered further investigation and one of the two cases of NRH identified in this study prior to development of NCPH would have been missed. Furthermore, splenomegaly is not uncommon in HIV positive patients[16].

We opted to use TE together with ALT and platelet counts on the basis of ready availability of blood tests and the ease of use of FS in the outpatient ambulatory setting. The most common histological abnormality we found on liver biopsy was steatosis, in association with fibrosis in 2 cases. We are unlikely to have missed cases though cannot exclude NRH in those who declined biopsy, but is unlikely that they had underlying NRH in a greater frequency that that seen in patients who did agree to undergo biopsy. Whilst DDI-associated NRH and NCPH is a serious condition with potentially life-threatening complications, this prospective study suggests a relatively low prevalence in treated cohorts. A previous study has identified non-alcoholic steatohepatitis (NAFLD) as a significant cause of unexplained serum aminotransferase elevation[12] in HIV positive people on ART and we also showed a significant rate of hepatic steatosis in association with hepatic fibrosis in our patients.

In this cross sectional study, we found a low prevalence of previously undiagnosed DDI-associated NRH using a screening strategy that combines TE, serum aminotransferase and platelet measurements followed by an U/S. We did, however, demonstrate a higher prevalence of NAFLD, which requires active management to address risk factors and prevent progression to fibrosis in HIV-positive patients.

ACKNOWLEDGMENTS

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COMMENTS

Background

Human immunodeficiency virus positive patients are at increased risk of liver disease. This is multifactorial and includes co-infection with hepatitis viruses, prescribed and recreational drug use and alcohol. The anti-retroviral drug Didanosine (DDI) has been implicated in the aetiology in some patients, particularly if the type of liver damage is nodular regenerative hyperplasia (NRH) or a patient has non-cirrhotic portal hypertension.

Research frontiers

The authors used a combination of liver enzyme level (with a lower upper limit of normal) and transient elastography (TE) (which measures the liver stiffness) as an initial screen of patients exposed to DDI. This highly sensitive approach identified 42% who required further investigation with an ultrasound scan and 17% who subsequently were offered a transjugular liver biopsy. The authors, therefore, believe that the prevalence rate of 2% NRH in this DDI exposed asymptomatic cohort is accurate.

Innovations and breakthroughs

The prevalence study is the first to systematically screen asymptomatic patients exposed to DDI. Other groups have looked at the association between the drug and liver disease but have not screened a large cohort of exposed but asymptomatic patients to establish a prevalence of disease.

Applications

Use of a simple screening strategy in patients previously exposed to DDI will allow clinicians to identify liver disease which if left undiagnosed may present with the complications of portal hypertension such as variceal bleeding.

Terminology

TE: A technique combining ultrasound waves and a pressure transducer to assess the stiffness of the liver. This has been validated as a tool to measure liver fibrosis and steatosis; NRH is characterized by small (less than 3 mm) regenerative nodules in the absence of fibrous septa on biopsy. The nodules cause obliteration of the portal veins which leads to portal hypertension.

Peer-review

It is an interesting study.

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ORIGINAL ARTICLE

Observational Study

Enzyme pattern of biliary colic: A counterintuitive picture

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Abstract

AIM

To evaluate the diagnostic value of serial biochemical blood tests in the diagnosis of biliary colic.

METHODS

Files were reviewed of 1039 patients who were admitted to the Share'e Zedek Medical Center emergency department between the years 2012-2013, and received the coding of acute biliary disease. Of these, the first 100 cases were selected that met the following criteria: (1) a diagnosis of biliary colic or symptomatic cholelithiasis; (2) at least two biochemical blood tests performed; and (3) 18 years of age or older. Patients with other acute biliary diseases were excluded. The biochemical profile of the patients was analyzed as were their clinical and radiological findings.

RESULTS

Three-quarters of the patients were women, whose average age of 37 years was younger than the average of the men, at 50 years. According to their histories, 47% of the patients had previously known cholelithiasis. Pain in either the right upper quadrant or the epigastrium was the presenting symptom in 93% cases. The greatest change in serum biochemical results was seen during the first day of the patients' admissions. Alanine aminotransferase (ALT) showed the highest initial rise above the reference range, followed by aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), bilirubin and alkaline phosphatase (ALKP) - all these increases were statistically significant ($P < 0.05$). AST showed the sharpest decline followed by bilirubin and ALT. GGT and ALKP did not fall. A sharp rise and fall in liver enzymes, especially during the first day, most prominently in AST and ALT, was seen in 70% percent of cases. In 65% of cases trans-abdominal sonography did not give diagnostic findings.

CONCLUSION

Serial serum liver enzyme measurements are helpful in the initial diagnosis of acute biliary colic.

Key words: Biliary colic; Symptomatic cholelithiasis; Gallstones; Liver enzymes; Aspartate aminotransferase; Alanine aminotransferase; Enzyme pattern; Diagnostic tool; Emergency department

Core tip: Gallstones are prevalent in affluent countries, more so in women than in men, and their prevalence increases with age. A large proportion of patients presenting to the emergency department with epigastric or right upper quadrant (RUQ) pain present a diagnostic challenge, especially when they belong to the older age group. We found that serial liver and biliary enzyme measurements reveal a characteristic pattern that helps the clinician determine quickly, cheaply and safely that the cause of RUQ/epigastric pain is biliary colic, in 71% of the patients. Serial enzyme testing is a useful adjunct to other diagnostic tools, for the diagnosis of acute upper abdominal pain.

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INTRODUCTION

Gallstones are prevalent in affluent countries, more so in women than in men, and their prevalence increases with age[1]. Gallstones usually produce symptoms when they migrate into the cystic duct or common bile duct (CBD), causing obstruction that increases intraluminal pressure and distends the viscus. The most characteristic symptom of gallstone disease is biliary colic[2], *i.e.*, pain arising from the cystic duct or CBD.

A patient presenting to the emergency department (ED) complaining of epigastric or right upper quadrant (RUQ) pain, with a classic history and physical examination compatible with biliary colic does not present a great diagnostic challenge. A large proportion of patients presenting to the ED with epigastric or RUQ pain do present a diagnostic challenge, especially when they belong to the older age group (in which both a wide array of underlying diseases and polypharmacy are prevalent) which has become more common in Western society over recent decades[1]. Unfortunately, "classic" cases are the exception rather than the rule[3,4]. The differential diagnosis in an older patient with many co-morbidities includes: An acute coronary syndrome, pericarditis, an aortic dissection, peptic ulcer disease including a perforated ulcer, pulmonary embolism, lower lobe pneumonia, renal colic, pyelonephritis, partial colonic obstruction, diverticulitis, appendicitis, pancreatitis, acute cholecystitis, cholangitis, diabetic ketoacidosis, porphyria and biliary colic[2]. The diagnostic pathways are not straightforward, consume time and resources and are subject to pitfalls and misleading findings.

The confirmatory diagnostic test for biliary colic in the ED is the demonstration of gallstones in the cystic duct or CBD by trans-abdominal sonography[4-6]. However the demonstration of cholelithiasis alone is not diagnostic[1], and unfortunately, the demonstration of gallstones in the cystic duct or CBD is very difficult even for an experienced radiologist under optimal conditions, namely in a lean, cooperative and fasted patient, when time is not limited. In practice, it is often very difficult to demonstrate gallstones in the cystic duct or CBD[7]. Due to these limitations, and in the majority of cases, ultrasonography is usually not diagnostic in the first hours or days of the patient's admission, and a negative ultrasound scan may be misleading[1,5,7-9]. A simple, rapid, cheap, non-invasive, reproducible and reliable test is needed to diagnose biliary colic quickly and efficiently.

Laboratory testing of serum "hepatocellular" and cholestatic liver enzymes [namely: Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and alkaline phosphatase (ALKP) and gamma-glutamyl transferase (GGT), respectively] is performed almost routinely in patients presenting with epigastric or RUQ pain, as the clinician searches for diagnostic patterns. For example: A patient with or without pain who has elevated "cholestatic" enzymes, ALKP and GGT, is considered a potentially "surgical" patient who is suffering from a chronic mechanical obstruction of the biliary tract. And indeed, it is rare to find such an obstruction without elevation of these enzymes[10]. In this type of obstruction, the hepatocellular enzymes, AST and ALT, will be only mildly elevated. Conversely, a patient with RUQ fullness, malaise, and AST and ALT in the hundreds and even thousands of international units per liter (IU/L), with or

without jaundice, and with only mildly elevated ALKP and GGT is considered a "medical" patient, who is suffering from viral, autoimmune or drug-induced hepatitis. These patterns that have served so well for so many years represent entrenched dogma.

We and others[11-13] have also observed another distinct pattern of a sharp (up to 100-fold above the upper limit of the normal reference range), short-lived (usually less than a week) rise in AST and ALT, and only a mild rise in ALKP, bilirubin and GGT, which we think is characteristic of acute "biliary colic". Our clinical impression is that this pattern has high specificity for the diagnosis of "biliary colic" especially in the first hours of the patient's admission, which is paradoxically counterintuitive in a patient, who has "surgical" pain with a "medical" enzyme pattern.

Our observations are mentioned (unreferenced) in the two latest editions of the leading textbooks in internal medicine, the leading textbook in gastroenterology as well as in a respected textbook on laboratory tests[2,8,14,15]. It is noteworthy that this pattern is not mentioned in the latest versions of two leading textbooks in general surgery[4,6].

Our current study will examine the existence and the utility of this paradoxical "biliary colic" enzyme pattern in our own patient population.

MATERIALS AND METHODS

Study design and patients

This was an observational retrospective chart review, which was approved by the Medical Center's Helsinki committee (number: p 92/13). We reviewed the medical records of 1039 patients, who were admitted to the Share'e Zedek Medical Center ED between December 1st 2013 and January 1st 2012, and who were assigned the coded diagnosis of "acute biliary disease". Inclusion criteria were: (1) 18 years of age and older; (2) two or more blood tests including a liver profile; and (3) a diagnosis of biliary colic or symptomatic cholelithiasis. Symptomatic cholelithiasis was also included as it was clear that this term was used interchangeably with biliary colic. It was used to differentiate this entity from acute cholecystitis. Exclusion criteria were: (1) acute cholecystitis; (2) ascending cholangitis; (3) ultrasound-confirmed choledocholithiasis; and (4) sepsis. The search for cases meeting the inclusion and exclusion criteria was closed once the first sequential 100 suitable patients (out of 1039 during 23 mo) were identified. The case acquisition flow chart is shown in Figure 1.

Data were collected from the Medical Center computerized database. Clinical data were derived from the either the ED file (if the patient was not hospitalized) or the hospital chart. Laboratory data were collected from the computerized laboratory records. Laboratory tests were performed using the standard techniques used for all biochemical tests at the hospital. Ultrasound interpretations were retrieved from the diagnostic imaging computerized database in written or dictated format.

Primary and secondary end points

The primary variables we examined were the serum biochemical laboratory results, including: Bilirubin, ALKP, AST, ALT and GGT, throughout the patient's stay in the ED or hospital, relating to the particular ED visit with biliary colic.

The secondary variables examined were the location of the patient's pain, radiation of the pain, whether it was post-prandial, and associated symptoms such as anorexia, nausea, vomiting, fever and chills, and physical examination findings. The interpretation of the radiologic tests performed was also evaluated.

Statistical analysis

Statistical analysis was performed by Tali Bdolah-Abram from The Hebrew University, Jerusalem, Israel. Paired *t*-tests as well as non-parametric Wilcoxon signed-rank tests were used to assess the differences between pairs of quantitative variables. The one-sample *t*-test was used to test the significance of percent changes between two measurements. Repeated measures ANOVA models were applied to quantitative variables in order to simultaneously test trends over time, the difference between subgroups of patients and the interaction between time and group. The significance of the trends and the interactions were tested using the Greenhouse-Geisser test. The Friedman non-parametric test was used for testing a trend over time for quantitative variables when the data was not normally distributed.

All tests applied were two-tailed, and a *P*-value of 5% or less was considered statistically significant.

RESULTS

Demographic characteristics

The basic demographic characteristics of the 100 cases studied are presented in Table 1. The mean age of the patients in our study was 40 years, 76% of cases were women. On average, the women were younger than the men with mean ages of 37.4 years and 50.0 years respectively. In 47% of cases, cholelithiasis was already known from the patient's history.

Clinical characteristics

Clinical presentations were extracted from the patients' files and are reported in Table 2. The clinical variables analyzed were: Location of pain and tenderness, presence of peritoneal irritation, Murphy sign, fever, chills, nausea or vomiting and whether the pain was post-prandial. The location of the pain was noted to be in the RUQ or epigastrium in the large majority of cases (92.8%).

Analysis of blood biochemistry tests

The primary endpoint of our study was the result of the analysis of the serial blood biochemistry laboratory tests, notably: Bilirubin, AST, ALT, ALKP and GGT. All tests were done according to the standard routine of the hospital laboratory. We retrieved the sequential laboratory results

Table 1 Demographic characteristics

Age (yr)	
Mean ± SD	40.5 ± 19.1
Median	37
Gender (#)	
Men	24
Women	76
Age by gender (yr)	
Men	
Mean ± SD	50.0 ± 17.0
Median	49
Women	
Mean ± SD	37.4 ± 18.8
Median	31.5
Cholelithiasis known previously (from history)	
Yes	47
No	53

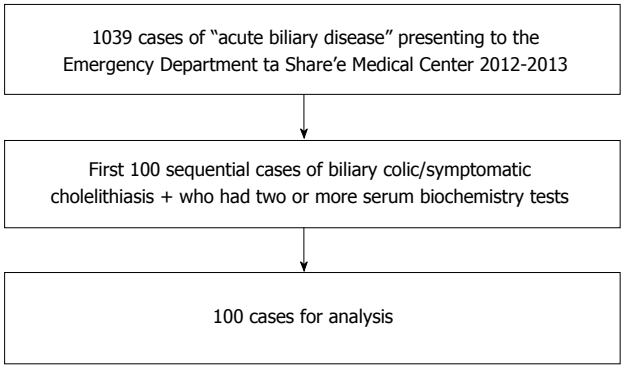

Figure 1 Flow chart illustrating case selection process. Of 1039 patients who presented to the emergency department (ED) of Share'e Zedek Medical Center during 23 mo (2012-2013) with various forms of acute biliary disease, 100 who were designated in the ED as having Biliary Colic and who fulfilled study criteria, were selected sequentially for analysis of concomitant serum bilirubin and enzyme temporal patterns.

for the 100 cases of interest, and analyzed the results from up to four sequential tests in each patient. Some patients had more than four tests done, but as this subset was small, we limited our analysis to four tests. The goal of the study was to differentiate which enzyme variable was most significantly indicative of the clinical event, namely biliary colic. To facilitate comparison between the four enzyme patterns, we normalized the data as percent changes per hour. For example, a change in AST from 50 to 150 units over a 10 h interval, would calculate to a change of 10 units per hour, representing a 20% per hour increase from 50 units. Figure 2 shows the time intervals between the sequential measurements tests, which allows a visual appreciation of the time course of the changes in enzyme levels. The average time between tests grew longer from the first to the last test, at 15.8, 25.3 and 29.8 h, respectively.

We compared changes in all five primary variables to one another at three different points, *i.e.*, percent changes per hour between tests 1 and 2, 2 and 3, and 3 and 4, as is shown in Figure 3.

It can be readily seen that the largest percent changes in enzyme levels, which were also statistically significant,

Table 2 Clinical features, *n* (%)

Pain location	
RUQ	41 (42.3)
Epigastric	43 (44.3)
RUQ and/or epigastric + other region	6 (6.2)
Not RUQ or epigastric	7 (7.3)
No data	3
Fever	
Yes	2 (2.1)
No	95 (97.9)
No data	3
Nausea	
Yes	28 (60.9)
No	18 (39.1)
No data	54
Vomiting	
Yes	20 (31.7)
No	43 (68.3)
No data	37
Tenderness to palpation	
RUQ	47 (49.0)
Epigastric	20 (20.8)

RUQ: Right upper quadrant.

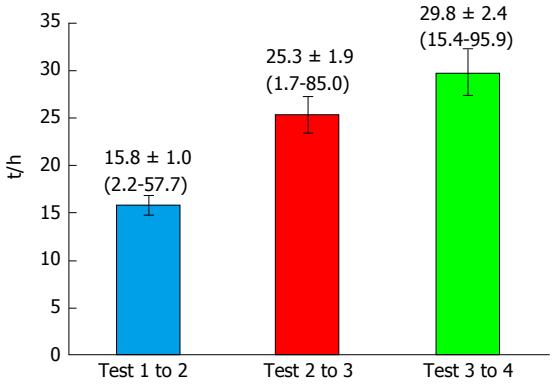

Figure 2 Time intervals between serological liver tests (hours). Time interval data in hours, are shown as means. Means ± SDs, and ranges (*i.e.*, minima to maxima), above the bars.

were seen between the first and second tests, and that ALT changed the most followed by AST, GGT, bilirubin and ALKP. Between tests two and three, the effects were far smaller with AST being the only variable with greater than one percent change. Between tests three and four effect percent changes were of similar magnitude to the changes between test two and three. AST still showed the largest absolute change, but this was smaller than between the previous tests, at 1.2%.

The enzyme pattern

In order to demonstrate the enzyme pattern we plotted the percent change over time as seen in Figure 4. As this is a retrospective study and not protocol-driven, blood was drawn for testing at different intervals in each case. Therefore, the time between tests is represented as the average time interval between tests. To permit a graphic presentation of the different enzyme patterns, the hourly percent changes are multiplied by the corresponding

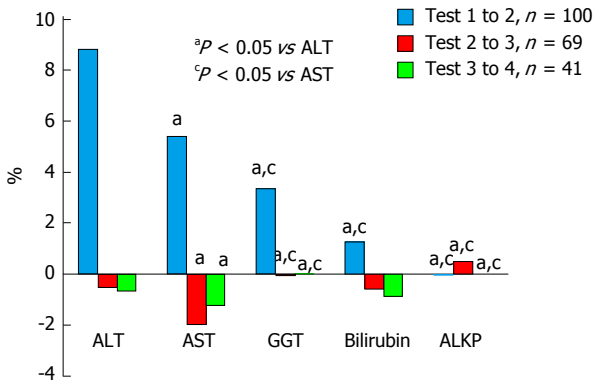

Figure 3 Comparison of relative percent changes (per hour) in bilirubin and liver enzymes, between serial tests. Percent change per hour is shown for these five variables. [a,c]Denote significant statistical difference from ALT and AST respectively in the same time frame (*i.e.*, ALT between test one and two compared with AST between test one and two). The number of cases in the analysis in the different time frames is shown on the top right. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; ALKP: Alkaline phosphatase.

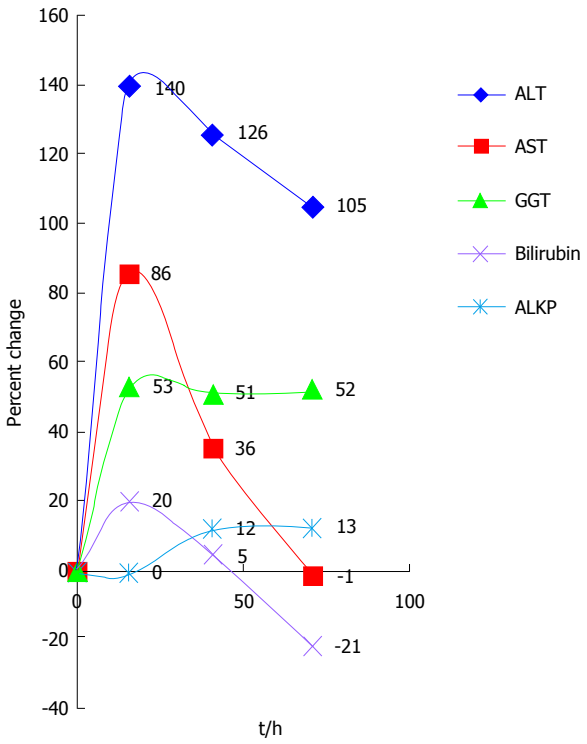

Figure 4 Time trends in percent changes of bilirubin and liver enzymes with serial testing. Time between tests is represented using the average time interval between tests (as seen in Figure 2). The hourly percent change between each test was multiplied by this interval, to provide a graphic presentation of the enzyme patterns. The intersection of the axes represents the first test and the following points represent each change from the previous result. For example, bilirubin increased by 20% between tests one and two and decreased by 15% between tests two and three; therefore, the next point is 5%. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; ALKP: Alkaline phosphatase.

average time intervals.

As shown above, the largest initial change was in ALT, at 140% between the first and second tests. AST rose less dramatically (86%) but showed the most dramatic average fall (50%). GGT rose initially by 53% but then hardly changed at all, while bilirubin and ALKP showed only minor fluctuations.

To determine how many of the 100 patients studied presented with the aforementioned enzyme pattern that is characteristic for biliary colic, we devised two criteria to separate patients into those either positive or negative for the pattern; positivity for either criterion was considered a characteristic pattern.

The first criterion was that a patient had at least doubled the levels of AST or ALT between the first two tests, compared to any subsequent test. The second criterion was that a patient had at least halved the first test results for AST or ALT in any one of the following three tests. Additionally, to be counted positive for the second criterion, only patients who had a first test result of more than double the upper limit of normal (ULN) for AST or ALT were counted. We used more than twice the ULN of normal as a cut-off as this has been used for many years in the field of chronic hepatitis B surveillance and in treatment algorithms (between 1-2 times the ULN is considered minimally raised)[16,17]. ULN for AST and ALT in our medical center are 36 and 52 units, respectively.

We found 33 (33%) patients positive for the first criterion (of a doubling in aminotransferases) all of whom showed a rise in ALT and 18 (54.5%) of whom had a rise in AST. Fifty-two (52%) patients were positive for the second criterion (of a significant subsequent fall in enzymes) - all had a fall in AST and 14 (26.9%) of whom also showed a fall in ALT. All told, 71 (71%) patients were positive for either one or the other of the criteria and 14 (57.6%) out of the 71 were positive for both criteria.

Ultrasonography findings

Trans-abdominal ultrasonography was performed in all but one case, a woman who had had sonography performed the previous day, when she was found to have gallbladder stones. The sonography reports given by the hospital radiologists, in either written or dictated form, were reviewed. We divided the findings into two categories, namely (1) highly suggestive or compatible with a cause for biliary colic; and (2) questionable or possibly non-contributory to the diagnosis of biliary colic. The results and the categorization of the different findings are displayed in Table 3. As can be seen, in only a third of the cases were the sonographic findings highly suggestive or compatible with a cause for biliary colic. In other words there was definite, albeit somewhat indirect, evidence of CBD disease. In the remaining 64 cases, whereas there was no evidence of CBD disease, it is conceivable, nonetheless, that gallstones could have migrated from the gallbladder in 62 cases without causing visible CBD injury.

DISCUSSION

Biliary colic is a common symptom that is defined as

Table 3 Ultrasound findings

	Frequency
Non contributory	64
Contracted gallbladder	2
Cholelithiasis	58
Contracted gallbladder + cholelithiasis	4
Compatible or highly suggestive	35
Dilated CBD	5
Dilated CBD + filling defect	1
Thickened gallbladder wall	6
Distended gallbladder + cholelithiasis	13
Distended gallbladder	2
Stone in cystic duct	4
Distended gallbladder + pericystic fluid	2
Thickened gallbladder wall + cholelithiasis	2
Total valid	99
Missing	1
Total	100

CBD: Common bile duct.

pain caused by spasm of the cystic duct or CBD, usually caused by intraluminal calculi that have migrated from the gallbladder, or occasionally that form *in situ*[1]. Patients diagnosed with biliary colic present with symptoms of RUQ and/or epigastric pain. In some cases, diagnosis is straightforward but in some the differential diagnosis is wide and the evaluation is time and resource-consuming and occasionally potentially harmful.

Trans-abdominal sonography is readily available, but has low sensitivity and a low negative predictive value for choledocholithiasis. Indeed, in our study all but one of the patients were finally diagnosed as having biliary colic due to the passage of a stone through the CBD despite, the fact that choledocholithiasis was not proven directly in 99% of them. Ultrasonic findings, *i.e.*, CBD abnormalities, were compatible or highly suggestive of biliary colic, in only 35% of the patients. But even in this minority, sonography did not clinch the diagnosis, since there seemingly was enough uncertainty to prevent the clinician from discharging the patient or referring him/her for laparoscopic cholecystectomy without additional tests. The same is truer for those 62 patients with typical biliary colic in whom cholelithiasis was present, but there was no visible CBD abnormality. The ED staff in Galveston, Texas, made similar observations on the utility of ultrasound scans, and this led to overuse of computed tomography scanning, especially at night[18].

Jafari *et al*[7] also point out that passage of stones through the CBD can be fast, so that by the time the patient is transported to the radiology department the diagnostic picture - a dilated CBD containing a stone, can be missed. They advocate that ultrasound scans should be performed in the ED, and for CBD diameter to be measured routinely. It is in this situation that the characteristic enzyme elevation and fall pattern comes into its own.

As opposed to these limitations of ultrasound scanning, we found that serial liver and biliary enzyme tests (2-4 tests performed during the first 80 h from admission) increase and decrease with statistical significance, and reveal a characteristic liver enzyme pattern that helps the ED clinician determine quickly, cheaply and safely, in concert with simultaneous other diagnostic maneuvers, that the cause of RUQ/epigastric pain is biliary colic. Thus, our findings confirm and extend what is already known. We assume that in most of the 29% of patients in whom the characteristic temporal pattern was not seen, the first blood sample was taken too late for the sharp rise and fall to be fully appreciated.

The most statistically significant changes in the variables we examined were seen between the 1st and the 2nd test. The most prominent effect is seen with ALT followed by AST. However, GGT and ALKP, the classic "obstructive" biliary enzymes, and bilirubin, contribute little, if at all, to the diagnosis. This finding is compatible with our assumption that ALT and AST, the "medical" hepatocellular enzymes, are deranged early in biliary colic, for which the most plausible hypothesis is that high biliary pressures lead to impairment of bile secretion and retention of bile acids with accompanying hepatocyte apoptosis, necrosis or leakage of enzymes[19]. The classic rise in the "biliary" enzymes, alkaline phosphatase and GGT, in prolonged obstruction is thought to reflect increased enzyme synthesis rather than cholangiocyte damage and leakage[20]. Moreover, the greatest changes in enzyme levels occur early and up to the first 24 hours of the patient's admission, which makes this a diagnostic tool particularly useful in the ED setting.

In our study many patients were seen to have both the rise and the fall of hepatocellular enzymes, unlike the findings in two earlier studies that showed mainly the down-sloping phase of the temporal enzyme pattern[12,21]. This can be explained by lesser availability of liver enzyme testing in the late 1980s, when these studies were published. And indeed, in a later study, published in 2010, an enzyme pattern almost identical to ours was found, in which both the ascending and descending phases were detected. This latter study was performed on subjects in whom choledocholithiasis was proven invasively, which, admittedly, is a different and far less common situation[22].

The pattern of a short term (less than a week), sharp rise and sharp (but to a lesser degree) fall in ALT and AST is classically recognized in two clinical scenarios, namely ischemic liver injury - so-called Hypoxic Hepatitis - and acetaminophen intoxication[23-25]. It is our opinion that this pattern is also typical of a third clinical scenario - biliary colic due to passage of a biliary stone that causes transient biliary obstruction.

Our impression is that this pattern is not widely appreciated by general clinicians although it is mentioned in several articles and in some leading textbooks[2,8,12-15,21-22,26]. One explanation for this pattern's relative anonymity is the dogma that elevated cholestatic enzymes mean biliary obstruction has served so well for prolonged biliary obstruction. The corollary has been assumed, that all biliary obstruction is accompanied by a cholestatic enzyme pattern. This is clearly not the case, as transient

biliary obstruction due to passage of a stone through the CBD causes elevation of ALT and AST - the medical or hepatocellular enzymes.

Limitations of this enzyme temporal analysis include the fact that this is a retrospective study, with its unavoidable selection bias, and that the exact timing of the clinical event, *i.e.*, the passage of the CBD stone, is unknown. Hence, the appropriateness of the time of first blood drawing is also uncertain. A third limitation of this study is that we only included patients who had two or more blood tests performed, which introduces further selection bias of patients who had initial enzyme elevations. Irrespective, the more prominent abnormal enzymes were the hepatocellular. Another bias is that our subjects were already diagnosed as suffering from biliary colic. It remains to be tested, therefore, whether the characteristic "biliary colic" pattern will be useful in the more real life scenario of a patient presenting to the ED with abdominal pains. We hope to be able to resolve this question in a prospective study, in which all patients presenting with abdominal pain will undergo serial liver enzyme measurement at protocol-defined intervals, along with a precise history of the time of onset of the abdominal pain. Finally, although we were careful to exclude cases of cholecystitis, it must still be acknowledged that aminotransferase elevation occurs in active gallbladder inflammation, usually associated with fever and leukocytosis (both absent in our 100 cases) and of slower resolution than seen in our cases. In gallbladder inflammation, the mechanism of aminotransferase rise is usually attributed to cytokine release and other mediators of the Systemic Inflammatory Response Syndrome[27].

Conclusions and practical implications

Based on a retrospective statistical analysis of 100 patients who were diagnosed as having biliary colic - which is defined as upper abdominal pain due to passage of a gallstone through the cystic duct or CBD, we found that a sharp, short term rise and fall of ALT and AST, typically thought to be indicative of ischemic hepatic injury and acetaminophen intoxication, is also typical of biliary colic. Whereas the current observations are not entirely novel, they are worth emphasizing and bringing to general attention as they do not appear to be widely appreciated. Thus, in the ED, when a patient presents with an appropriate history and physical exam, we recommend adding liver enzymes to the list of blood tests already ordered. If liver enzymes are found to be elevated, we recommend repeating these tests twice or thrice at intervals over the next 24 h. If the pattern that is characteristic of biliary colic is seen, concomitant with the resolution or even only marked improvement of the pain, and no other diagnosis is suspected (*e.g.*, an acute coronary event, pancreatitis, cholecystitis, *etc.*), then the patient can be discharged home or admitted for laparoscopic cholecystectomy according to local practice[28].

ACKNOWLEDGMENTS

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COMMENTS

Background

Patients complaining of abdominal pain are frequently seen in the emergency department. The differential diagnosis is vast and therefore the clinical workup is resource and time consuming. However, some clinical observations led us to the thought that a few cheap and routine blood tests could greatly aid the clinician in this diagnostic challenge.

Research frontiers

A few studies, mainly from the 1980's and 1990's described the enzymatic profile of biliary colic and reached. However this very useful profile did not become common knowledge.

Innovations and breakthroughs

In recent years more patients were tested for liver enzymes as part of the routine workup of acute abdominal pain. Additionally many patients had more than one blood sample taken. This has allowed us to follow the level of liver enzymes and observe a unique pattern.

Applications

This pattern helps clinicians in the clinical scenario of acute abdominal pain diagnosis. It is cheap, rapid, readily available, accurate and non-invasive.

Terminology

Biliary colic is a term used to describe pain arising from stones obstructing the cystic duct or the passage of a stone through the common bile duct.

Peer-review

This manuscript provides the updated evidence to the readers. The topic is an important one and deserves a practical value.

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SYSTEMATIC REVIEWS

Isolated bilateral Tapia's syndrome after liver transplantation: A case report and review of the literature

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Abstract

AIM

To describe one case of bilateral Tapia's syndrome in a liver transplanted patient and to review the literature.

METHODS

We report a case of bilateral Tapia's syndrome in a 50-year-old man with a history of human immunodeficiency virus and hepatitis C virus child. A liver cirrhosis and a bi-nodular hepatocellular carcinoma, who underwent liver transplantation after general anesthesia under orotracheal intubation. Uneventful extubation was performed in the intensive care unit during the following hours. On postoperative day (POD) 3, he required urgent re-laparotomy due to perihepatic hematoma complicated with respiratory gram negative bacilli infection. On POD 13, patient was extubated, but required immediate re-intubation due to severe respiratory failure. At the following day a third weaning failure occurred, requiring the performance of a percutaneous tracheostomy. Five days later, the patient was taken off mechanical ventilation and severe dysphagia, sialorrea and aphonia revealed. A computerized tomography and a magnetic resonance imaging of the head and neck excluded central nervous injury. A stroboscopy showed bilateral paralysis of vocal cords and tongue and a diagnosis of bilateral Tapia's syndrome was performed. With conservative management, including a prompt establishment of a speech and swallowing rehabilitation program, the patient achieved full recovery within four months after liver transplantation. We carried out MEDLINE search for the term Tapia's syndrome. The inclusion criteria had no restriction by language or year but must provide sufficient

available data to exclude duplicity. We described the clinical evolution of the patients, focusing on author, year of publication, age, sex, preceding problem, history of endotracheal intubation, unilateral or bilateral presentation, diagnostic procedures, type of treatment, follow-up, and outcome.

RESULTS

Several authors mentioned the existence of around 70 cases, however only 54 fulfilled our inclusion criteria. We found only five published studies of bilateral Tapia's syndrome. However this is the first case reported in the literature in a liver transplanted patient. Most patients were male and young and the majority of cases appeared as a complication of airway manipulation after any type of surgery, closely related to the positioning of the head during the procedure. The diagnosis was founded on a rapid suspicion, a complete head and neck neurological examination and a computed tomography and or a magnetic resonance imaging of the brain and neck to establish the origin of central or peripheral type of Tapia's syndrome and also the nature of the lesion, ischemia, abscess formation, tumor or hemorrhage. Apart from corticosteroids and anti- inflammatory therapy, the key of the treatment was an intensive and multidisciplinary speech and swallowing rehabilitation. Most studies have emphasized that the recovery is usually completed within four to six months.

CONCLUSION

Tapia's syndrome is almost always a transient complication after airway manipulation. Although bilateral Tapia's syndrome after general anesthesia is exceptionally rare, this complication should be recognized in patients reporting respiratory obstruction with complete dysphagia and dysarthria after prolonged intubation. Both anesthesiologists and surgeons should be aware of the importance of its preventing measurements, prompt diagnosis and intensive speech and swallowing rehabilitation program.

Key words: Liver transplantation; Follow-up; Outcome; Postoperative complications; Bilateral Tapia's syndrome

Core tip: Tapia's syndrome is a rare entity characterized by the concomitant extracranial injury of the hypoglossal nerve (Ⅻ) and the recurrent laryngeal branch of the vagus nerve (Ⅹ) at the base of the tongue and the pyriform fossa. Anesthesiologists, surgeons and otorhinolaryngologist should be aware of its presentation at any type of surgery as in the present case, after liver transplantation. The purpose of this study is to present our even rarer presentation of bilateral Tapia's syndrome to the liver transplant community and to review the literature to update the current management and treatment. The most relevant common feature in most cases of bilateral syndrome was orotracheal intubation prolonged for more than 14 d.

Bilbao I, Dopazo C, Caralt M, Castells L, Pando E, Gantxegi A, Charco R. Isolated bilateral Tapia's syndrome after liver transplantation: A case report and review of the literature. *World J Hepatol* 2016; 8(36): 1637-1644 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i36/1637.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i36.1637

INTRODUCTION

Tapia's syndrome was described for the first time by the Spanish otorhinolaryngologist Antonio García Tapia in 1904[1]. It is characterized by the unilateral paralysis of the tongue and the vocal cord caused by extracranial injury to the hypoglossal nerve (Ⅻ) and the recurrent laryngeal branch of the vagal nerve (Ⅹ) at the base of the tongue and the pyriform fossa)[1-6]. Although the Tapia's syndrome refers to the extracranial lesion of the hypoglossal and recurrent laryngeal nerves, some authors also describe a central type of Tapia's syndrome, referring to those patients with the same symptoms, but whose damage has occurred in the nucleus ambiguous, the nucleus of the hypoglossal nerve, and the pyramidal tract in the central nervous system. We describe one case of bilateral Tapia's syndrome in a liver transplant patient, which is not previously reported in the literature.

MATERIALS AND METHODS

We report herein a case of bilateral Tapia's syndrome together with a review of the literature. We carried a literature research in the MEDLINE database through the PubMed search service for the term Tapia's syndrome. The inclusion criteria had no restriction by language or year but must provide sufficient available data to exclude duplicity. We described the clinical evolution of the patients, focusing on author, year of publication, age, sex, preceding problem, history of endotracheal intubation, unilateral or bilateral presentation, diagnostic procedures, type of treatment, follow-up, and outcome.

Case report

A 50-year-old man with a history of human immunodeficiency virus (HIV) and hepatitis C virus positive serology, with class A of Child-Pugh classification liver cirrhosis and a bi-nodular hepatocellular carcinoma underwent liver transplantation after general anesthesia under orotracheal intubation. Body mass index at time of transplantation was 21 kg/m^2. An 8.0 endotracheal tube was placed. The cuff was inflated with 3 mL of air and verified with a manual manometry to reach a filling pneumotamponade of 20 cm water. Surgery lasted 375 min. The procedure was well tolerated and required a low dose of inotrops (noradrenalin 0.5 mL/h) during surgery. Immunosuppression therapy during induction was based on mycophenolate mophetil and tacrolimus. Patient was transferred to the intensive care unit (ICU) under mechanical ventilation, sedated with remifentanyl. Uneventful weaning was performed during the following

hours. On postoperative day (POD) 3, he required urgent re-laparotomy due to a perihepatic hematoma and was transferred to the ICU under mechanical ventilation, sedated with propofol and remifentanyl. Extubation was postponed due to a respiratory gram negative bacilli infection and agitation after several attempts of decreasing sedation. On POD 13, patient was extubated and required immediate re-intubation after severe respiratory failure. A third weaning failure occurred the following day requiring re-intubation for the third time. Then percutaneous tracheostomy was performed with no events. Five days later, patient was taken off mechanical ventilation progressively and oral diet was started the day after, appearing severe dysphagia and important sialorrhea, being hardly able to swallow a pureed diet. Aphonia was another significant symptom presented at that time. At POD 28 patient was decanulated and persisted with swallowing difficulty, requiring parenteral nutrition. A computerized tomography (CT) of the head and neck and a magnetic resonance imaging (MRI) of the brain and neck were then performed to exclude central nervous injury. Both explorations did not show pathological findings.

At POD 34, patient was transferred to the ward and enteral nutrition was initiated *via* nasogastric tube. He was evaluated by speech and swallow theraphysts and diagnosis of a bilateral tongue paralysis and aphonia was made. Evaluation by otorhinolaryngologist excluded a recurrent laryngeal nerve injury. Detailed neurological examination revealed bilateral tongue paralysis, severe dysarthria and dysphagia for liquids and solids. A stroboscopy was performed showing bilateral paralysis of vocal cords in addition to the bilateral tongue paralysis. Cervical electromyography was also performed. Bilateral Tapia's syndrome was then diagnosed; a bilateral hypoglossal and laryngeal recurrent nerve neuroapraxia. At three months post-transplant, subjective improvement in aphonia and dysphagia were observed and the patient was discharged with enteral nutrition.

Outpatient neurological follow-up regarding speech and swallow training was performed twice weekly. Satisfactory recovery of his aphonia and dysphagia were observed. At four months post-transplant, videofluoroscopy was performed with no significant findings; however, laryngeal stroboscopy showed severe hypomotility of cricoaritenoideal articulations, cordal atrophy and minimal adduction movements with severe longitudinal hiatus. Despite that, the patient presented no problems during intake, being able to take out the nasogastric feeding tube. At that time, the nasogastric tube was preferred to the percutaneous gastrostomy to avoid invasive procedures in a patient with a complex postoperative.

RESULTS

In total around 70 cases were initially described in the literature, but only 53 fulfilled the inclusion criteria: To have patients with sufficient available data in the description of cases in order to rule out duplicity. Table 1[1-2,7-51] summarizes the 54 cases (including ours) of Tapia's syndrome, focusing on author, year of publication, age, sex, preceding problem, history of endotracheal intubation, unilateral or bilateral presentation, diagnostic procedures, type of treatment, follow-up and outcome.

The majority were young. Only 13 cases were older than 50 years (range 16-95). All cases except 10 were males. Two cases were attributed to a central cause (metastatic hemangiosarcoma in the medulla oblongata[2] and infiltration of a large B-cell lymphoma[14]), but the remaining 53 patients were peripheral type. Six patients[8,22,24,36,42,43], apart from ours, had a bilateral presentation of the syndrome; four with complete deficit of hypoglossal and recurrent laryngeal nerves and three[22,24,43] incomplete with bilateral paralysis of the hypoglossus nerves and unilateral recurrent laryngeal nerve palsy. All the cases, except one[36], followed to a prolonged oro-tracheal intubation for more than 14 d. In the systematic review, we have found two other cases of isolated bilateral hypoglossal paralysis without other nerve involvement after oro-tracheal intubation[52,53].

All, except seven of peripheral cases[9,15,29,39,40,47,51], have been attributed to orotracheal intubation for surgery or respiratory failure. The most frequently involved operations were: Osteoarticular surgery of the shoulder, mandible and cervical spine in 14 cases, otorhinolaryngology surgical procedures in 11 cases, cardiac surgery in 4 cases, thoracic surgery in 2 cases, abdominal surgery in 2 cases, and direct traumatic nerve injury in 2 cases. However, several causes have been described in the literature such as: Vascular (vertebral artery dissection, carotid artery aneurism); metastasic or primary neoplasia (lymphoma, hemangiosarcoma, prostate, pseudotumor of the neck, nasopharyngeal carcinoma, neurilemoma, neurofibrome, *etc.*); infectious of the neck (bacterial, viral, fungal), *etc*.

The diagnosis and management of Tapia's syndrome in the majority of cases was based on a complete neurological examination, including laryngeal endoscopy and a head and neck CT or MRI. Some authors have advocated for the use of video-fluoroscopic swallowing and electromyography to confirm the diagnosis and to predict prognosis.

The treatment was supportive in all cases with a prompt establishment of a swallowing rehabilitation program. The administration of intravenous or oral steroids in combinations with B1, B6, B12 vitamins or hialuronic acid injection has been proposed by many authors in the acute setting. At least 4 patients[8,17,23] required percutaneous endoscopic gastrostomy and 2 a naso-gastric tube insertion[20,42] to ensure nutritional requirements while the oro-esofageal route was unable to be used. In two cases (Takimoto[43] and ours), where bilateral paralyses were discovered, reintubation with subsequent tracheotomy was necessary to prevent respiratory failure.

Recovery was excellent for the majority of non-tumour peripheral cases after a duration of 3 to 6 mo, ranging from 15 d to 3 years. In 9 cases the patients reported only

Table 1 Cases of Tapia's syndrome reported in the literatura to date (including our case): 54 peripheral type and 2 central type

Ref.	Age	Sex	Clinical procedure	OTI	Bil	Diagnosis	Treatment	Follow-up	Recovery
Bilbao 2016	50	M	Liver transplantation due to HCV cirrhosis coinfected with HIV and hepatocellular carcinoma	Yes	Yes	N eurological examination Electromiography Laryngeal endoscopy Head and neck CT and MRI Video fluoroscopic examination	Temporary tracheotomy for airway management Nasogastric tube feeding Speech and swallowing therapy	4 mo	Yes
Cariati *et al*[7] 2016	36	M	Neck abscess drainage	Yes	No	Neurological exam Barium swallow X-ray Swallowing endoscopy	Rehabilitation program	3 mo	Yes
	61	M	Neck abscess drainage	Yes	No	Neurologic exam Airway endoscopy	Rehabilitation program	3 mo	Yes
	42	M	Shoulder fracture reduction	Yes	No	Neurologic exam Airway endoscopy	Rehabilitation program	3 mo	Yes
Coninckx *et al*[8] 2015	64	M	Liver cirrhosis. Pneumonia and respiratory failure	Yes	No	Neurological examination Lumbar puncture Laryngeal endoscopy Head and neck CT and MRI Chest CT	Speech and swallowing therapy Percutaneous endoscopic gastrostomy	22 mo	Yes
	49	M	Myocardial infarction. Percutaneous coronary intervention. Penumonia	Yes	Yes	Neurologic examination Brain CT	Corticosteroid therapy 8 wk Speech and swallowing therapy Percutaneous endoscopic gastrostomy	4 mo	Yes
Yilmaz *et al*[9] 2015	61	M	Bone metastatic prostate cancer	No	No	Neck CT and MRI	-	-	-
Paramalingam *et al*[10] 2015	38	M	Eagle syndrome. Pneumonia	Yes	No	Head and neck CT			
Brandt *et al*[11] 2015	23	M	Otorhinolaryngology surgical procedure	Yes	No	-	-	-	-
	67	-	Arthroscopic intervention of left shoulder	Yes	No	-	-	-	-
Ghorbani *et al*[12] 2014	27	M	Septorhinoplasty	Yes	No	Neurological examination Head and neck MRI	Systemic corticosteroids	6 mo	Yes
Ulusoy *et al*[13] 2014	19	F	Nasoseptal deformity	Yes	No	Neurological examination Head and neck MRI Airway endoscopy	Systemic corticosteroids	6 mo	Yes
Cantalupo *et al*[14] 2014	16	M	Large B-cell Lymphoma	No	No	-	-	-	-
Lo Casto *et al*[15] 2013	42	F	Inflammatory pseudotumor of the neck	No	No	Neurological examination Electromiography Laryngeal endoscopy Head and neck MRI Chest and abdomen CT	Corticosteroid therapy	-	-
Kang *et al*[16] 2013	47	M	Cervical spine surgery	Yes	No	Head and neck CT and MRI	Corticosteroid therapy Speech therapy rehabilitation	8 mo	Partially
Emohare *et al*[17] 2013	17	M	Artrodesis T1-L1	Yes	No	Barium swallow X-ray Head and neck MRI Airway endoscopy	Percutaneous endoscopic gastrostomy Hialuronic acid inyection Rehabilitation program	1 mo	Yes
Varedi *et al*[18] 2013	27	M	Zygomatic complex fracture	Yes	No	Neurological examination Head and neck CT and MRI Laringoscopic examination	Systemic corticosteroids Vitamin B complex Rehabilitation program	9 mo	Yes
Gevorgyan *et al*[19] 2013	48	F	Liposuction 3 yr previously rhinoplasty 25 yr previously	Yes	No	Neurological examination Head and neck CT and MRI Laringoscopic examination	Vocal cord injection Rehabilitation program	3 yr	Partially
Lim *et al*[20] 2013	64	M	Cervical spine surgery	Yes	No	Neurological examination Head and neck CT and MRI Laringoscopic examination Video fluoroscopic examination	Systemic corticosteroids Electrical stimulation therapy Nasogastric tube feeding	3 mo	Yes
Park *et al*[21] 2013	53	M	Posterior cervical spine surgery Posterior cervical spine surgery	Yes	No	Head and neck CT and MRI Laryngeal electromyography	-	6 mo	Yes
	56	M		Yes	No	-	-	2 mo	Yes

Sønnichsen *et al*[22] 2013	-	-	Legionella infection	Yes	Yes	-	-	2 mo	Partially
Nalladuru *et al*[23] 2012	49	M	Cardiac surgery	Yes	No	Neurological examination Head and neck CT and MRI	Systemic corticosteroids Percutaneous endoscopic gastrostomy	2.5 mo	Yes
Turan *et al*[24] 2012	15	M	Acute lymphoblastic leukemia pneumonia	Yes	Yes	Neurological examination Laringoscopic examination	Systemic corticosteroids	0.5 mo	Partially
Wadelek *et al*[25] 2012	57	M	Arthroscopic shoulder	Yes	No	Neurological examination Head and neck MRI Laryngeal endoscopy	Rehabilitation program	+ 2 mo	Yes
Lykoudis *et al*[26] 2012	32	M	Rhinoplasty	Yes	No	Head and neck CT Laryngeal endoscopy	Oral corticosteroid therapy Speech and swallowing therapy	4 mo	Yes
Park *et al*[27] 2011	42	M	Anterior cervical spine surgery	Yes	No	Neurological examination Electromyography Video fluoroscopic swallowing Laryngeal endoscopy Head and neck MRI	Rehabilitation program	7 mo	Yes
Torres-Morientes *et al*[28] 2011	32	M	Tracheostomy and right thoracostomy	[1]	No	Neurological examination	Speech and swallowing therapy	4 mo	Yes
Al-Sihan *et al*[29] 2011	63	M	Vertebral artery dissection	No	No	-	Clopidogrel for 6 wk Speech and swallowing therapy	-	Partially
Kashyap *et al*[30] 2010	41	M	Mandibular fracture	Yes	No	-	None	16 mo	Partially
Rotondo *et al*[31] 2010	-	-	Cardiac surgery	-	-	-	-	-	-
Boğa *et al*[32] 2010	35	M	Septorhinoplasty	Yes	No	-	Systemic corticosteroids	0.5 mo	Yes
Dursun *et al*[33] 2007	-	-	Hunting rifle-shot	-	-	-	-	-	-
Sotiriou *et al*[34] 2007	-	-	Coronary bypass grafting surgery	Yes	-	-	-	-	-
Tesei *et al*[35] 2006	30	F	Rhinoplasty	Yes	No	Neurological examination Head and neck MRI	Systemic corticosteroids Speech and swallowing therapy	4 mo	Yes
Cinar *et al*[36] 2005	20	M	Open rhinoplasty	Yes	Yes	-	Systemic corticosteroids	1 mo	Yes
Yavuzer *et al*[37] 2004	42	F	Septorhinoplasty	Yes	No	-	Oral corticosteroid therapy	6 mo	Yes
Krasnianski *et al*[2] 2003	77	M	Metastasic hemangiomasarcoma in the medulla oblongata	-	No	-	None	-	-
Boisseau *et al*[38] 2002	42	M	Shoulder surgery	Yes	No	Vertebral and carotid ultrasonography Head and neck CT and MRI	Systemic corticosteroids Speech and swallowing therapy	6 mo	Yes
Johnson *et al*[39] 1999	44	M	Surgical repair of a shoulder injury	No[1]	No	Head and neck CT and MRI	None	2 mo	Partially
Shimohata *et al*[40] 1994	61	F	Aneurism of extracranial internal carotid artery	No	No	Carotid angiography Head and neck CT and MRI	-	-	-
Millán Guevara *et al*[41] 1993	-	-	Viral etiology?	-	-	-	-	-	-
McCleary *et al*[42] 1993	95	F	Fracture of the odontoid process	-	Yes	-	Naso-gastric tube	12 mo	Partially
Takimoto *et al*[43] 1991	18	F	Nasopharyngeal carcinoma radiation	-	Yes	-	Temporary tracheotomy for airway management during pregnancy	4 yr	No
de Freitas *et al*[44] 1991	37	F	Paracoccidiodomycosis fungus in the nasal mucosa	-	-	-	Oral Ketoconazol	2 yr	No
Quattrocolo *et al*[45] 1986	24	M	Neurilemoma of vagus and hypoglossal nerves	-	-	-	-	-	-
Gelmers *et al*[46] 1983	41	M	Thoracotomy	Yes	No	-	-	12 mo	No
	36	M	Thoracotomy	Yes	No	-	-	12 mo	No
Andrioli *et al*[47] 1980	25	M	Neurofibrome of X and XII nerves below the nodose ganglion	No	No	-	Surgery: Resection of the two nerves	-	No
Mayer *et al*[48] 1974	51	M	Hiatus hernia repair. Pneumonia	Yes	No	-	None	0.5 mo	Partially
Ruhrmann *et al*[49] 1963	-	-	Congenital	-	-	-	-	-	-
Babini *et al*[50] 1961	-	-	Obstetrical trauma	-	-	-	-	-	-

Symonds *et al*[51] 1923	35	F	Chronic otitis media	No	No	-	-	2 yr	Partially
Tapia *et al*[1] 1905	-	M	Bullfighter injury behind the angle of the jaw		No				

Interscalene brachial plexus block [1]Tracheostomy. OTI: Orotracheal intubation; BIL: Bilateral; F: Female; M: Male; CT: Computed tomography; MRI: Magnetic resonance imaging; HCV: Hepatitis C virus.

partial recovery.

DISCUSSION

The case described above, is the first reported case of complete bilateral Tapia's syndrome (paralysis of the tongue' muscles and vocal cords because of an extracraneal injury of the Ⅹ and Ⅻ cranial nerves) occurring after liver transplantation and oro-tracheal general anaesthesia requiring re-intubation for three times. There are many causes of Tapia's syndrome, including general anaesthesia, fungal infections[44], neoplasms[2,9,14,15,24,43,45,47], vascular[29,40] and traumatic problems[1,33,50], being general anaesthesia the main cause. Intubation tube or its cuff and motion of the head during surgery can lead to injury to the pharyngeal wall and its underlying neurovascular structures (Ⅹ and Ⅻ cranial nerves)[32]. Excessive dorsiflexion of the head during laryngoscopy, excessive cuff pressure, malposition of the cuff in the larynx rather than the trachea, or extubation while the cuff is still inflated is the most likely cause[18]. The tracheal tube and its cuff may press on a localized area just at the crossing of the vagal and hypoglossal nerves, compressing the anterior branch of the inferior laryngeal nerve against the posteromedial part of the thyroid cartilage and this can lead to a recurrent laryngeal paralysis[6]. Hypoglossal nerve damage can be caused by a stretching of the nerve against the greater horn of the hyoid bone by an orotracheal tube or compression of the posterior part of the laryngoscope or orotracheal tube[35]. There was no clear mechanism for injury to the hypoglossal and recurrent laryngeal nerves in our patient. Intracraneal pathology was unlike because of negative CT scan and MRI. We postulate that low blood pressure during surgery and post-operatively due to intrabdominal hemorrhage requiring reintervention and the need of several orotracheal reintubations (3 times), 2 of them in emergency conditions, in addition to prolonged intubation with probable unnoticed overinflation and malposition of the endotracheal cuff, might have been the source of the bilateral nerve compression. A change in the position of the neck at some point, compression by the endotracheal tube and pressure to the lateral roots of the tongue with the McIntosh blade during intubation could be additional mechanisms. The caquexia of the patient and some degree of lypodistrophya due the HIV coinfection at time of transplant could also play a role. Liver transplantation is usually a long lasting surgical procedure, which could contribute, along with other factors to the development of Tapia's syndrome. This fact should be taken into account by all clinicians involved in the liver transplantation care: Liver surgeons, anesthetists, intensivists, hepatologists, gastroenterologists, *etc*.

Although most patients were male and young, there is no an explanation to relate the syndrome to sex or age. We believe that this syndrome is more related to anatomical, positional and lasting-time issues than to other characteristics.

The diagnosis is founded on a rapid suspicion, a complete history around the paralysis and a complete head and neck neurological examination. A computed tomography and or a magnetic resonance imaging of the brain and neck is essential to establish the diagnosis of central or peripheral type of Tapia's syndrome and also the nature of the lesion, ischemia, abscess formation, tumor or haemorrhage.

Tapia's syndrome classification and a treatment protocol have been proposed by Aktas and Boğa[32]: Grade Ⅰ/mild type, unilateral cord and tongue paralysis, no uvula distortion, minimal slowdown in speaking, no swelling in tongue and no trouble in swallowing, Corticosteroid treatment is not recommended; GradeⅡ/moderate type, unilateral cord and tongue paralysis, no uvula distortion, mild slowdown in speaking, swelling in tongue, dryness in pharynx, trouble in swallowing, cracked speech and normal feeding and drinking, 15 d of corticosteroid treatment is recommended; Grade Ⅲ/severe type, unilateral cord and tongue paralysis, significant uvula distortion, significant difficulty in speaking, swelling in tongue, dryness in pharynx, trouble in swallowing and difficulties in feeding and drinking, endovenous corticosteroid is recommended for 1 wk.

To our knowledge, only six cases[8,22,24,36,42,43] of isolated bilateral Tapia's syndrome have been reported in the literature and all of them were related to transoral intubation during general anaesthesia. The most relevant common feature was the prolonged oro-tracheal intubation for more than 14 d in all the cases except one[36]. Our patient was reintubated three times, two of them as an urgent procedure, and remained ventilated for more than 18 d.

The majority of all reported cases, even unilateral or bilateral, recovered in 4-6 mo and this progressive recovery of function suggests nerve damage of a neuropraxic type, which is typical of compression injury. But there are some reports in the literature regarding its irreversible form[43,44,46,47] or partially reversible form[16,19,22,24,29,30,39,42,48,51].

Apart from corticosteroids and anti-inflammatory therapy described above as key of the therapy, other support treatments recommended are speech and swallow therapy and warm air inhalation. Most studies

have emphasized that the recovery is usually completed within 6 mo, but with an intensive and multidisciplinary approach the patients' recovery time could be reduced. In our case, despite no corticosteroids were administered, the recovery was complete four months post-transplant after intensive speech and swallow training.

In conclusion, Tapia's syndrome is mainly a rare complication of airway manipulation. It can occur after any type of surgery under endotracheal general anesthesia. Clinicians should be aware of its preventive strategies, diagnosis, treatment and almost always transient outcomes. Although bilateral Tapia's syndrome after general anaesthesia is exceptionally rare, this complication should be recognized in patients reporting respiratory obstruction with complete dysphagia and dysarthria after extubation. Special attention should be paid to correct positioning of the head during surgery to avoid such problems.

COMMENTS

Background

Tapia's syndrome is an extracraneal ipsilateral palsy of the recurrent laryngeal and the hypoglossal nerves. It is a very rare complication with few cases reported in the literature. The predisposing factors are most commonly orotracheal intubation for general anesthesia but also other etiologies.

Research frontiers

This study tries to collect all articles published to date, emphasizing the common aspects of all reported cases.

Innovations and breakthroughs

The rarity in the presentation of Tapia's syndrome makes its incidence probably underestimated if clinicians are not aware of its symptoms. The publication of this review will help the scientific community to keep in mind Tapia's syndrome and to establish common guidelines for diagnosis, management and treatment.

Peer-review

This is a very interesting case report and a good literature review about the topic.

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