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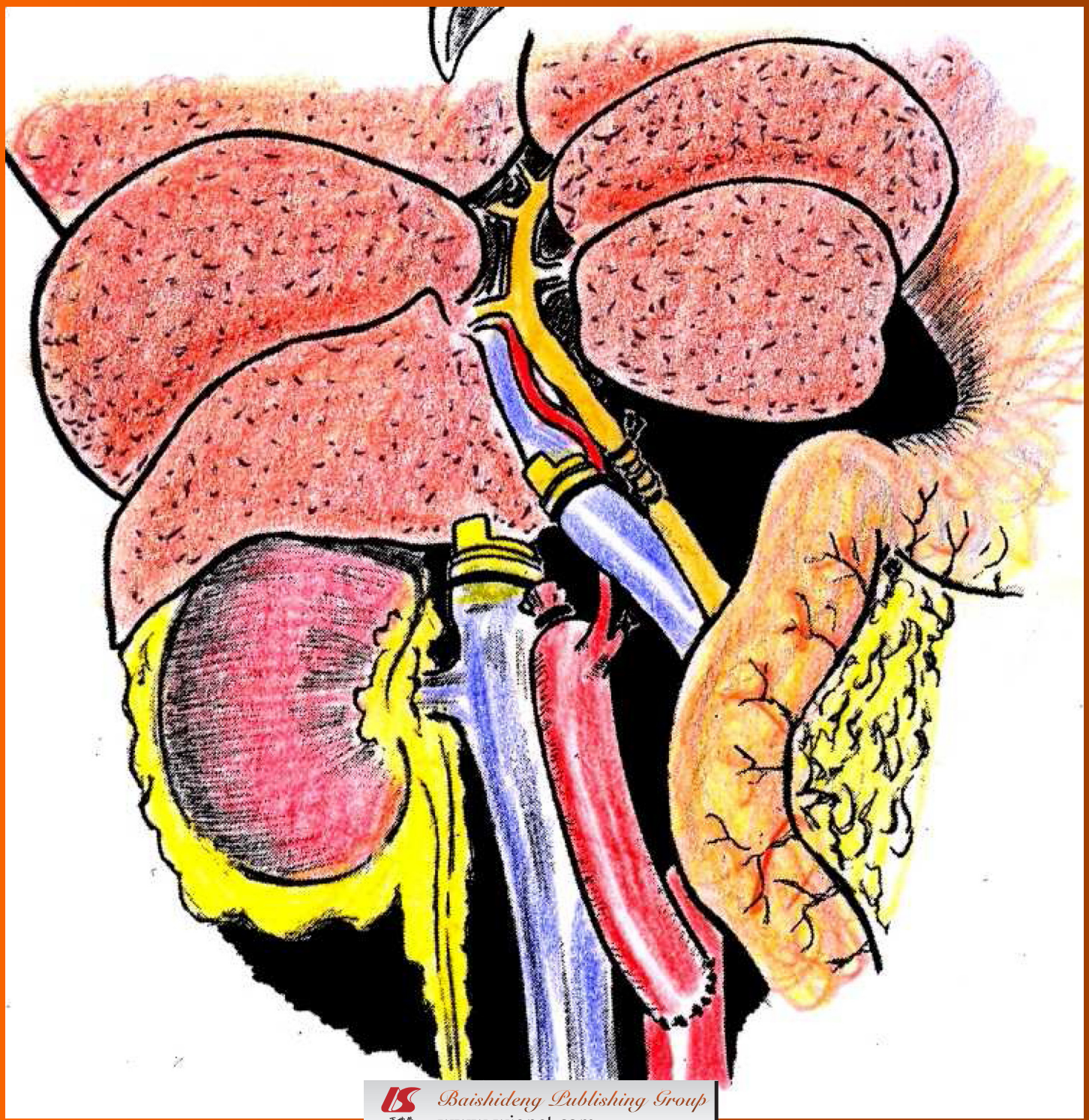

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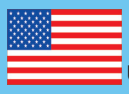

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REVIEW

A half century (1961-2011) of applying microsurgery to experimental liver research

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Abstract

The development of microsurgery has been dependent on experimental animals. Microsurgery could be a very valuable technique to improve experimental models of liver diseases. Microdissection and microsutures are the two main microsurgical techniques that can be considered for classifying the experimental models developed for liver research in the rat. Partial portal vein ligation, extrahepatic cholestasis and hepatectomies are all models based on microdissection. On the other hand, in portacaval shunts, orthotopic liver transplantation and partial heterotopic liver transplantation, the microsuture techniques stand out. By reducing surgical complications, these microsurgical techniques allow for improving the resulting experimental models. If good experimental models for liver research are successfully developed, the results obtained from their study might be particularly useful in patients with liver disease. Therefore experimental liver microsurgery could be an invaluable way to translate laboratory data on liver research into new clinical diagnostic and therapeutic strategies.

Key words: Microsurgery; Portacaval shunts; Cholestasis; Hepatectomies; Liver transplantation; Portal hypertension

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INTRODUCTION

The development of microsurgery has been dependent on experimental animals and driven by the demands of human surgery for more sophisticated techniques. It has also been driven by the need for novel laboratory models in biomedical research. Therefore, in this context, microsurgery could be a very valuable technique for improving the experimental models of hepatic insufficiency[1].

Microsurgery is a specific surgical field that requires the use of specialized microsurgical instruments, as well as dissection and suture microsurgical techniques. Microsurgical material is fundamental for performing microsurgical techniques. Microsurgical material is made up of means of magnification and microsurgical instruments[2].

The means of magnification or amplification systems are the basic and fundamental material for performing microsurgical techniques. In fact, the name of this kind of surgery is based on the use of magnification means. The amplification system par excellence in microsurgery is the surgical or operating microscope. The operating microscope is a binocular instrument with an optical lens system that provides stereoscopic or three-dimensional vision[2].

Microsurgical instruments have special characteristics for increasing the surgeon's ability in handling small structures while reducing tissue damage as much as possible. The micro instruments are designed to adapt to the surgeon's hand movements when using the operating microscope. The ergonomic characteristics or adaptation between hand and micro instruments are very important in microsurgery[2].

Microsurgical instruments correspond to the fundamental time phases of a surgical operation: cut or incision, hemostasia, exposure, dissection and suture.

Hemostasia is carried out with hemostatic forceps and clamps. Clamps and vascular clips are instruments used for hemostasis and for bringing small vessels closer together without causing too much tissue damage[3]. New vascular clips have been designed to reduce trauma on the vascular wall[4]. Exposure to the surgical field is made up of separation, aspiration and traction. Forceps without teeth for prehension are the fundamental instrument for dissection. The fundamental instrument for suture is the needle holder.

Microsuture material is made up of microsutures and microsurgical atraumatic needles. Microsurgical materials can be reabsorbable in the long-term or non-absorbable, i.e. nylon or silk, and their gauge ranges between 6/0 (75 μ diameter) and 11/0 (14 μ diameter). Non-absorbable monofilament polypropylene and polyester synthetic suture material are also used[1,2,5].

In the beginning, surgeons should learn microsurgical techniques mentally and physically in order to perform them successfully. The maneuvers are usually slow and precise and last a long time. Therefore, the microsurgeon needs to be calm and rested when starting the procedure. During the operation, the setting should also be calm and relaxing. Distractions, such as noise and movements, should be avoided. To minimize fatigue, microsurgeons should work sitting down. The complete stability of the surgeon and the surgical field are essential to perform microsurgical techniques correctly[2].

MICROSURGICAL TECHNIQUES FOR RESEARCHING LIVER DISEASES

Microsurgery applied to the rat and mouse liver makes it possible to obtain new experimental models and improve the already existing macrosurgical models. From the pioneer works of Lee *et al*[1,6-8] in the early 1960s through today, microsurgery has gained acceptance as an integral component of liver research.

Microdissection and microsutures are the two main surgical techniques that can be considered for classifying the experimental models developed. Based on this classification, simple and triple partial portal vein ligation, extrahepatic cholestasis and hepatectomies are all surgical models based on microdissection techniques, while in portacaval shunts and orthotopic and heterotrophic liver transplantation, the microsuture techniques stand out (Figure 1).

Microsurgical dissection techniques

The techniques of microsurgical dissection are mainly used for performing partial portal vein ligation, extrahepatic cholestasis and hepatectomies[8].

PARTIAL PORTAL VEIN LIGATION

For the experimental study of portal hypertension (PH), the prehepatic type is usually chosen since it produces the least degree of hepatic insufficiency. The most frequently used experimental model of prehepatic PH is that achieved by simple partial portal vein ligation in the rat[9-11]. This surgical technique was first described by Chojkier and Groszmann in 1981[12]. In brief, after laparotomy, the portal vein is dissected and isolated. A 20 gauge blunt-tipped needle is placed alongside the portal vein and a ligature (4/0 silk) is tied around the needle and the vein. The needle is immediately removed, yielding a calibrated stenosis of the portal vein[10,12].

If it is taken into account that the intensity of the PH, as well as its posterior evolution, are conditioned by the resistance to the inflow produced by the constriction of the portal vein, this experimental model of prehepatic PH could be improved by increasing the initial resistance to the blood flow. With this objective in mind, we have modified the surgical technique by increasing the length of the stenosed portal tract with three equidistant partial ligatures. In brief, three partial ligatures are performed in the superior, medial and inferior portion of the portal vein and maintained in position by the previous fixation of the ligatures to a sylastic guide (Figure 2). The stenoses are calibrated by a simultaneous ligation (4/0 silk) around the portal vein and a 20 G needle. The midline abdominal incision is closed in two layers with an absorbable suture (polyglycolic acid) and 3/0 silk. The

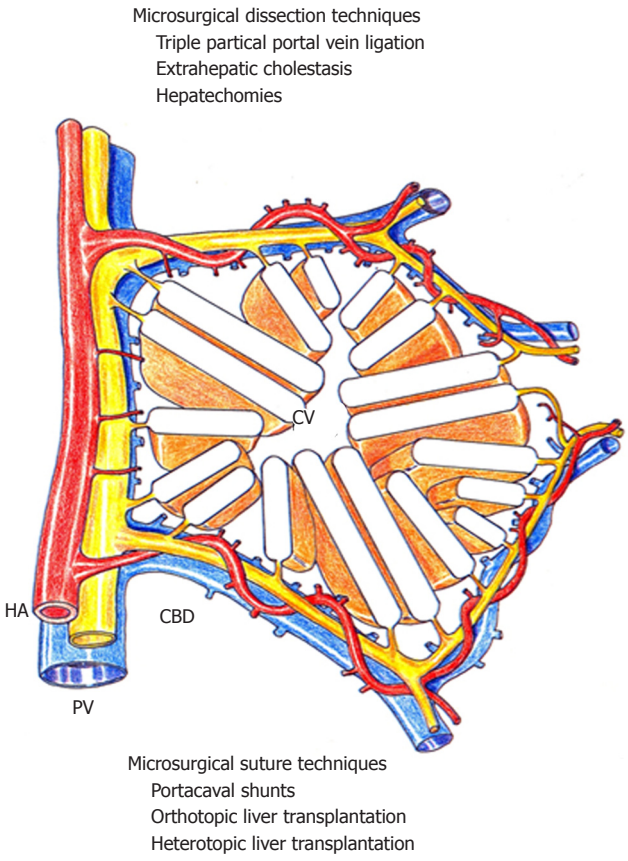

Figure 1 Microsurgical techniques used in liver research. The liver has been schematically represented as a hepatic functional unit formed by parenchyma (hepatic trabeculae) and stroma (portal space with vascular and biliary structures). The microsurgical experimental models are listed around this hepatic functional unit, grouped according the predominantly surgical technique used. Thus, hilar and parenchymal microdissection and vascular and biliary microsutures are the two fundamental microsurgical techniques used to obtain experimental models of liver diseases. Therefore, both microsurgical techniques can be considered for classifying the experimental models developed. HA: Hepatic artery; CBD: Common bile duct; PV: Portal vein; CV: Centrolobular vein.

microsurgical technique allows a better dissection of the portal vein in its initial tract, in which this vein establishes a close adhesion with the proper hepatic artery[8,13].

One of the reasons that the prehepatic PH experimental model presents great evolutionary variability could be based on its inflammatory nature[13,14]. Several of the early and late morphological and functional disorders presented by the splanchnic and systemic tissues and organs in experimental prehepatic PH make it possible to suspect that inflammatory-type mechanisms participate in their etiopathogeny[13-15]. In this way, mast cells could develop a key role in experimental PH because their mediators have the ability to produce all of the alterations associated with inflammation, both at the splanchnic, i.e. portal hypertensive enteropathy and liver steatosis[14], as well as at the systemic level, i.e. portal hypertensive encephalopathy and metabolic syndrome[14,16-18].

EXTRAHEPATIC CHOLESTASIS

Obstructive jaundice causes a high rate of morbidity and mortality in the human clinical field[19]. The serious repercussions of cholestasis on the liver and at the systemic level have led to the creation of many experimental models in order to better understand its pathogenesis, prophylaxis and treatment.

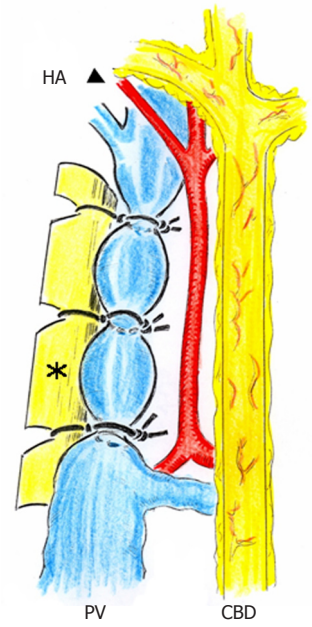

Figure 2 Prehepatic portal hypertension in the rat by triple partial portal vein ligation. Three partial ligatures are performed in the superior, medial and inferior portion of the portal vein and maintained in position by the previous fixation of the ligatures to a sylastic guide (*). PV: Portal vein; HA: Hepatic artery; CBD: Common bile duct.

Several surgical techniques for developing extrahepatic cholestasis have been described, especially in the rat, based on the section of the common bile duct between ligatures[20,21]. These macrosurgical techniques of extrahepatic cholestasis, called common bile duct ligation (BDL), cause the development of infected hilar biliary pseudocysts and multiple systemic abscesses and as a result, rats die during the early postoperative period due to sepsis[22].

The hepatic parenchyma in the rat has four lobes, the right lateral, middle, left lateral and caudate lobes, which in turn have independent portal and arterial vascularization and a separate biliary drainage. This anatomic feature makes it possible to resect the bile ducts that drain the four lobes of the liver in continuity with the common bile duct up to the beginning of its intrapancreatic segment by means of a microsurgical technique[23] (Figure 3). An advantage of the microsurgical technique of extrahepatic cholestasis in the rat is the absence of biliary pseudocyst formation, hepatopulmonary infection, and thus the prevention of mortality related to sepsis[22,23]. In rats with microsurgical extrahepatic cholestasis, the weekly administration of antibiotics and vitamin K allows rodents to survive for more than 8 wk[24,25].

In the long-term evolution, both macrosurgical (BDL) and microsurgical experimental cholestasis models develop hepatomegaly with a marked ductular proliferation and fibrosis, but the loss of normal liver architecture, typical of cirrhosis, is seldom found[24-26]. In relation to extrahepatic alterations, jaundice, choluria, PH with enlarged spleen and collateral portosystemic circulation, hepatic encephalopathy and ascites, stand out[25,27]. Therefore, experimental extrahepatic cholestasis is not only a good model for studying the hepatic pathology related

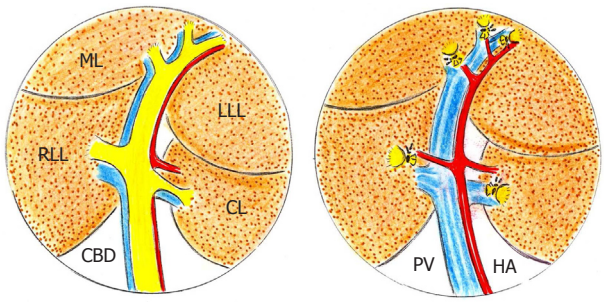

Figure 3 Microsurgical technique of extrahepatic cholestasis in the rat. This microtechnique consists of resecting the bile ducts that drain the four lobes of the liver in continuity with the common bile duct up to the beginning of its intrapancreatic segment (left side). The biliary branches of the caudate lobe, right lateral lobe, middle lobe and left lateral lobe are dissected, ligated and sectioned close to the hepatic parenchyma (right side). ML: Middle lobe; LLL: Left lateral lobe; RLL: Right lateral lobe; CL: Caudate lobe; CBD: Common bile duct; PV: Portal vein; HA: Hepatic artery.

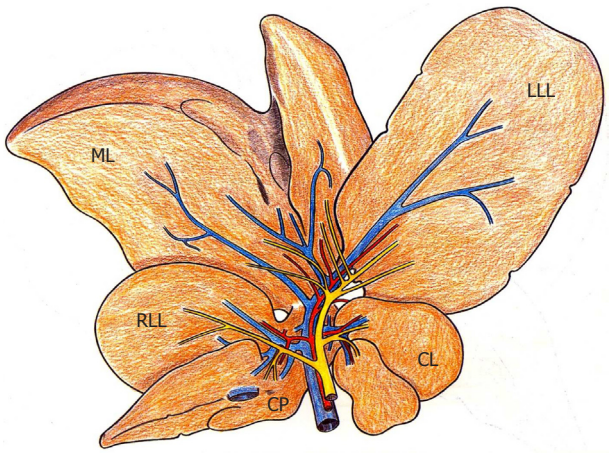

Figure 4 The hilar anatomy of the rat liver. Drawing showing the multilobulated rat liver with lobe-independent portal (blue) and arterial (red) vascularization and biliary (yellow) drainage. ML: Middle lobe; LLL: Left lateral lobe; RLL: Right lateral lobe; CL: Caudate lobe; CP: Caudate process.

to biliary obstruction, but also for studying extrahepatic complications[25,28].

The hypothetical comparison of the characteristics of amniotic and ascitic fluid in extrahepatic cholestatic rats would again compel raising the role of peritoneal mesothelial cells in the etiopathogeny of ascites that arise in the portal hypertensive syndrome. The functional comparison of both fluids would imply that in the decompensated portal hypertensive syndrome, the abdominal mesothelium acquires properties of the amniotic membranes or amnion. This hypothesis would suggest several suppositions or suggestions. For example, ascitic fluid could be a source of powerful therapies for using its supposedly amniotic-like beneficial properties[28].

HEPATECTOMIES

Hepatectomies in the rat allow for obtaining experimental models to study important aspects of hepatic physiopathology, such as liver regeneration or acute liver failure. Hepatectomies in the rat have benefited from the application of microsurgery because it reduces the limits to and the complications inherent in macrosurgical techniques[8,29].

Knowledge of the rat's liver anatomy has been widened through the use of the operating microscope. In the classical descriptions of the rat liver, four lobes were normally considered: two big cranial or anterior lobes, the middle and the left lateral lobes, and two small caudal or posterior lobes, the right lateral and the caudate lobes[30]. However, the findings obtained from the anatomical study using the operating microscope of the parenchyma located between the right lateral and caudate lobes allows for individualizing it as the caudate process[31] (Figure 4).

The study of the distribution of the portal and venous branches of the rat liver makes it possible to know its most frequent variations that must be considered when partial hepatectomies are performed. Furthermore, the distribution of these branches constitute the basis for the description of the functional anatomy of the rat liver as it defines the common site of the portal pedicles and the hepatic veins[32,33]. Thus, anatomical-functional description is based on Couinaud's description of the human liver[34].

The unique aspects of rat liver anatomy allow for various degrees of resections and they are highly reproducible[8,29,35,36]. Hepatectomies ranging from 5% to 95% of total liver weight can be easily performed with high reproducibility using microsurgical techniques because the parenchymal mass of each lobe is relatively constant[35]. However, depending on the extent of the resection, the resulting experimental model has different degrees of usefulness. Therefore, depending on the rat liver lobe or segment that can be microsurgically resected, the most used techniques are those that allow for obtaining adequate models for studying liver regeneration and fulminant hepatic failure[8,35-37]. Surgical removal of two-thirds (70%) of the liver in the white rat represents the most valuable and most extensively studied animal model of liver regeneration[30,37]. In turn, subtotal (90%) hepatectomy represents an experimental model for studying acute liver failure[35-37].

MICROSURGICAL 70% HEPATECTOMY

Commonly, regeneration of the liver is studied by performing a surgical procedure that removes two-thirds of the liver mass in rodents (rats and mice), a technique known as two-thirds partial hepatectomy[30,38]. This technique, which consists of the resection, after in "bloc" ligature, of the middle and left lateral lobes, was first described by Higgins and Anderson in 1931 to study the regeneration of the remaining parenchyma, the right lateral and caudate lobes[30].

Nowadays, this 70% hepatectomy technique can be improved with an operating microscope as the individualized dissection and ligation of the vascular and biliary branches of the middle and left lateral lobes can be done

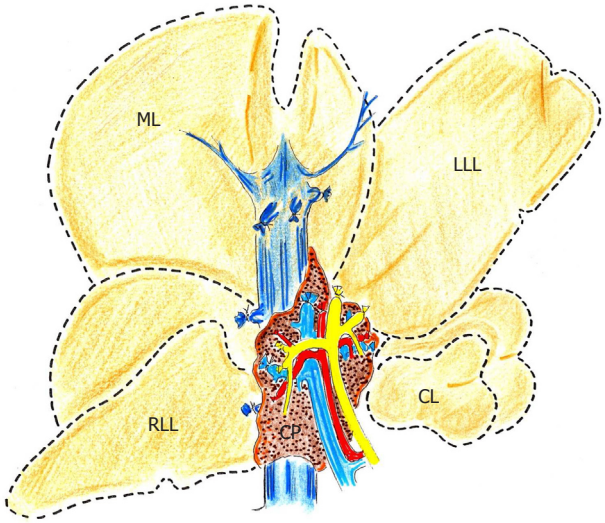

Figure 5 Hepatectomies in the rat. Drawing showing the microsurgical 70% (middle lobe (ML) and left lateral lobe (LLL)) and 95% [ML, LLL, right lateral lobe (RLL) and caudate lobe (CL)]. The microsurgical technique left the caudate process (CP) intact after 70%, 90% and 95% hepatectomies.

without damaging the right lateral and caudate lobes or the caudate process[39] (Figure 5).

The residual parenchyma after 70% hepatectomy grows in size to restore the original mass of the liver[30,38]. In both rodents and humans, the process known as "liver regeneration" after partial hepatectomy does not regenerate the resected hepatic lobes. Twenty-one days after the two-thirds microsurgical hepatectomy in the rat, the liver weight completely recovers its original volume[39].

After 70% hepatectomy, the sudden reduction in the liver vascular bed causes intrahepatic portal hypertension with the development of portosystemic collateral circulation[40]. The compensatory liver growth developing after partial hepatectomy is a phenomenon that is associated with an inflammatory response in which both the innate cellular immunity and growth factors participate[41]. The intestinal persistence of mast cell hyperplasia after compensatory hepatic growth could be associated with a late splanchnic remodeling process[42,43]. The 70% hepatectomy is also involved in heterotrophic liver transplantation in the rat, which in turn serves to study the host inter-liver competition phenomenon[44,45].

MICROSURGICAL 90% HEPATECTOMY

Subtotal (90%) hepatectomy invariably results in the death of rats if regeneration is not produced. However, this surgical model of hepatic failure does not reproduce the clinical setting of severe acute liver failure i.e. massive liver necrosis and cerebral edema[46].

The 90% hepatectomy consists of the resection of the middle lobe, left lateral lobe and right lateral lobe. Short-term survival is possible when using a macrosurgical technique, with mass ligation[47] or with a vessel-oriented approach[35]. With this last technique and the subcutaneous administration of glucose immediately after the operation and the addition of glucose to drinking water to prevent hypoglycemia, a 1 wk 100% survival rate is possible[35].

This 90% hepatectomy technique can also be improved using an operating microscope[37]. The microsurgical technique consists of the individualized dissection and ligation of the vascular and biliary branches from middle, left lateral and right lateral lobes without damaging the caudate process and caudate lobe branches[37] (Figure 5).

In a 95% hepatectomy, the survival rate is one week (66% of the animals) and in a 97% hepatectomy it does not exceed 4 d[35]. These techniques of subtotal or extended hepatectomies, when performed by microsurgery, reduce the injury to the caudate process parenchyma[36,37]. These models of 90%, 95% and 97% liver resection are of great interest in studying the physiopathological mechanisms leading to the failure of the remnant liver[35], as well as in assaying new therapies that favor liver regeneration[48] and subsequently increase the survival of the rat.

MICROSURGICAL TOTAL HEPATECTOMY

The total removal of the liver, while maintaining the portal and inferior vena cava circulation, provides a reproducible experimental model of acute liver failure. Total hepatectomy in the rat requires constructing a microsurgical portacaval shunt because this animal can only tolerate portal flow interruption for a maximum of 20 min[49].

Various techniques for creating an anhepatic rat have been described[46,49,50]. Total hepatectomy in the rat can be performed in one, two or three stages[46,69]. One-stage procedures consist of portacaval shunting and hepatectomy with prosthetic or vascular grafts[46,49,50]. To avoid grafting, multistage procedures have been devised, where initially partial constriction of both the portal vein and the vena cava is carried out to establish an adequate collateral circulation[46]. Microsurgical techniques can be used to obtain an anhepatic rat, not only to perform the portacaval shunt, but also to perform a total hepatectomy, avoiding damage to the intrahepatic vena cava[37].

MICROSURGICAL SUTURE TECHNIQUES

The techniques of microsurgical suturing are mainly used to make portacaval shunts, orthotopic liver transplants and heterotopic liver transplants.

Portacaval shunts

The splanchnic venous circulation flows into the portal vein towards the liver. The portal venin (*"porta"* means door in Latin) gives this venous system its name[51]. The portal venous system creates a functional unit between the organs that drain and vascularize.

Microsurgery allows for carrying out different types

of portosystemic shunts in the rat and mouse. The most frequently used have been end-to-side portacaval shunt, side-to-side portacaval shunt, mesentericocaval shunt and portacaval transposition[9,13].

End-to-side portacaval anastomosis in the rat is a shunt procedure that has become increasingly widespread since it was first performed by Lee *et al*[1,6] in 1961. Its value mainly lies in the fact that it is an appropriate technique for microsurgical training and for researching liver diseases, particularly hepatic encephalopathy[1,9,13].

Lee's description of end-to-side portacaval shunt in the rat has served as a reference for authors to make modifications[13]. Thus, we have described a simplified end-to-side portacaval shunt in rats, which consequently favors its widespread use[52,53]. In brief, the portal vein is clamped at the confluence with the splenic vein and ligated (7/0 silk) at the level of its hilar bifurcation. Next, a partial venotomy is performed just below its hilar ligature. A 9/0 nylon thread with a loop at its distal end is used to perform the end-to-side shunt. The first stitch in the vascular suture is made before completing the portal vein venotomy. This stitch is passed through the upper angle of the hole in the vena cava. On completing the section of the portal vein, the loop brings the two vessels closer together and the shunt is continued without requiring a first knot to be made. On finishing the anastomoses by continuous running suture, the suture is tied when it reaches the end corner, the loop can be untied by pulling on the end and then the suture is tied to it[52,53] (Figure 6).

Eck's fistula has been a model of liver atrophy for more than 100 years. The arterialization of the portal stump after portacaval shunt in the rat prevents hepatic atrophy[54]. In this model, the metabolic, neurological and behavioral alterations could be similar to those found in human type B hepatic encephalopathy, which concerns encephalopathy related to the portosystemic shunt[55]. It has been proposed that the alterations produced in hepatic encephalopathy could be of an inflammatory nature[56].

To study these portacaval shunt experimental models, sham-operated rats are usually used as a control. The sham operation consists of portal vein clamping during a similar period of time to that of the portacaval shunt[57].

Orthotopic liver transplantation

The orthotopic liver transplant technique in the rat is very laborious, which is why it requires prior training. In the description by Lee *et al*[58], they use the manual suture to perform the anastomosis, although the application of the cuff technique by Kamada and Calne in 1979[59] has been more widely accepted as it simplifies the orthotopic liver transplantation[60-62].

DONOR OPERATION

The dissection of the infrahepatic inferior vena cava (IH-IVC) includes the sectioning between ligatures of the right lumbar adrenal veins, as well as the right renal vein. To dissect the entire length of the portal vein, the gastroduodenal vein is ligated with 10/0 nylon suture and sectioned. Hypothermic perfusion of the liver is carried out through the portal vein. The operation is completed with a sectioning of the common bile duct hepatic artery, the portal vein, the IH-IVC and the suprahepatic-inferior vena cava (SH-IVC), which is carried out at the intrathoracic level, respecting a diaphragmatic flap around it[60,61,63].

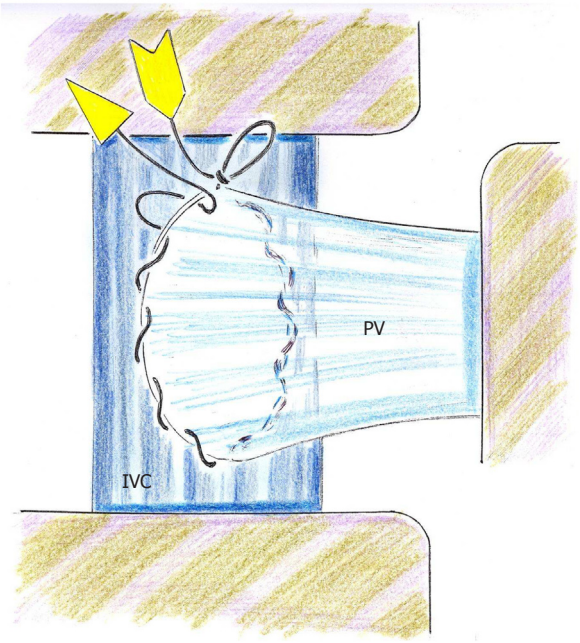

Figure 6 End-to-side portacaval shunt in the rat. The construction of a loop at the end of the suture facilitates the early approximation between the portal vein and the inferior vena cava, as well as its ending. Thus, when it reaches the end corner, the loop is untied by pulling on the end and the suture is tied to it. PV: Portal vein; IVC: Infrahepatic inferior vena cava.

In vitro preparation or *"bench"* surgery

Once the donor liver exeresis is performed, it is placed in a container with a hypothermic solution. Firstly, the common bile duct is perfused with 2 or 3 mL of cold preservation solution. Subsequently, a cuff is placed on the IH-IVC and then tied with a circumferential 6/0 silk ligature. Also, a cuff is tied on the portal vein with a circumferential 6/0 silk ligature.

RECIPIENT OPERATION

The recipient operation consists of a hepatectomy and transplant. A laparotomy is performed and the gastrointestinal tract is pushed downward towards the left of the animal. The hepatic artery is dissected and ligated, while the common bile duct is cannulated distally with a catheter and tied with a circumferential 6/0 silk ligature. Next, the portal vein is clamped and its right and left branches are ligated with 6/0 silk, sectioning the ligatures distally. The SH-IVC is clamped with a small Satinsky clamp. The caudal traction of the liver facilitates this maneuver. The sectioning of the SH-IVC at the level of the hepatic parenchyma finalizes the hepatectomy in the recipient.

The transplant beginning with an end-to-end anastomosis of the SH-IVC is performed by manual suture with Prolene® 6/0 thread. Later, the portal end-to-end anastomosis is performed using the cuff technique. Once the anastomosis has been ended, the portal vein and the SH-IVC are unclamped, while the liver progressively begins to regain normal coloring. The end-to-end anastomosis of the IH-IVC is also performed using the cuff technique. Next, the recipient IH-IVC is unclamped[60-63]. Lastly, the cannula of the donor common bile duct is introduced into the lumen of the recipient common bile duct, thus performing an end-to-end cholecocholedochostomy, over which the greater omentum is placed to avoid fistulas[60,61].

The arterial blood supply of the transplant could be restored through an aortic segment of the donor in continuity with the celiac axis and the hepatic artery. This donor aortic segment is sewn end-to-side with 10/0 monofilament thread to the recipient infrarenal aorta[64] (Figure 7).

A mechanical microvascular anastomosis for orthotopic liver transplant in the rat using a quick-linker technique has been also proposed[65].

One of the most serious complications after orthotopic liver transplantation is primary non-function from ischemia-reperfusion injury. Hepatic microcirculatory disturbance secondary to inflammation after graft reperfusion produce hepatocyte damage[66].

Microsurgical models of orthotopic liver transplantation in the rat are an indispensable component of transplantation research[67]. These experimental models serve to study new preservation methods, tolerance induction, rejection mechanisms and novel immunosuppressor therapies. The use of these microsurgical methods in liver transplantation avoids complications related to the surgical technique. When the surgical complications are minimized, complications associated with preservation, tolerance induction, rejection and new immunosuppressive drugs can be better studied[8].

HETEROTOPIC LIVER TRANSPLANTATION

Heterotopic liver transplantation (HLT) is an alternative to orthotopic liver transplant in patients. The liver transplant is not performed in the same anatomical place. In most cases, the recipient liver is respected and, if so, the transplanted heterotopic liver is called the "auxiliary liver". The HLT is a valid alternative to orthotopic liver transplant in both acute hepatic insufficiency and chronic hepatic insufficiency. However, better knowledge is still necessary in terms of postoperative complications to facilitate its diffusion to the human clinical area.

The development of microsurgical techniques was the decisive factor for disseminating the realization of the HLT in rats[45,68-70]. In this animal, several techniques have been described for HLT that are differentiated by the vascularization of the graft (portal, arterial or arterio-portal), the venous drainage (through the SH-IVC or the IH-IVC), the type of portal blood (splanchnic or systemic), the biliary drainage (by choledochoduodenostomy or choledochojejunostomy), the localization of the graft (intra- or extra-abdominal) and the mass of hepatic pa-

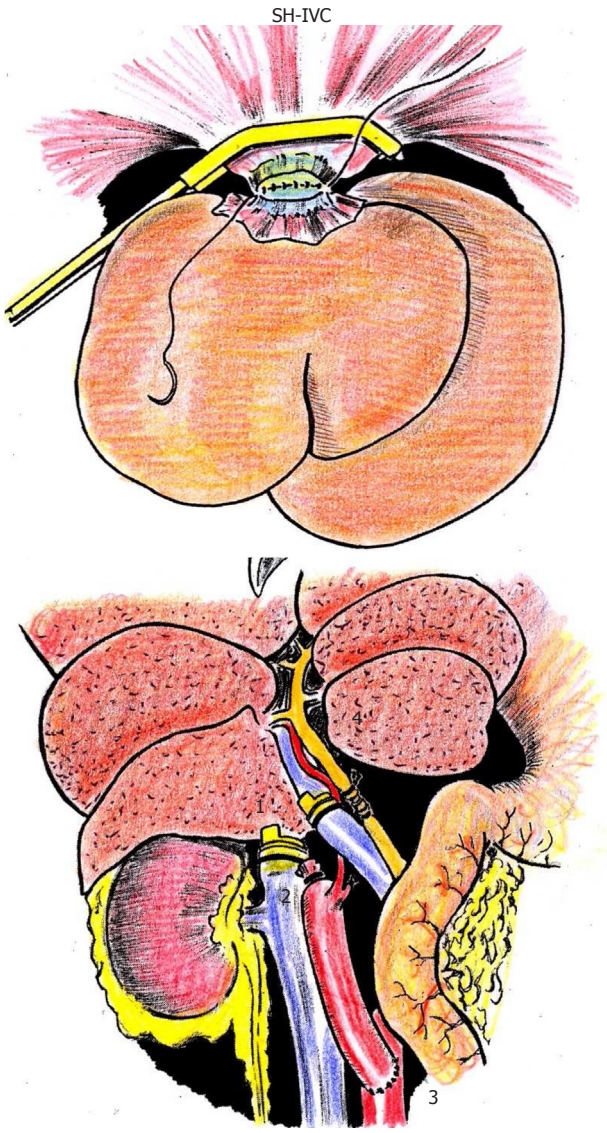

Figure 7 Orthotopic liver transplant in the rat. The first anastomosis that is carried out in the orthotopic transplant of the rat's liver is end-to-end between the suprahepatic inferior vena cava of the donor and recipient is represented on the top. The suture of the posterior semi circumferences of both vessels is intraluminal and from left to right of the animal. Next, the extraluminal suture is made on the anterior semi circumferences in the same way. Before finishing this anastomosis, saline is perfused inside to eliminate air that can cause a gas embolism when revascularizing the transplanted liver. The end-to-end portal anastomosis is made second, using the cuff technique. It is convenient to perfuse the liver through the portal route before the anastomosis is completed to eliminate potassium and acid catabolites, which accumulated during hypothermic preservation, from its circulation. Once proceeding with portal revascularization of the liver, the end-to-end anastomosis of the infrahepatic inferior vena cava of the donor and recipient is performed using the cuff technique. Then, arterial revascularization using the donor aorta, in continuity with the celiac trunk and hepatic artery, is made. End-to-side anastomosis is performed with the infrarenal abdominal aorta of the recipient. Lastly, the choledococholedochostomy is performed (bottom). 1, portal vein anastomosis; 2, infrahepatic inferior vena cava anastomosis; 3, arterial anastomosis; 4, choledococholedochostomy.

renchyma (total liver or partial liver transplant).

PARTIAL HETEROTOPIC LIVER TRANSPLANTATION

The donor operation is similar to that described previously in orthotopic liver transplant. But, in the hepatic hilum, the vascular structures and the bile ducts corresponding to the middle and the left lateral lobes are ligated when a partial heterotopic liver transplant is performed. In this case, the interlobular IVC, between the cranial and caudal lobes, is also ligated[45]. The abdominal aorta is clamped at its proximal end and is cannulated at its distal end. Then, hepatic arterial perfusion is initiated with a hypothermic preservation solution. The effluent drains through a venotomy carried out in the IH-IVC. Next, the donor liver perfusion is initiated through the portal vein. Lastly, the graft is explanted[45,60,70].

The *in vitro* preparation, or "bench surgery", of the graft consists of placing a cuff on the portal vein and a ligature or suture on the proximal end of the abdominal aorta.

In the recipient, the portal vein is dissected along its whole length and the gastroduodenal vein is ligated and sectioned. The IH-IVC is dissected between the drainage of the renal veins. The ends of the dissected IH-IVC are clamped. On the anterior wall of the clamped IH-IVC, a 4mm long oval venotomy is carried out. The graft is removed from the hypothermic container and placed in the abdominal cavity of the recipient with the right lateral lobe cranially and the caudate lobe caudally. An end-to-side anastomosis is carried out by microsurgical suture technique between the IH-IVC of the donor and the IH-IVC of the recipient using 8/0 monofilament thread[69].

Once the anastomosis is completed, a bull-dog is placed on the donor IH-IVC to avoid reflow of venous blood into the graft when the recipient IH-IVC is unclamped to avoid hypovolemic shock. Next, the end-to-end portal anastomosis is carried out using the cuff technique[45]. Once the graft is revascularized *via* the portal vein, the end-to-side aortic anastomosis is carried out by a continuous running suture with 10/0 monofilament thread[8,45]. Once arterial revascularization of the graft is carried out, a choledochoduodenostomy is performed[45,68]. The functional competence with the recipient liver determines the evolution of the graft[45,69] (Figure 8).

CONCLUSION

It could be concluded that the microsurgical techniques, when applied to experimental liver surgery, improve the experimental models obtained since they reduce the complications inherent in macrosurgical techniques. If good experimental models of liver research are successfully developed, the results obtained from their study might be particularly useful in patients with acute and chronic liver diseases.

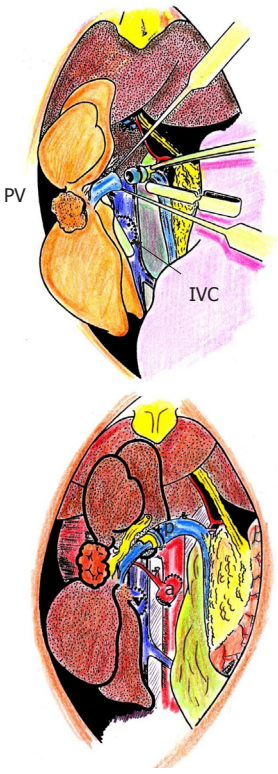

Figure 8 Heterotopic partial liver transplantation in the rat. First, an end-to-side anastomosis is carried out by microsurgical suture technique between the infrahepatic-IVC of the donor and the infrahepatic-IVC of the recipient. Next, the end-to-end portal anastomosis is carried out using the cuff technique (top). Once the graft is revascularized via the portal vein, the end-to-end aortic anastomosis is carried out by continuous suture (bottom). p: Portal vein anastomosis; a: Aortic anastomosis; v: Inferior vena cava anastomosis. IVC: Inferior vena cava anastomosis; PV: Portal vein.

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ORIGINAL ARTICLE

Life style modification improves insulin resistance and liver histology in patients with non-alcoholic fatty liver disease

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Abstract

AIM: To study the effect of regular aerobic exercise on insulin resistance, serum aminotransferase and liver histology in nonalcoholic fatty liver disease (NAFLD) patients.

METHODS: Sixty (mean age 40.0 ± 8.5 years, 75% male) NAFLD patients were included in the study. After baseline anthropometric measurement i.e., body mass index (BMI), waist circumference (WC); all patients were advised regular aerobic exercise for 30 min/d, for at least 5 d/wk and trained to achieve around 70% of his maximal heart rate. In addition, moderately energy restricted diet was advised to patients with high BMI (> 25 kg/m^2). Monthly follow up was done by measuring BMI, WC, aspartate aminotransferase, and alanine aminotransferase (ALT). Insulin resistance was calculated using homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) model, at baseline and after 6 mo. Insulin resistance was arbitrarily considered altered when it was ⩾ 2. Liver biopsy was done in a section of patients at baseline and after 6 mo.

RESULTS: Seventy percent (42/60) patients were overweight or obese; 95% (57/60) had central obesity (WC > 90 cm in men, > 80 cm in women). In the 45 exercise compliant patients insulin resistance decreased from 6.4 ± 6.1 to 1.3 ± 1.0, BMI from 26.7 ± 3.3 kg/m^2 to 25.0 ± 3.3 kg/m^2, WC from 95.7 ± 8.9 cm to 90.8 ± 7.3 cm and ALT from 84.8 ± 43.5 U/L to 41.3 ± 18.2 U/L (P < 0.01). In 15 exercise noncompliant patient's insulin resistance, BMI, WC and ALT did not show significant change at 6 mo follow up. Six of 8 patients in compliant group on repeat liver biopsy showed significant change in steatosis and necroinflammation. Nonalcoholic steatohepatitis scores improved form 5.3 ± 1.5 to 3.35 ± 1.5. The decline in insulin resistance correlated with decline in ALT (P = 0.01, r_s = 0.90) and liver histology (P = 0.03, r_s = 0.73).

CONCLUSION: Life style modification improves insulin resistance resulting in improvement in ALT and liver histology in NAFLD patients.

Key words: Lifestyle changes; Insulin resistance; Metabolic syndrome; Nonalcoholic steatohepatitis; Liver histology

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INTRODUCTION

Obesity and insulin resistance related health hazards (metabolic syndrome) has become major health burden in the present century. In the United Stated, around 30% of population is obese and three fourth of them have fatty liver disease[1]. Nonalcoholic fatty liver disease (NAFLD) is a spectrum of clinicopathologic liver disease ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and is being increasingly recognized as a major cause of liver related morbidity and mortality[2,3]. With emerging clinical and epidemiological evidence, NAFLD is considered as hepatic component of metabolic syndrome[4].

At present, there is no established means for treating or preventing NASH. Clinical trials of potential treatments have mostly been conducted in uncontrolled settings on small number of subjects and extrapolation of these observations to large populations of patients with NAFLD is unreasonable. Available clinical and epidemiological data indicates that obesity, hyperlipidemia and diabetes are associated with NASH[5-8].

As insulin resistance has been recognized as major mechanism for development of NAFLD (proposed to be the "first hit" leading to hepatic steatosis), improving insulin-sensitivity has been considered as a strategy in the treatment of NAFLD[9-11]. Oxidative stress with subsequent lipid peroxidation and generation of reactive oxygen species seems to be prominent in NASH ("second hit") and has been identified as therapeutic target for antioxidants[9,12]. As NASH is often categorized as part of metabolic syndrome, drug treatment carries risk of significant side effects and probably requires life long therapy resulting in poor compliance.

Lifestyle modifications with dietary restriction of calorie and exercise have shown to improve insulin sensitivity and help in reducing the risk of chronic illnesses like coronary artery disease and maturity-onset diabetes[13,14]. In the United States, at any given time, it has been estimated that around 30% men and 45% women are on lifestyle changes to lose weight. Short term weight loss resulting from dietary modification and exercise have been shown to improve obesity and aminotransferase, however its ultimate effect on liver histology and the natural course of NASH is not well documented[15,16,17]. We performed a prospective study aimed to asses the effect of regular aerobic exercise and dietary modification on insulin resistance liver histology in patients with NASH.

MATERIALS AND METHODS

Patients and methods

Sixty patients diagnosed as NAFLD attending Liver clinic were included in the study. Diagnosis of NAFLD was based on the presence of fatty liver on ultrasonography and an elevated serum alanine aminotransferase (ALT) > 1.5 times the upper limit of normal for a minimum period of 3 mo. Patients with other causes of chronic hepatitis including viral hepatitis, autoimmune hepatitis, cholestatic liver disease, hemochromotosis, Wilson's disease and alcoholic liver disease (alcohol use > 20 g/d) were excluded from study.

This study was carried out in accordance with the principles of the Helsinki declaration and was formally approved by institutional ethical committee. All patients gave a written, informed consent before participation in the study.

Clinical and anthropometric data

Detailed history including use of drugs, particularly oral contraceptives, corticosteroids and antituberculosis, antidiabetics, insulin sensitizers was obtained and clinical examination to look for any evidence of chronic liver disease was done at initial screening. Bodyweight was measured using self-zeroing weight scale with light clothing without shoes to the nearest half-kilogram. Height was measured to the nearest 2 mm with patient standing on bare feet closely apposed to each other and against the wall with patient looking straight. Body mass index (BMI) was calculated as follows: body weight (kg/m^2)[18]. Waist circumference (WC) in centimeters was measured at a level midway between the lower rib margin and iliac crest and hip circumference at the widest portion of buttocks. Waist-hip ratio (WHR) was calculated by dividing waist circumference by hip circumference. Increased WHR was defined as $\geqslant$ 0.90 in men and $\geqslant$ 0.85 in women[19].

Metabolic syndrome

Metabolic syndrome was defined according to National Cholesterol Education Program (NCEP) adult treatment panel III (ATP III) guidelines as the presence of 3 or more of the following 5 risk factors: (1) Waist circumference > 102 cm (men) and > 88 cm (women); (2) Fasting triglycerides $\geqslant$ 150 mg/dL; (3) High density lipoprotein cholesterol < 40 mg/dL (men) and < 50 mg/dL (women); (4) blood pressure $\geqslant$ 130/$\geqslant$ 85 mmHg; (5) fasting glucose > 110 mg/dL[20]. The cut off for normal waist circumference in adult Indians has been found to be lower than Caucasians; hence we used a cut off of > 90 cm (men) and > 80 cm (women) in our study[21].

Laboratory tests

Overnight fasting blood sample were obtained for measurement of plasma glucose and serum lipids. Plasma glucose 2 h after 50 gm of glucose load was also done in all patients. Plasma glucose was measured with an automated analyzer using glucose oxidase and peroxidase method. Fasting lipid profile for total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, very low-density lipoprotein and triglycerides was obtained (RA-XT random access clinical chemistry analyzer, Bayer Diagnostics, Tarrytown, NY, USA). Serum iron studies, albumin, aspartate aminotransferase (AST) and ALT were done on all patients at baseline.

To exclude hepatitis B and hepatitis C, HBsAg

(Hepanostika, Biomerieux bv, Boxtel, NL, US) and anti-hepatitis C virus (UBI, United Biochemicals Inc, Houppauge, NY, US) and to exclude autoimmune hepatitis, anti nuclear antibody, anti smooth muscle antibody, anti mitochondrial antibody and anti liver kidney microsomal antibody were done in all patients. Patients less than 40 years of age also underwent slit lamp examination to rule out Wilson's disease.

Insulin resistance

Fasting samples of serum obtained after centrifugation were stored at -70 ℃ until assayed. Fasting insulin levels (mU/L) were measured using radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The hinsulin resistance was calculated on the basis of fasting values of plasma glucose and insulin according to homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) model formula,

HOMA-IR = fasting insulin (mU/L) × fasting glucose (mmol/L) ÷ 22.5[22].

As previously recommended, insulin resistance was arbitrarily considered altered when it was > 2[23].

Ultrasonography

Ultrasonography of liver was performed in all participants in fasting state by trained radiologist blinded for laboratory and anthropometric data. Brightness and posterior attenuation were considered indices of the extent of fatty infiltration. The diagnosis of fatty liver was made based on findings of echogenicity: graded as: grade 0: normal echogenicity; grade 1: slight, diffuse increase in fine echoes in liver parenchyma with normal visualization of diaphragm and intrahepatic vessel borders; grade 2: moderate, diffuse increase in fine echoes with slightly impaired visualization of intrahepatic vessels and diaphragm; and grade 3: marked increase in fine echoes with poor or non-visualization of the intrahepatic vessel borders, diaphragm, and posterior right lobe of the liver[24].

Liver biopsy

In patients who gave consent, liver biopsy was done as indoor procedure using Menghini's needle. All biopsies were reported by one pathologist blinded for clinical data using the Brunt's scoring system for non-alcoholic steatohepatitis[25]. Steatosis was graded according to percentage of cells with fatty droplets (grade I, 0%-33%; grade II, 34%-66%; grade III > 66%). Necroinflammation was graded 0-3 (0, absent; 1, occasional ballooning and no or minimal inflammation; 2, ballooning of hepatocytes with mild to moderate portal inflammation; 3, intra-acinar inflammation and portal inflammation). Fibrosis was graded as 0-4: (0, no fibrosis; 1, perisinusoidal/ pericellular fibrosis; 2, periportal fibrosis; 3, bridging fibrosis; 4, cirrhosis.

Study design

All patients were counseled to an aerobic exercise regimen. Moderately energy-restricted diet was advised by a qualified dietician to those with high BMI (> 25 kg/m^{2}).

Exercise program and diet regimen

All patients were given training by professionally qualified physical instructor from "Health Zone" Lucknow about exercise programme at beginning and at regular interval. During workshop two physical trainers assessed and individualized the exercise program for each patient, and supervised their performance during workshops and were trained to achieve target heart rate. The exercise program included brisk walking; jogging or rhythmic aerobic exercises set to beat music, for a minimum period of 45 min/d, for at least 5 d/wk. They were counseled to achieve approximately 70% of their maximal heart rate for minimum period of 20 min. Maximum heart rate was estimated from the formula 220 - age (years) with a standard deviation of 10-12 beats/min[26]. All patients were asked to maintain records about exercise programme and MHR in the provided Performa to assess their compliance. Those patients who exercised on less than 4 d/wk (16 d/mo) were considered as exercise non-compliant.

All patients received standardized nutritional counseling by registered Dietician, who supervised them regularly. Moderate energy restricted diet containing 60% carbohydrate, 20% fat, 20% protein and 200 mg cholesterol (National cholesterol education program step I diet, (25 kcal/kg ideal body weight) was advised to patients with high BMI[20]. Ideal body weights were calculated in kg using the formula: Ht (cm) - 100 × 0.9[27].

Follow-up

All patients were followed up monthly by measuring anthropometric data and laboratory assessment of serum albumin, ALT and AST levels. Fasting insulin levels were taken at baseline and after 6 mo of exercise program. Care was taken so that none of them took any other pharmacological intervention except for coexisting condition like hypertension.

Statistical analysis

Statistical analysis was performed using SPSS 10.0.1 software (SPSS Inc., Chicago, IL, US). All data was expressed as mean ± SD. Baseline parameters were compared between groups using Mann-Whitney *U* test and for comparing the variables before and after therapy, Wilcoxon signed ranks test was used. The degree of association between the decline in insulin resistance, ALT and liver histology; before and after exercise was done using Spearman's correlation coefficient (r_s). A significance level of $P < 0.05$ was considered as statistical significance.

RESULTS

Seventy-five patients were initially screened for study of which 60 agreed to participate. Forty five of 60 complied with the exercise program (compliant group) while 15 did not (non-compliant group). Majority of our patients had non-specific minor symptoms like dyspepsia, right upper quadrant heaviness or had been referred for incidental detection of fatty liver on ultrasonography(USG) and elevated transaminases on routine blood tests.

Baseline parameters

Majority of study population were male (70%, 42/70) with mean age being 40.0 ± 8.9 years. While 70% (42/60) patients were overweight, 95% (57/60) had central obesity (WC ⩾ 0.90 in men and ⩾ 0.80 in women). Two of our patient had essential hypertension and none of our patients were diabetic. All of our patients were nonalcoholic and had negative viral markers. On USG examination 19 (32%) patients had grade 1, 30 (50%) had grade 2 and 11 (18%) had grade 3 fatty liver.

Histopathology

Thirty-two (53%) patients gave consent for liver biopsy and findings were given in Table 1. All liver biopsies showed fatty infiltration predominantly macro vesicular. Grade 1 fatty infiltration was present in 12 patients, while grade 2 in 15 and grade 3 in 5. Ballooning degeneration was present in 8 (25%) and glycogenated nuclei in 11 (34.3%). Majority of the biopsies had mild to moderate necroinflammatory activity (grade 1 in 10, grade 2 in 17). Only 5 biopsies showed severe (grade 3) necroinflammatory activity. None of the biopsies showed cirrhotic changes, while grade 1 fibrosis was seen in 14 patients, grade 2 in 4 and grade 3 in 3 patients. Eleven patients had no fibrosis on liver biopsy.

Insulin resistance and metabolic syndrome

Mean insulin resistance level in our patients was 7.1 ± 5.1. Fifty-six (93%) patients had HOMA-IR > 2. The details of the presence of the components of metabolic syndrome according World Health Organization (WHO) criteria and according to modified Indian criteria are given in Table 2. 17% (10) patients had metabolic syndrome according to WHO criteria, but when the modified Indian criterion for WC was used 37% (22) fulfilled the criteria. Only one patient in our study population did not have any component of metabolic syndrome when Indian criteria were used. The most common feature observed was high WC (modified) in 57 (95%) followed by low HDL in 38 (63%).

Baseline comparison of compliant and non-compliant groups

Exercise compliant and non-compliant groups had no significant difference in baseline characteristics as shown in Table 3. Demographic profile, anthropometric parameters and baseline biochemical results were not different in two groups.

Effect of exercise on BMI, WC, aminotransferases

Comparison of anthropometric measurements before after 6 mo of intervention is given in Table 4. Compliant group showed significant decrease in BMI [26.7 ± 3.3 kg/m^2 *vs* 25.0 ± 3.3 kg/m^2 (P < 0.001)] and WC [95.7 ± 8.9 cm to 90.8 ± 7.3 cm (P < 0. 001)]. ALT also showed significant improvement from 84.8 ± 43.5 U/L to 41.3± 18.2 U/L, (P < 0.001) respectively.

Among compliant group, there was 2.9 kg mean weight loss in patients who were advised both exercise and dietary restriction, while 0.3 kg decrease in weight was seen in patients who were advised only exercise (normal BMI group). Noncompliant group had increase of 0.4kg weight at the end of 6 mo. Patients who were advised exercise only showed no significant change in BMI [23.0 ± 1.4 kg/m^2 *vs* 21.7 ± 1.6 kg/m^2; P = not significant (NS)], but they had significant improvement in waist circumference (88.0 ± 7.0 cm *vs* 85.4 ± 7.2 cm, P = 0.001). Patients who were advised exercise and diet restriction (high BMI) showed significant improvement in BMI, waist circumference (28.5 ± 2.4 kg/m^2 *vs* 26.6 ± 2.7 kg/m^2, P < 0.001 and 99.5 ± 7.2 cm *vs* 93.6 ± 5.5 cm, P < 0.001, respectively).

Table 1 Histological characteristics of nonalcoholic steatohepatitis on liver biopsy

Histological finding	n = 32
Steatosis	
Grade 1	12
Grade 2	15
Grade 3	5
Ballooning degeneration	8
Glycogenated nuclei	11
Necroinflammatory activity	
Grade 1	10
Grade 2	17
Grade 3	5
Staging fibrosis	
Stage 0	11
Stage 1	14
Stage 2	4
Stage 3	3

Table 2 Components of metabolic syndrome seen in our patients n = 60 (%) according to National Cholesterol Education Program adult treatment panel Ⅲ and waist modified by Indian criteria

Components present	NCEP ATP III	Indian criteria
None	7 (12)	1 (2)
1	20 (34)	13 (22)
2	23 (38)	24 (40)
3	8 (13)	18 (30)
4	2 (4)	4 (7)

NCEP ATP III: National Cholesterol Education Program adult treatment panel III.

Effect of exercise on insulin resistance and correlation with ALT

Insulin resistance showed a significant decline at the end of exercise program in the compliant group. Insulin resistance decreased significantly in both combined group and exercise alone group (6.5 ± 4.8 to 1.4 ± 1.1 and 6.2 ± 4.6 to 1.2 ± 0.8 respectively) (Figure 1). In the noncompliant group there was no significant change in insulin resistance level before and after exercise (Table 4). Using Spearman's correlation, the decline in ALT correlated with decline in insulin resistance levels in the compliant group (P = 0.01, r_s = 0.903), but there was no correlation in noncompliant group (P = NS), r_s = 0.321), (Figure 2).

At the end of follow-up, 24 compliant patients had

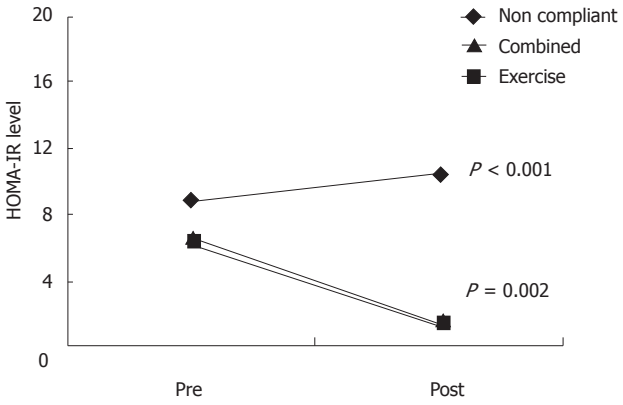

Figure 1 Diagrammatic representation of homeostasis model assessment-estimated insulin resistance levels of all the patients in the 3 groups before and after exercise. HOMA-IR: Homeostasis model assessment-estimated insulin resistance.

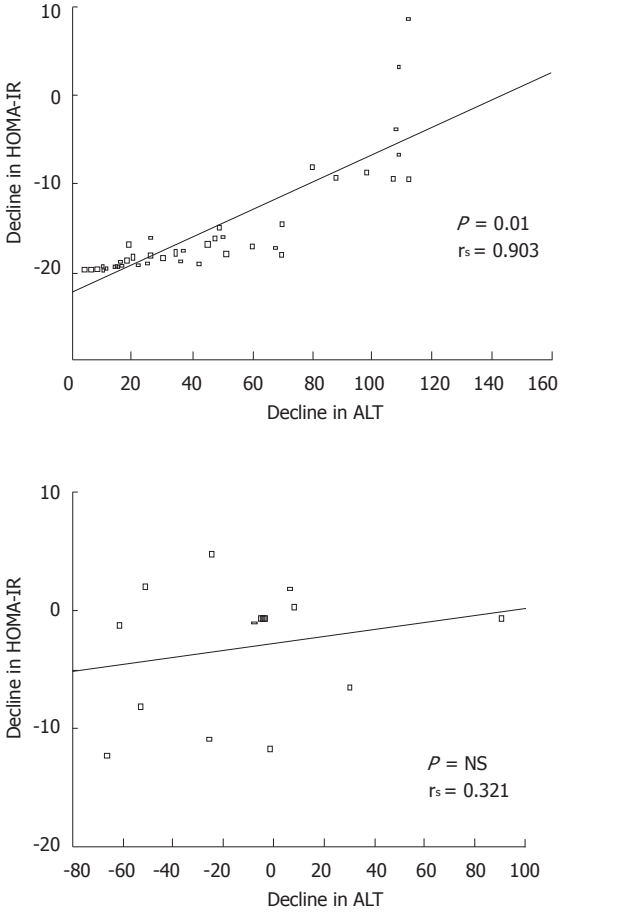

Figure 2 Correlation of decline in homeostasis model assessment-estimated insulin resistance levels to serum alanine aminotransferase in the compliant and non-compliant group using Spearman's test. NS: Not significant.

normal ALT levels while in none of the noncompliant patients ALT normalized.

Effect of exercise on liver histology and correlation with insulin resistance and anthropometry

Eight patients had repeat liver biopsy in compliant group,

Table 3 Baseline demographic, anthropometric and biochemical characteristics of patients

Characteristic	Non-compliant (n = 15)	Compliant group (n = 45)	P value[1]
Age (yr)	39.6 ± 8.9	40.1 ± 9.0	NS
Gender (Males)	9	37	
BMI (kg/m^2)	27.6 ± 3.8	26.7 ± 3.3	NS
< 25	6	15	
25-30	6	20	
> 30	3	10	
WC	98.0 ± 8.6	95.7 ± 8.9	NS
Fasting glucose (mg/dL)	86.4 ± 11.3	86.8 ± 14.3	NS
2 h glucose (mg/dL)	128.2 ± 43.5	123.5 ± 41.8	NS
Serum albumin (g/dL)	3.9 ± 0.4	4.0 ± 0.4	NS
AST (U/L)	78.3 ± 460.2	76.2 ± 46.2	NS
ALT (U/L)	82.0 ± 45.0	84.8 ± 43.5	NS
Total cholesterol (mg/dL)	202.2 ± 45.0	194.6 ± 62.0	NS
Triglycerides (mg/dL)	178.5 ± 102.6	186.4 ± 112.5	NS
LDL (mg/dL)	114.5 ± 43.5	120.4 ± 56.4	NS
HDL (mg/dL)	38.5 ± 18.5	37.4 ± 8.6	NS

[1]Mann Whitney *U* test. BMI: Body mass index; WC: Waist circumference; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDL: Low-density lipoprotein; HDL: High-density lipoprotein. NS: Not significant.

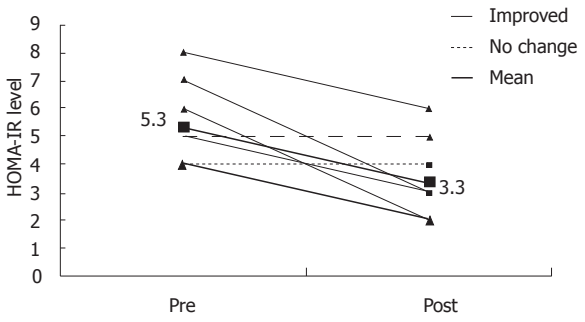

Figure 3 Change in nonalcoholic steatohepatitis score pre and post intervention (n = 8).

of which six showed improvement in steatosis, necroinflammatroy score with no change in fibrosis score and two had no change of the NASH score (Table 5 and Figures 3 and 4). Total NASH score in eight patients decreased from 5.3 ± 1.5 to 3.3 ± 1.5 (P = 0.02).

Using spearman's correlation decline in HOMA-IR correlated with decline in NASH score in these 8 patient with repeat histology (P = 0.03, r_s = 0.73). Improvement in NASH score also correlated with decline in WC (P = 0.04, r_s = 0.65), BMI (P = 0.05, r_s = 0.62) and ALT (P = 0.05, r_s = 0.54) (Table 6.)

DISCUSSION

Our study showed that regular aerobic exercise by promoting weight loss resulted in improvement in insulin resistance, aminotransferase level and liver histology after 6 mo. All these patients had insulin resistance at baseline and persistent elevation of serum aminotransferase be-

Table 4 Sub-group analysis of the compliant group - exercise alone and both exercise and diet modification and comparison with non-compliant group

Variable	Non-compliant (n = 15)			Exercise (n = 12)			Exercise and diet (n = 33)		
	Pre	Post	P value[1]	Pre	Post	P value[1]	Pre	Post	P value[1]
BMI (kg/m^2)	27.6 ± 3.8	27.7 ± 3.5	NS	23.0 ± 1.4	21.7 ± 1.6	NS	28.5 ± 2.4	26.6 ± 2.7	< 0.001
WC (cm)	99.2 ± 7.00	98.7 ± 8.4	NS	88.0 ± 7.0	85.4 ± 7.2	0.001	99.5 ± 7.2	93.6 ± 5.5	< 0.001
WHR	0.9 ± 0.3	0.9 ± 0.1	NS	0.9 ± 0.3	0.9 ± 0.1	0.006	0.9 ± 0.6	0.9 ± 0.1	0.001
ALT (U/L)	82.0 ± 45.0	78.2 ± 18.6	NS	69.8 ± 32.3	34.6 ± 13.1	0.001	90.3 ± 46.1	43.8 ± 19.4	0.001
HOMA-IR	8.7 ± 4.1	10.5 ± 9.8	NS	6.2 ± 4.6	1.2 ± 0.8	0.002	6.5 ± 4.4	1.4 ± 1.1	< 0.001

[1]Wilcoxon signed rank test. BMI: Body mass index; WC: Waist circumference; WHR: Waist-hip ratio; ALT: Alanine aminotransferase; HOMA-IR: Homeostasis model assessment-estimated insulin resistance. NS: Not significant.

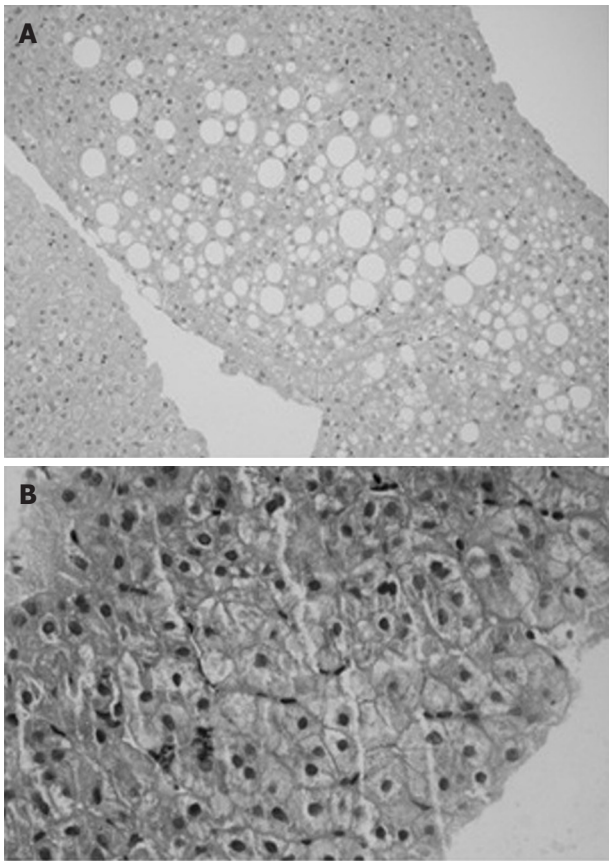

Figure 4 Liver histology pre (A) and post (B) intervention in compliant group.

Table 5 Anthropometric, biochemical and histological characteristics of patients with paired liver biopsy (n = 8)

Variable	Pre-intervention	Post-intervention	P value[1]
BMI (kg/m^2)	27.6 ± 3.8	26.0 ± 2.4	0.012
WC (cm)	96.3 ± 7.6	91.0 ± 6.3	0.01
ALT (U/L)	99.8 ± 37.2	34.0 ± 16.8	0.01
HOMA-IR	6.1 ± 3.8	1.04 ± 0.5	0.01
NASH score	5.3 ± 1.5	3.3 ± 1.5	0.02

[1]Wilcoxon sign rank test. BMI: Body mass index; WC: Waist circumference; ALT: Alanine aminotransferase; HOMA-IR: Homeostasis model assessment-estimated insulin resistance; NASH: Nonalcoholic steatohepatitis.

Table 6 Correlation between improvement in nonalcoholic steatohepatitis score and anthropometry, biochemical characteristics

Variable	r_s	P value[1]
BMI (kg/m^2)	0.62	0.05
WC (cm)	0.68	0.04
ALT (U/L)	0.54	0.05
HOMA-IR	0.73	0.03

[1]Spearman correlation. BMI: Body mass index; WC: Waist circumference; ALT: Alanine aminotransferase; HOMA-IR: Homeostasis model assessment-estimated insulin resistance.

fore entering into the study. Previous studies have shown that weight reduction results in normalization of alanine aminotransferase[16,17]. In the present study, improvement in insulin resistance and aminotransferase was seen in all exercise compliant subjects.

In an earlier study, we have shown that lifestyle modification adapted by previously sedentary patients resulted in improvement aminotransferase levels after 3 mo[17]. In the present study we demonstrated that decline in aminotransferase correlated with improvement in insulin resistance and in a small proportion of exercise compliant patient we documented histological improvement with paired liver biopsy.

There was significant correlation in the decline of ALT to improvement insulin resistance which shows the causal relation of insulin resistance in NAFLD. This proves that exercise improves insulin sensitivity resulting to decline in ALT level. Insulin resistance, with the other features of the metabolic syndrome, is now regarded major mechanism for development of NAFLD, even in the absence of obesity and diabetes mellitus[10]. Regular aerobic exercise has been shown to improve insulin sensitivity and alter substrate use in skeletal muscle. This effect is by up -regulation of insulin receptor substrate (IRS-1) which promotes GLUT4 transporter protein necessary for the uptake of glucose by muscle[28]. Exercise has also been shown to increase oxidative capacity of muscle cells and utilization of fatty acids for oxidation. This decreases fatty acids and triglyceride accumulation in the myocytes and thereby improves insulin sensitivity[29].

In this study exercise compliant patients had an average 2.9 kg weight reduction in high BMI group compared to 0.4 kg weight in noncompliant group. Previous studies

have shown benefit of weight reduction in improving serum aminotransferases, but optimum rate and amount of weight loss needed to achieve this benefit are still unclear. In obese children reduction of around 0.5 kg/wk with dietary modification had been shown to improve serum aminotransferase level[30]. We found around 5% decline in weight and BMI is sufficient to achieve significant decline in insulin resistance. Earlier studies have shown that around 10 % weight reduction results in significant improvement in clinical condition[31].

Majority patients in our study had visceral adiposity. While 30% patients had normal BMI (< 23 kg/m^2), visceral adiposity was present in 95%. Earlier studies have shown that Asian people have higher visceral fat compared to western population for a given BMI[32]. The normal BMI and WHR were also found to be lower in Asian Indians. The key finding in our study was significant improvement in IR with decline observed in waist circumference and WHR in the normal BMI group without significant reduction in their BMI. This decrease in waist circumference in these groups is likely to be due to decrease in abdominal fat stores. Regular aerobic exercise seems to redistribute the fat stores in the body, which ultimately leads to decrease in visceral obesity and heightens the insulin responsiveness in adipose tissue[33]. Data from other studies suggest that regular physical exercise reduces the risk of developing non-insulin dependent diabetes mellitus and improves blood cholesterol (LDL and HDL) level, both of which are significant risk factors for NASH[33,34]. The use of life style modification has shown more encouraging results than metformin in prevention of diabetes (58% *vs* 31%) in Diabetes Prevention Program[35]. As type 2 diabetes is the ultimate outcome of severe insulin resistance and NAFLD is considered as another consequence of insulin resistance, should also preventable by lifestyle modification. Obesity and insulin resistance with all known health consequences is now considered high priority, it is reasonable to think widespread community adoption of lifestyle modification will help to overcome present century epidemic of obesity related health hazard including NAFLD.

There is enough data to suggest reduction in visceral adiposity is an important step in improving insulin resistance. In a previous study with 32 obese patients, improvement in insulin resistance correlated with change in regional adiposity[33]. Previous data suggest exercise induced reduction in total and regional adiposity is positively associated with intensity of exercise[36]. These data corroborate our finding of improvement in IR correlates with improvement in visceral adiposity.

There is very limited data available on the efficacy of non-pharmacological and pharmacological interventions on liver histology, particularly necroinflammation with NAFLD. Two previous studies with paired liver biopsies showed improvement or stable liver histology (both necro-inflammation and fibrosis score)[37,38]. In our study eight patients had repeat liver biopsy, six had significant improvement in necroinflammatory score, 2 had stable score and none had worsening of NASH score over 6 mo period.

Improvement in NASH score correlated with improvement in insulin resistance, waist circumference, BMI and ALT. Our study highlights the fact serum aminotrasferase levels correlates with histological improvement in NAFLD patients. In a study by Gomez *et al*[38], addition of antioxidants to lifestyle modification intensifies improvement in insulin resistance and liver histology. This gives scope for future research about addition of pharmacological treatment (antioxidants and insulin sensitizers) to life style changes and their synergistic benefit on liver histology.

As this study was short-term, the greatest improvement in liver histology was seen in steatosis, and necroinflammatory score. Decrease in necroinflammatory score by $\geqslant$ 2 was observed in 2 patients and by 1 point in 4 patients indicating that life style changes was effective in decreasing hepatocyte inflammation. There was no change in fibrosis score, which is likely related to the short duration of the study.

Pharmacological treatment of NAFLD has shown variable results [11,12,39]. At present, very limited data available on impact of pharmacological agent on histological progression in NAFLD. Most promising among them are insulin sensitizer (thiazolidendiones and metformin) and antioxidants. However weight gain is known side effect of thiazolidendiones and the long-term effects in patients with NAFLD of these drugs are currently unknown.

We agree that our study was short term study, and none of noncompliant subjects had second liver biopsy as these patients were less motivated about life style changes and reluctant for repeat procedure. Despite these limitations we feel that aerobic exercise and dietary modifications improves insulin resistance resulting in histological improvement.

To summarize, based on these results, lifestyle modification improves insulin resistance and liver histology. All patients with NAFLD should be encouraged to continue moderate intensity aerobic exercise; improvement in serum ALT and insulin resistance can be used as laboratory parameters for effective treatment. We acknowledge that our study was short term and involved highly motivated patients, and confirming these results requires larger randomized controlled trials. Despite these limitations, we feel it is important to recommend lifestyle changes as the first line therapy in the treatment of NAFLD.

COMMENTS

Background

With the global epidemic of obesity, the problem of Nonalcoholic fatty liver disease (NAFLD) is going to be increasingly encounterd by clinicians. Most clinicians with emerging clinical epidemiological evidence regard NAFLD as part of metabolic syndrome. At present there is no established mode for treatment or prevention of NAFLD and metabolic syndrome. Short term weight losing measures has shown to improve obesity and aminotransferase levels, but its effect on insulin resistance and liver histology has not been fully established.

Research frontiers

Insulin resistance has been recognized as major mechanism (first hit) for the development of NAFLD. In this study author demonstrated improvement in in-

sulin sensitivity with life style modification and its benefit on liver histology.

Innovations and breakthroughs

Recent studies have shown that life style modification shows improvement in liver histology. In this study, authors showed improvement in insulin sensitivity correlates with improvement in liver histology. Improvement in aminotransferase level and insulin resistance with life style modification can be used as a laboratory parameter for effective treatment.

Applications

Future studies needed to evaluate the effect of life style modification with pharmacological treatment (insulin sensitizer) on insulin sensitivity and liver histology.

Terminology

Inulin resistances play key role in the pathogenesis of NAFLD. homeostasis model assessment-estimated insulin resistance method, even though not so accurate, can be used as simple method to calculate insulin resistance in clinical practice.

Peer review

In this paper, the authors found that in exercise compliant individuals decline in insulin resistance correlated with decline in alanine aminotransferase levels and liver histology. An increase in serum ferritin level is feature of NAFLD and authors have to discuss effect on ferritin levels. An intake of fish is associated with insulin resistance and ALT levels, authors' needs to investigate the change in dietary habits. Inflammation is associated with insulin resistance and authors needs to demonstrate data for inflammation including C - reactive protein and interleukin-6. One of the key issues is how to treat NAFLD patient with poor compliance for lifestyle modification. The authors have to discuss the point by referring the new exercise device.

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ORIGINAL ARTICLE

Prevalence and virological profiles of hepatitis B infection in human immunodeficiency virus patients

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Abstract

AIM: To determine the prevalence of hepatitis B virus (HBV) in adult human immunodeficiency virus (HIV) patients with CD4+ T-cell count less than 500/mm^3 and without antiretroviral therapy; to describe different HBV-HIV coinfection virological profiles; and to search for factors associated with HBs antigen (HBsAg) presence in these HIV positive patients.

METHODS: During four months (June through September 2006), 491 patients were received in four HIV positive monitoring clinical centers in Abidjan. Inclusion criteria: HIV-1 or HIV-1 and 2 positive patients, age $\geqslant$ 18 years, CD4+ T-cell count < 500/mL and formal and signed consent of the patient. Realized blood tests included HIV serology, CD4+ T-cell count, quantitative HIV RNA load and HBV serological markers, such as HBsAg and HBc antibody (anti-HBcAb). We performed HBeAg, anti-HBe antibody (anti-HBeAb), anti-HBc IgM and quantitative HBV DNA load in HBsAg positive patients. Anti-HBsAb had been tested in HIV patients with HBsAg negative and anti-HBcAb-positive. HBV DNA was also tested in 188 anti-HBcAb positive patients with HBsAg negative status and without anti-HBsAb. Univariate analysis (Pearson χ^2 test or Fischer exact test) and multivariate analysis (backward step-wise selection logistic regression) were performed as statistical analysis.

RESULTS: Mean age of 491 patients was 36 ± 8.68 years and 73.3% were female. Type-1 HIV was found in 97% and dual-type HIV (type 1 plus type 2) in 3%. World Health Organization (WHO) clinical stage was 1, 2, 3 and 4 respectively in 61 (12.4%), 233 (47.5%), 172 (35%) and 25 patients (5.1%). Median CD4+ T-cell count was 341/mm^3 (interquartile range: 221-470). One hundred and twelve patients had less than 200 CD4+ T-cell/mm^3. Plasma HIV-1 RNA load was elevated ($\geqslant$ 5 log$_{10}$ copies/mL) in 221 patients (45%). HBsAg and anti-HBcAb prevalence was respectively 13.4% and 72.9%. Of the 66 HBsAg positive patients, 22 were inactive HBV carriers (33.3%), 21 had HBeAg positive hepatitis (31.8%) and 20 had HBeAg negative hepatitis (30.3%). HBeAg and anti-HBeAb were indeterminate in 3 of them. Occult B infection prevalence (HBsAg negative, anti-HBcAb positive, anti-HBsAb negative and detectable HBV DNA) was 21.3%. Three parameters were significantly associated with the presence of HBsAg: male [odds ratio (OR): 2.2; P = 0.005; 95% confidence interval (CI): 1.3-3.8]; WHO stage 4 (OR: 3.2; P = 0.01;

95% CI: 1.3-7.9); and aspartate aminotransferase (AST) level higher than the standard (OR: 1.9; P = 0.04; 95% CI: 1.02-3.8).

CONCLUSION: HBV infection prevalence is high in HIV-positive patients. HBeAg positive chronic hepatitis and occult HBV infection are more frequent in HIV-positive patients than in HIV negative ones. Parameters associated with HBsAg positivity were male gender, AIDS status and increased AST level.

Key words: Hepatitis B virus-human immunodeficiency virus coinfection; Prevalence; Virological profiles; Black Africa

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INTRODUCTION

Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) infections are real public health problems, particularly in high-prevalence areas such as sub-Saharan Africa. In the world, there are more than 30 million people living with HIV/AIDS (two thirds of which live in sub-Saharan Africa) and about 350 to 400 million chronic HBV carriers[1-3]. HBV infection was reported to increase mortality and morbidity in HIV patients. Indeed, HIV infection increases chronic HBV infection risk and promotes faster progression to cirrhosis and its complications, especially when HBV replication is important[4-9]. If HBV-HIV coinfection is very common in sub-Saharan Africa, there are few data on HBV infection virological aspects in HIV Black African patients[10-14]. This study's aims were to estimate HBV infection prevalence among an adult population, with HIV infection, less than 500 CD4+ T-cell/mm^3 and without antiretroviral therapy (ART), to describe the different profiles of virological B co-infected subjects and to search for HBs antigen (HBsAg) presence associated factors in these HIV patients.

MATERIALS AND METHODS

This is a multicenter cross-sectional study conducted in Abidjan, Cote D'Ivoire and approved by the Ivorian Ministry of Health ethics committee. We included all adult patients who had a consultation in one of the four sites identified for the study over the period of June 1 to September 31, 2006 and who met the following inclusion criteria: HIV-1 or HIV dual (1 and 2) infection, no history of ART, last available CD4+ T-cell count less than 500/mm^3, unknown previous HBV status and informed consent to participate in the study. These four recruitment centers (Integrated Center of Bioclinical Research of Abidjan, General Medicine Department of Yopougon Teaching Hospital, Department of Infectious and Tropical Diseases of Treichville Teaching Hospital, Integrated Center for Bio-Clinical Research in Treichville, Abidjan) were regular monitoring and support centers for HIV-infected people. Socio-demographic data, clinical history and physical examination data (including clinical manifestations of liver disease) were collected on a standardized basis. Blood samples were obtained from all patients after they signed a written agreement. The following tests were routinely performed: blood count (MaxM® Coulter Beckman Coulter, Fullerton, CA, United States), a measurement of serum transaminase assays (Cobas Integra 400 plus® Roche Diagnostics, Mannheim, Germany), a CD4+ T-cell count by flow cytometry (FACSCalibur®Becton Dickinson, San Jose, CA, United States), plasma HIV-1 RNA levels quantification (Generic HIV, viral load assay threshold detectability of 300 copies/mL, Biocentric, Bandol, France) and a search for HBsAg and anti-HBc antibody (anti-HBcAb) (Mini Vidas®, Biomerieux, Marcy l'Etoile, France). All HBsAg positive patients were tested for HBeAg, anti-HBe antibody (anti-HBeAb) and IgM anti-HBc. We searched for anti-HBs Ab in all HBsAg negative and anti-HBcAb positive patients. We also conducted a plasma HBV DNA quantitative determination test in all HBsAg positive patients and in the first 188 patients with HBsAg negative, anti-HBcAb positive and anti-HBs Ab negative (Cobas® Amplicor HBV Monitor assay threshold detectability of 35 copies/mL or 6 IU/mL, Roche Diagnostics). We detected HBV infection in different virological profiles in these patients, according to laboratory tests results. HBeAg positive patients were considered infected with wild-type virus (HBeAg positive hepatitis); HBeAg negative patients with normal transaminases and viremia under 2000 IU/mL were considered inactive HBV carriers; HBeAg negative patients with elevated transaminases and viremia higher than 2000 IU/mL were considered infected with precore mutant virus (HBeAg negative hepatitis); occult HBV infection was diagnosed when HBV DNA was detectable in patients with HBsAg negative, anti-HBcAb positive and anti-HBsAb negative status.

Statistical analysis

In univariate analysis, we compared the differences between HBsAg positive and HBsAg negative patients using the Pearson χ^2 test or the Fisher exact test. A multivariate analysis (backward stepwise logistic regression) was performed to identify factors likely to be associated with the

presence of HBsAg positivity in HIV patients. Variables included in the univariate analysis were: age, gender, body mass index (BMI), World Health Organization (WHO) stage, CD4+ T-cell count, liver enzymes and HIV viral load. All variables with "*P*" value under 0.25 in univariate analysis were included in the multivariate analysis initial model. Statistical analysis was performed using WSTATA version 9.0 software.

RESULTS

Of 608 HIV patients contacted during the study period, 506 (83.2%) met inclusion criteria. Finally, 491 of them (97%) were included in the study. Figure 1 represents the flow chart of the study population distribution according to HBV serological markers. Mean age was 36.1 ± 8.68 years (range 18-66 years) and 73.3% were women. Overall, 98 (20%) were illiterate, 171 (34.8%) had primary school education and 222 (45.2%) had at least secondary school level of education. Thirty-three patients (6.7%) had reportedly received an HBV vaccine, 17 patients (3.5%) had an accidental blood exposure history and 39 patients (7.9%) a blood transfusion history. The distribution, by WHO clinical stage, was 61 patients (12.4%), 233 patients (47.5%), 172 patients (35%) and 25 patients (5.1%), respectively, in stage 1, 2, 3 and 4. Jaundice and hepatomegaly were found respectively in 6 (1.2%) and 18 (3.7%) patients. Table 1 shows clinical and laboratory features of 491 patients. HBV DNA was present in 59 out of 66 HBsAg positive patients (89.4%). Among HBsAg positive 66 patients, 21 (31.8%) had HBeAg positive hepatitis, 20 (30.3%) had HBeAg negative hepatitis and 22 (33.3%) had a profile of HBV inactive carrier. For 3 patients with HBsAg positive (4.6%), HBeAg and anti-HBeAb were negative. Biochemical and virological profile of these three patients was comparable to the 22 HBV inactive carriers (normal transaminases and viral DNA B less than 2000 IU/mL). Anti-HBc IgM was present in 2 of 66 HBsAg positive patients (3%). In both patients, transaminases were normal, HBeAg positive, anti-HBeAb negative and very high viral load (6 090 000 IU/mL and 110 000 000 IU/mL). Occult B infection was found in 40 of 188 patients (21.3%). Table 2 summarizes HBV DNA quantitative values according to HVB infection type. Thirty-three previously vaccinated patients were all positive for anti-HBcAb. Six of them were HBsAg positive and of the 27 remaining patients negative for HBsAg, 13 had anti-HBsAb. The relationship between HBsAg presence and baseline patient characteristics are summarized in Table 3. In multivariate analysis, male gender, WHO stage 4 and elevated aspartate aminotransferase (AST) level were found to be significantly associated with HBsAg positivity.

DISCUSSION

Our study results confirm the high prevalence of HBV infection among HIV patients in Côte d'Ivoire, and more generally in sub-Saharan Africa, as evidenced by most studies on the subject[10,13,15-22]. Indeed, the prevalence of HBsAg and that of anti-HBcAb were respectively 13.4% and 72.9% in our patients. In African studies comparing HBV infection prevalence in HIV-positive and HIV-negative patients, rates of HBsAg and anti-HBcAb did not differ significantly by HIV status[10,11,15,16]. In areas with high HBV endemicity, such as sub-Saharan Africa, two main contamination modes are perinatal or vertical transmission and horizontal transmission within the family in early childhood[23-28]. In these areas, contamination

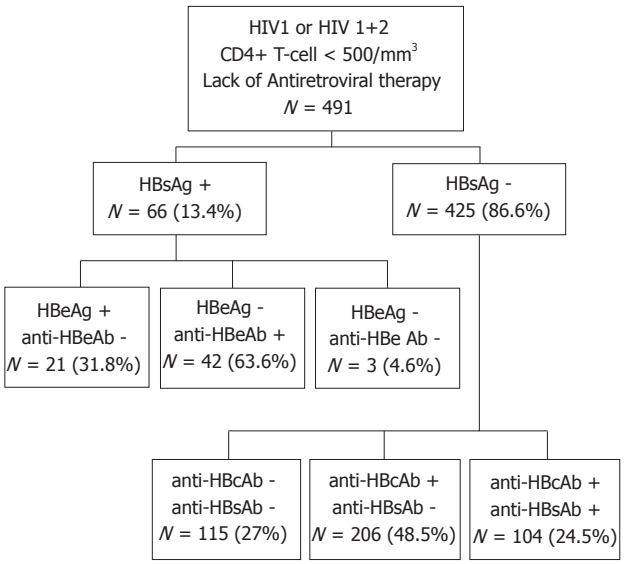

Figure 1 Study population distribution flow chart through hepatitis B virus serological markers. HBsAg: HBs antigen; anti-HBcAb: anti-HBc antibody; anti-HBeAb: anti-HBe antibody.

Table 1 Study population baseline characteristics (491 patients)

Patients baseline characteristics	
Female gender, *n* (%)	360/491 (73.3)
Median age, yr (IQR)	35 (30-41)
BMI (kg/m^2), *n* (%)	
< 18.5	115 (23.4)
18.5-25	281 (57.2)
> 25	95 (19.4)
WHO clinical stage, *n* (%)	
1 or 2	294/491 (60)
3 or 4	197/491 (40)
HIV serology, *n* (%)	
HIV-1	476 (97)
HIV-1 and HIV-2	15 (3)
Median CD4+ T-cell count (/mm^3) (IQR)	341 (221-470)
CD4+ T-cell < 200/mm3, *n* (%)	112/491 (22.8)
Median plasma HIV RNA (Log$_{10}$ copies/mL) (IQR)	4.87 (4.15-5.45)
Plasma HIV-1 RNA > 5 log$_{10}$ copies/mL, *n* (%)	221/491 (45)
Serum transaminase level, *n* (%)	
AST > UNV	81/491 (16.5)
ALT > UNV	41/491 (8.4)

IQR: Interquartile range; BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; UNV: Upper normal value (50 UI/mL); WHO: World Health Organization; HIV: Human immunodeficiency viruses.

Table 2 Hepatitis B virus DNA quantitative values by hepatitis B virus infection type

Type of viral B infection	Viral infection B DNA (UI/mL)		
	Median	Interquartile range	Range
HBeAg positive hepatitis	2.1×10^7	1.1×10^7-1.1×10^8	27200-1.1×10^8
HBeAg negative hepatitis	139 000	8760-1.1×10^8	3030-1.1×10^8
Viral B inactive chronic carriers	331	62-641	8-1540
Occult B infection	46	16-149	7-258

by HIV occurs generally late in adolescents and adults because of sexual transmission predominance. By contrast, in areas of low HBV endemicity, such as western countries, most infections occur in adolescents and young adults. Vertical and horizontal transmissions within the family in early childhood are marginal. HBV infection transmission is mainly parenteral and sexual[9,29]. Contamination of both viruses generally occurs in the same period in young adults and sexually active adolescents. Thus, in these areas, HBV infection among HIV-positive patients is ten times more common than among HIV-negative ones[9,29].

In our study, 33 patients reported having received a complete HBV vaccine (Genhevac B or Euvax B recombinant vaccine). HBV vaccine was not systematic in HIV patients. Besides, vaccines were administered to these adults without preliminary assessment to eliminate previous viral B infection. Indeed, the assessment of these patients showed that 6 of them had active viral B infection (HBsAg positive and anti-HBcAb positive), 13 patients had past HBV infection with immunization status (HBsAg negative, anti-HBcAb and anti-HBs Ab positive) and 14 of them had past B virus infection with anti-HBs Ab clearance (HBsAg negative, anti-HBcAb positive without anti-HBs Ab). Several studies showed that HBV vaccination efficiency depends on the immunity status of HIV-positive patients[30-33]. Therefore, there is a good correlation between CD4+ T-cell count and vaccinal response[30-33]. This aspect has never been studied in our context.

Among HBsAg positive patients, the proportion of inactive carriers of HBV and that of patients with HBeAg positive hepatitis or HBeAg negative hepatitis were similar (about 30% for each of the three groups). The prevalence of patients with HBeAg positive hepatitis seemed higher in our study compared with HIV-negative data, confirming the results of previous African studies[10,25,26,28,34,35]. Moreover, our study reported a 21.3% prevalence for occult B infection. In a South African study[11], the authors compared occult B virus infection rates among HIV-positive and HIV-negative patients. Occult B infection prevalence was significantly higher among HIV-positive subjects (22.1% against 2.4% in HIV-negative subjects, $P < 0.001$). In contrast, B viremia of our patients with occult infection was not higher than values found in HIV-negative cases[11,13,36-39]. Most Western studies show that HIV infection reduces the likelihood of spontaneous recovery from HBV infection, promotes progression to chronicity, cirrhosis and its complications, HBV seroreversion, HBV reactivation and occult B infection[4-9]. WHO recommends that in countries with limited resources, if routine HBV DNA testing is not feasible, ART must start earlier in HIV patients carrying HBsAg, irrespective of CD4+ T-cell count or WHO clinical stage[40]. This treatment shall include a nucleosidic analogue (lamivudine or emtricitabine) and a nucleotidic analogue (tenofovir). In this context, the role of HBV DNA must be specified in HBV-HIV coinfected patients, especially for occult B infection diagnosis and treatment.

Three parameters were associated with the presence of HBsAg in our patients: male gender (OR: 2.2; $P = 0.005$; 95% CI: 1.3-3.8); WHO stage 4 (OR: 3.2; $P = 0.01$; 95% CI: 1.3-7.9); and increased level of AST (OR: 1.9; $P = 0.04$; 95% CI: 1.02-3.6). Male gender predominance has been reported in several HBV-HIV coinfection studies[12,19,41]. Moreover, it is now well admitted that in HIV patients with AIDS status and HBV coinfection, HBV infection is more likely to evolve to chronic disease compared to HIV-HBV co-infected patients with much higher level of CD4+ T-cell count or to HBV mono-infected patients[4-9]. Because of immunosuppressant, seroreversion and HBV reactivation are more likely to occur in them[4-9]. The most frequent elevation of AST in our HBsAg positive patients is more difficult to interpret as non-specific and probably of multifactorial origin (weight loss with muscle wasting, liver opportunistic disease localization, active hepatitis B disease, hepatitis due to another virus). Taking hepatotoxic drugs for opportunistic infections treatment and alcohol abuse were excluded by systematic search for these factors for the inclusion of our patients. Literature data show that, besides drug-induced liver toxicity (ART, anti-tuberculosis therapy or other treatments), promoted itself by the existence of a chronic viral liver disease, transaminases elevation is fairly well correlated with HBsAg presence in HIV patients[41,42].

HBV infection prevalence is elevated among our HIV patients. This prevalence seems similar to that observed in HIV-negative subjects. Among HBV serological profiles observed in our study, HBeAg positive chronic hepatitis and occult HBV infection are more frequent in HIV-positive patients than in HIV negative ones. Parameters associated with HBsAg positivity were male gender, AIDS status and increased AST levels. In HIV patients, HBV serological markers (especially HBsAg) must be part of the initial check-up. When HBV-HIV coinfection is diagnosed, ART must include molecules likely to be active on both viruses. Determination of HBV DNA load should be performed in HIV-infected patients with HBsAg negative, anti-HBcAb positive and anti-HBsAb negative in order to detect occult HBV infection cases that can also benefit from the same ART as HBsAg positive HIV patients.

Table 3 Relationship between HBs antigen positivity and baseline characteristics

Baseline characteristics	Univariate analysis			Multivariate analysis	
	HBsAg (+)[1] *n* = 66 (%)	HBsAg (-)[1] *n* = 425 (%)	*P* value	OR (95% CI) *n* = 491	*P* value
Age > 35 yr[2]	57.6	48	0.15	-	-
Gender, male	43.9	24	0.001	2.2 (1.3-3.8)	0.005
BMI < 18.5 kg/m^2	34.9	21.7	0.06	-	-
WHO stage 4[3]	13.6	3.8	0.003	3.2 (1.3-7.9)	0.01
AST > UNV	30.3	14.4	0.001	1.9 (1.02-3.6)	0.04
ALT > UNV	16.7	7.1	0.009	-	-
CD4+ T-cell < 200/mm3	34.9	20.9	0.01	-	-
HIV RNA ≥ 5 Log	59.1	42.8	0.02	1.5 (0.9-2.7)	0.12

[1]HBsAg positive and HBsAg negative. [2]median age. [3]Stage four of World Health Organization (WHO) clinical classification or AIDS. BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; UNV: Upper normal value (50 UI/mL); HBsAg: HBs antigen.

COMMENTS

Background

Hepatitis B virus (HBV) is the leading cause of chronic liver disease and liver-related death worldwide, with the majority of these cases occurring in African and Asian areas where HBV prevalence is high. Most of the countries affected by hepatitis B are also affected by a high human immunodeficiency viruses (HIV) burden, leading to frequent HIV-HBV co-infection. However, few data are available on HIV-HBV co-infection from regions with high chronic hepatitis B prevalence.

Research frontiers

Describe virological profiles of HBV-HIV co-infection in sub-Saharan Africa area and their particularity regarding worldwide data.

Innovations and breakthroughs

Our study results confirm the high prevalence of HBV infection among HIV patients in sub-Saharan Africa, as evidenced by most studies on the subject. Besides, among HBV serological profiles observed in our study, HBeAg positive chronic hepatitis and occult HBV infection are more frequent in HIV-positive patients than in HIV negative ones. These observations may be due to late consultation of our patients and an advanced stage of HIV disease (40.1% were at World Health Organization (WHO) stage 3 or 4 and 22.8% with less than 200 CD4+ T-cell/mm^3). At this stage, occult infections, HBV seroreversions and reactivations are more frequent.

Applications

In countries with limited resources, if routine HBV DNA testing is not feasible, antiretroviral therapy (ART) must start earlier in HIV patients carrying HBsAg, irrespective of CD4+ T-cell count or WHO clinical stage. This treatment should include a nucleosidic analogue (Lamivudine or Emtricitabine) and a nucleotidic analogue (Tenofovir). DNA VHB measurement should be a part of the initial checkup tests of the HIV positive patient carrier of Ag HBs as well as the patients who have a past HBV infection without immunization (HBsAg negative and anti-HBc positive without anti-HBs) in order to facilitate occult HBV infections diagnosis and management.

Terminology

HBeAg positive hepatitis (patients infected with wild-type virus): HBeAg positive patients with elevated transaminases and HBV DNA higher than 2000 IU/mL; HBeAg negative hepatitis (patients infected with precore mutant virus): HBeAg negative patients with elevated transaminases and HBV DNA higher than 2000 IU/mL; Inactive HBV carriers: HBeAg negative patients with normal transaminases and HBV DNA under 2000 IU/mL; Occult HBV infection: HBV DNA detectable in patients with HBsAg negative, anti-HBcAb positive and anti-HBs Ab negative status.

Peer review

In this descriptive and analytical study, the authors describe different HBV-HIV co-infection virological profiles and analyze the relationship between the patient's baseline characteristics and HBsAg positivity. The results are interesting and suggest that HBV infection diagnosis and ART start-up (Tenofovir with Lamivudine or Emtricitabine) should be earlier to improve HBV-HIV co-infection prognosis.

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BRIEF ARTICLE

Lifestyle intervention in non-alcoholic fatty liver disease in Chengyang District, Qingdao, China

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Abstract

AIM: To evaluate the effect of a 6 and 12 mo lifestyle modification intervention in nonalcoholic fatty liver diseases (NAFLD) in Chengyang District of Qingdao.

METHODS: Participants with NAFLD who had resided in Chengyang District for more than 5 years were enrolled in this study. After the 6 and 12 mo lifestyle modification intervention based on physical activity, nutrition and behavior therapy, parameters such as body weight, body mass index (BMI), waist circumference, serum alanine aminotransferase (ALT), aspartate aminotransferase values, serum cholesterol, triglycerides, fasting glucose, fasting insulin and visceral fat area (VFA), the liver-spleen ratio and the homeostasis model assessment of insulin resistance (HOMA-IR) were evaluated and compared between participants with and without the intervention.

RESULTS: Seven hundred and twenty-four participants were assigned to the lifestyle intervention group (LS) and 363 participants were assigned to the control group (CON). After the intervention, body weights in the LS group were significantly decreased compared to those in the CON group at 6 mo (11.59% ± 4.7% *vs* 0.4% ± 0.2%, $P = 0.001$) and at 12 mo (12.73% ± 5.6% *vs* 0.9% ± 0.3%, $P = 0.001$). Compared with the CON group, BMI was more decreased in the LS group after 6 and 12 mo ($P = 0.043$ and $P = 0.032$). Waist circumference was more reduced in the LS group than in CON ($P = 0.031$ and $P = 0.017$). After the 6 and 12 mo intervention, ALT decreased significantly in the LS group ($P = 0.003$ and $P = 0.002$). After 6 and 12 mo, the metabolic syndrome rate had decreased more in the LS group compared with the CON group ($P = 0.026$ and $P = 0.017$). After 12 mo, the HOMA-IR score decreased more obviously in the LS group ($P = 0.041$); this result also appeared in the VFA after 12 mo in the LS group ($P = 0.035$).

CONCLUSION: Lifestyle intervention was effective in improving NAFLD in both 6 and 12 mo interventions. This intervention offered a practical approach for treating a large number of NAFLD patients in the Chengyang District of Qingdao.

Key words: Non-alcoholic fatty liver disease; Lifestyle

intervention; Obese

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INTRODUCTION

In recent years, fuelled by rapid urbanization and abundant dietary and sedentary lifestyles, more chronic diseases have emerged with obesity in Chinese citizens. Nonalcoholic fatty liver (NAFLD) has appeared, accompanied with an obesity phenotype that is associated with several negative metabolic aberrations, including dyslipidemias, hypertension, insulin resistance and the metabolic syndrome[1]. NAFLD has been increasingly recognized as a condition strongly involved in the pathogenesis of the epidemically spreading metabolic diseases, type 2 diabetes and cardiovascular disease[2,3]. One study showed that women in the high liver fat group also had significantly higher fasting insulin, triglycerides, insulin resistance and blood pressure[4]. NAFLD could also lead to liver inflammation, fibrosis and even develop to cirrhosis. Recent studies indicated that in the general population in Shanghai and Guangdong, the prevalence of fatty liver were 17% and 15%, respectively[5]. Chengyang District is one of new developing downtown areas in Qingdao city. With the high calorie dietary as well as sedentary behaviors of Chengyang's residents, obesity has become common in our daily clinical practice and the number of NAFLD is escalating in this area.

It is apparent that weight loss and lifestyle modification should be the primary target for treating NAFLD. Previous studies have reported a beneficial effect of a recommended diet combined with an increase in physical activity on the progression of NAFLD[6]. One study showed that a 7% to 10% weight reduction through intensive lifestyle intervention would lead to improvements in biochemical and histological features of nonalcoholic steatohepatitis (NASH)[7]. At present, there are still no established methods for intensive lifestyle modification in NAFLD. The aim of our study was to evaluate a 12 mo lifestyle modification intervention for NAFLD patients in Chengyang District and evaluate its effect of ameliorating NAFLD.

MATERIALS AND METHODS

We recruited participants between January 2008 and October 2011 in this study. Participants were required to have elevated alanine aminotransferase (ALT) or aspartate aminotransferase (AST) values (ALT > 41 or AST > 34 U/L) and body mass index (BMI) between 25 and 40. All participants had resided in Chengyang District for more than 5 years (Figure 1).

All participants underwent abdominal ultrasonography and NAFLD was diagnosed according to the following criteria: (1) slight diffuse increase in bright homogeneous echoes in the liver parenchyma with normal visualization of the diaphragm and portal and hepatic vein borders and normal hepatorenal echogenicity contrast; (2) diffuse increase in bright echoes in the liver parenchyma with slightly impaired visualization of the peripheral portal and hepatic vein borders; and (3) a marked increase in bright echoes at a shallow depth with deep attenuation, impaired visualization of the diaphragm and marked vascular blurring[8]. Exclusion criteria were: (1) hepatitis B virus (HBV) infection (HBV surface antigen and anti-HBc antibodies) or hepatitis C virus (HCV) infection (anti-HCV antibodies); (2) autoimmune liver disease or alcoholic liver disease (20 g/d alcohol); (3) use of medications associated with steatosis or steatohepatitis; and (4) use of insulin-sensitizing medications.

Participants were randomly assigned to a lifestyle intervention group or a control group in a 2:1 ratio. Randomization was performed using a random number generator developed by the project statistician. Data collection was obtained by trained staff not aware of the group assignment or sequence of measurement. The ultrasonography operator was blinded to the groups. Participants were allowed to start a new medication for management of hyperglycemia if medically necessary. Sulfonylureas, meglitinides, insulin and insulin-sensitizing agents (thiazolidinediones and metformin *etc.*) were available options.

A total of 1533 NAFLD patients were enrolled in the screening phase of the study; 1087 subjects completed the screening evaluation and underwent randomization. The baseline characteristics of the participants who underwent randomization are shown in Table 1.

Participants in the control group were provided with basic education about NAFLD and principles of healthy eating, physical activity and weight control. The intervention group of lifestyle modification consisted of a diet tailored for the individual's requirements and increased physical exercise. The nutritional course was based on a fat- and sugar- reduced diet compared with the common everyday nutrition of people in Qingdao. The diet contained 30% fat, 15% proteins and 55% carbohydrates, including 5% sugar. The nutritional course was based on the prevention concept of the optimized mixed diet; the scientific recommendations were translated into food-based dietary guidelines with consideration of the dietary habits and customs in Qingdao Chengyang. The target dietary energy intake was defined as standard ideal body weight of 25-30 kcal/kg. The exercise therapy consisted of walking, jogging, stair climbing and instructions in physical exercise as part of everyday life, especially a re-

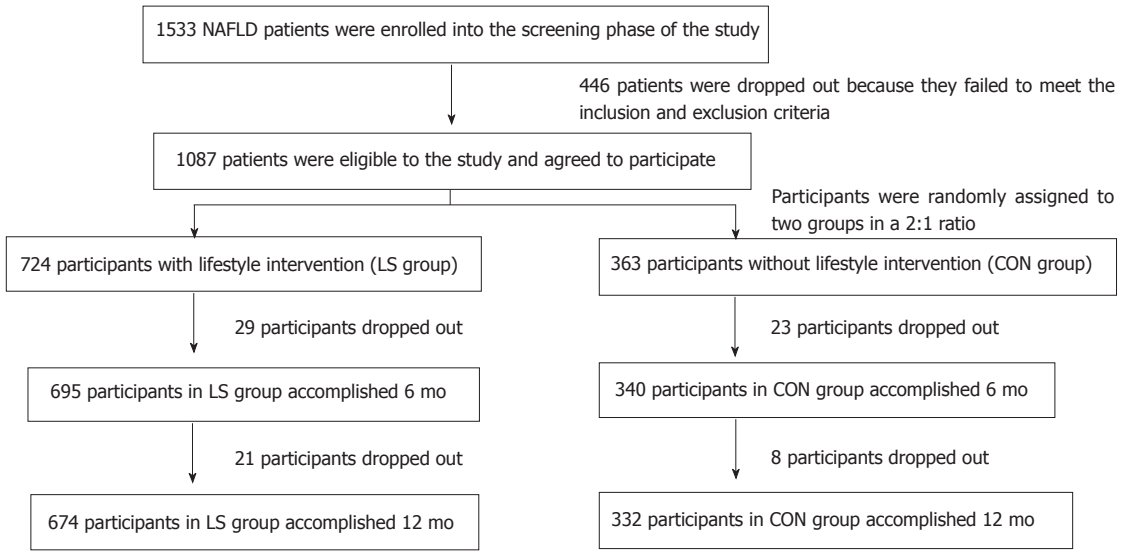

Figure 1 Recruitment process of the study. NAFLD: Non-alcoholic fatty liver disease. LS: Lifestyle intervention group; CON: Control group.

Table 1 Baseline characteristics of participants

Variable	LS (n = 724)	CON (n = 363)	P value
Gender (M/F)	464/260	229/134	0.632
Age (yr)	39.9 ± 13.1	37.4 ± 18.2	0.221
Weight (kg)	88.5 ± 18.4	84.3 ± 21.8	0.711
Waist circumference (cm)	106.3 ± 23.2	108.8 ± 15.7	0.954
BMI	37.7 ± 12.7	38.4 ± 17.1	0.842
ALT (IU/L)	59.3 ± 20.1	58.3 ± 18.8	0.513
AST (IU/L)	57.8 ± 26.6	59.3 ± 12.9	0.182
GGT (IU/L)	61.3 ± 11.2	59.8 ± 19.6	0.215
Cholesterol (mmol/L)	5.7 ± 0.9	5.8 ± 1.1	0.167
Triglycerides (mmol/L)	2.4 ± 0.4	2.2 ± 0.6	0.094
Fasting glucose (mmol/L)	7.5 ± 1.3	7.4 ± 1.6	0.332
Fasting insulin (μIU/mL)	17.7 ± 4.3	19.5 ± 5.7	0.185
HOMA-IR	2.4 ± 0.7	2.3 ± 0.9	0.231
L/S ratio	0.8 ± 0.3	0.7 ± 0.2	0.423
VFA, cm^2	135.6 ± 38.7	138.9 ± 39.4	0.172
Diabetes (%)	66 (6.1)	62 (5.7)	0.876
Metabolic syndrome (%)	201 (18.5)	186 (17.1)	0.563

Data as percentage or mean ± SE. BMI: Body mass index; ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: γ-glutamyltransferase; HOMA-IR: Homeostasis model assessment of insulin resistance; VFA: Visceral fat area; L/S: Liver-spleen.

duction in the amount of time spent watching television. Exercise therapy was performed to achieve a target of 23 metabolic equivalent tasks (METs)•h/week (physical activity) + 4 METs•h/week (exercise)[9-11].

Body weight (kg), height (m), BMI and waist circumference (cm^2) were measured. Venous blood samples were taken from all patients at around 8am. After a 12 h overnight fast, hepatic function and total cholesterol, triglyceride, fasting plasma glucose (FPG) and plasma insulin were determined by automatic biochemistry analyzer (Hitachi, Japan). Homeostasis model assessment (HOMA) was used to detect the degree of insulin resistance by the formula: (HOMA) = [insulin (mU/L) × glucose (mmol/L)]/22.5[12].

The metabolic syndrome was diagnosed according to the International Diabetes Federation (IDF) consensus worldwide definition[13]. The IDF definition requires central obesity (measured as ethnic group-specific thresholds for waist circumference; for Chinese people: ⩾ 80 cm for females and ⩾ 90 cm for males) plus any two of the following four components: (1) serum triglycerides 1.70mmol/L or more, or specific treatment for this lipid abnormality; (2) high density lipoprotein-cholesterol 1.03 mmol/L or less in males and 1.29 mmol/L or less in females, or specific treatment for this lipid abnormality; (3) blood pressure 130/85 mmHg or more, or treatment for previously diagnosed hypertension; and (4) FPG 6.16 mmol/L or more, or previously diagnosed type 2 diabetes[14,15].

The abdominal computed tomography (CT) protocol was as follows. Before starting the intervention, the patient's liver fat deposition was assessed by an abdominal CT scan to determine the liver-spleen ratio (L/S ratio) and visceral fat accumulation [visceral fat area (VFA), cm^2]. Images were reconstructed at 10 mm increments. All patients underwent abdominal CT in the morning after a 12 h overnight fast. VFA was measured at the level of the umbilicus by FatScan software version 3.0. Radiological assessments were made every 6 mo after starting the treatment. All patients provided written informed consent and the study protocol was approved by the Ethics Committee of Qingdao Chengyang People's Hospital.

At baseline, there were 724 patients in the lifestyle intervention group (LS) group and 363 patients in the control group (CON) group. After 6 mo, 29 patients in the LS group and 23 patients in the CON group were excluded from the results because they refused to continue the investigation, without any reasons. After 12 mo, a total of 50 patients in the LS group and 31 patients in the CON group were excluded, as shown in Figure 1.

Statistical analysis

Continuous variables are summarized as mean ± SD. Pearson χ^2 was used to measure the enumeration data between two groups. Proportions and categorical variables were tested by the χ^2 test. Analysis of variance (ANOVA) for repeated measurements was used to examine differences between two groups with and without lifestyle intervention. The overall within-subject effect of the intervention was estimated in a doubly-multivariate repeated-measures ANOVA (for repeated measurements). Post hoc tests compared means of different time points and were adjusted for multiple testing. All statistical analyses were conducted using SPSS 13.0. A two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the participants

For over 6 mo, 96.0% (695/724) of patients in the LS group and 93.7% (340/363) patients in the CON group were followed. There was no difference in the follow-up ratio between these two groups ($P = 1.457$). After 6 mo, there were no significant differences between these two groups for other factors of age, height, fasting glucose and fasting insulin (all $P > 0.05$).

After 12 mo, there were 93.1% (674/724) of patients in the LS group and 91.5% (332/363) patients in the CON group. There was no difference in the follow-up ratio between two groups ($P = 1.754$). After 12 mo, there were also no significant differences between these two groups for other factors of age, height, fasting glucose and fasting insulin (all $P > 0.05$).

Weight and waist circumference change

The initial body weight between the LS and CON groups did not differ significantly. Body weight at the end of 6 mo obviously decreased from 88.5 ± 18.4 kg to 83.5 ± 21.6 kg in the LS group, but in the CON group there was no difference in the body weight decrease (from 84.3 ± 21.8 kg to 85.7 ± 16.8 kg). There was obviously a difference between these two groups ($P = 0.036$) (Table 2). After 6 mo, percentage weight reduction of participants in the LS group was significantly greater than those in the CON group (11.59% ± 4.7% *vs* 0.4% ± 0.2%, $P = 0.001$). After 6 mo, compared with the CON group's BMI change (from 38.4 ± 17.1 at baseline to 39.7 ± 17.3), the BMI change in the LS group at baseline to 6 mo (from 37.7 ± 12.7 to 28.2 ± 11.5) was a more obvious decrease than in the CON group ($P = 0.043$).

In the LS group, the waist circumference reduced from the beginning 106.3 ± 23.2 cm to 99.4 ± 33.7 cm after 6 mo. Compared with the waist circumference in the CON group from 108.8 ± 15.7 cm to 109.3 ±21.8 cm, there was an obvious decrease in waist circumference in the LS group ($P = 0.031$).

After 12 mo, body weight in the LS group decreased from 88.7 ± 18.6 kg to 81.7 ± 19.3 kg, compared with the CON group's weight from 84.8 ± 21.3 kg to 86.1 ± 14.9 kg. There was a greater decrease in the LS group ($P = 0.036$). After 12 mo, the percentage weight reduction of participants in the LS group was significantly greater than those in the CON group (12.73% ± 5.6% *vs* 0.9% ± 0.3%, $P = 0.001$). After 12 mo, compared with the CON group's BMI change (from 38.8 ± 17.5 at baseline to 37.4 ± 14.8), BMI change in the LS group was from 37.6 ± 11.4 to 26.6 ± 9.3), more obvious than in the CON group ($P = 0.032$) (Table 3).

In the LS group, the waist circumference reduced from the beginning 107.2 ± 22.6 cm to 98.7 ± 28.4 cm after 12 mo. Compared with the CON group's from 107.3 ± 15.6 cm to 112.6 ± 19.4 cm, there was an obvious decrease in waist circumference in the LS group ($P = 0.017$).

Table 2 Change in physical and biochemical parameters after 6 mo of intervention

Variable	Group	Baseline	6 mo	*P* value
Gender (M/F)	LS	442/253	442/253	1.457
	CON	221/119	221/119	
Age (yr)	LS	39.9 ± 13.1	38.7 ± 12.4	1.658
	CON	37.4 ± 18.2	39.7 ± 14.6	
Weight (kg)	LS	88.5 ± 18.4	83.5 ± 21.6	0.036
	CON	84.3 ± 21.8	85.7 ± 16.8	
BMI	LS	37.7 ± 12.7	28.2 ± 11.5	0.043
	CON	38.4 ± 17.1	39.7 ± 17.3	
Waist circumference (cm)	LS	106.3 ± 23.2	99.4 ± 33.7	0.031
	CON	108.8 ± 15.7	109.3 ± 21.8	
ALT (IU/L)	LS	59.3 ± 20.1	49.1 ± 18.3	0.003
	CON	58.3 ± 18.8	59.3 ± 24.5	
AST(IU/L)	LS	57.8 ± 26.6	54.2 ± 25.3	0.614
	CON	59.3 ± 12.9	58.6 ± 14.5	
GGT (IU/L)	LS	67.8 ± 19.6	61.8 ± 15.2	0.071
	CON	68.3 ± 11.2	69.3 ± 14.1	
Cholesterol (mmol/L)	LS	5.7 ± 0.9	4.8 ± 1.4	0.046
	CON	5.8 ± 1.1	5.8 ± 1.8	
Triglycerides (mmol/L)	LS	2.4 ± 0.4	2.7 ± 0.7	0.785
	CON	2.2 ± 0.6	2.5 ± 0.5	
Fasting glucose (mmol/L)	LS	7.5 ± 1.3	6.2 ± 1.6	0.736
	CON	7.4 ± 1.6	7.1 ± 2.3	
Fasting insulin (μIU/mL)	LS	16.7 ± 4.3	13.4 ± 5.1	0.347
	CON	17.5 ± 8.7	18.3 ± 5.6	
HOMA-IR	LS	2.4 ± 0.7	1.17 ± 0.6	0.232
	CON	2.3 ± 0.9	2.74 ± 0.7	
L/S ratio	LS	0.8 ± 0.3	0.9 ± 0.2	0.894
	CON	0.7 ± 0.2	0.8 ± 0.3	
VFA, cm^2	LS	145.6 ± 38.7	132.0 ± 59.5	0.137
	CON	148.9 ± 39.4	149.2 ± 41.6	
Metabolic syndrome (%)	LS	371 (53.4)	142 (20.4)	0.026
	CON	182 (53.2)	159 (46.8)	

BMI: Body mass index; ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: γ-glutamyltransferase; HOMA-IR: Homeostasis model assessment of insulin resistance; VFA: Visceral fat area; L/S: Liver-spleen; CON: Control group; LS: Lifestyle intervention group.

Transaminases change

In the LS group, after 6 mo intervention, ALT decreased from 59.3 ± 20.1 U/L to 49.1 ± 18.3 U/L; however the change in ALT in the CON group was from 58.3 ± 18.8 U/L to 59.3 ± 24.5 U/L. ALT decreased significantly over time in the LS group with lifestyle intervention but not in the CON group after 6 mo (Table 2). The ALT decrease in the LS group was more obvious than in the CON group ($P = 0.001$). There was no difference of

Table 3 Change in physical and biochemical parameters after 12 mo of intervention

Variable	Group	Baseline	12 mo	*P* value[1]
Gender (M/F)	LS	431/243	431/243	1.754
	CON	215/117	215/117	
Age (yr)	LS	38.7 ± 14.2	37.9 ± 12.3	0.896
	CON	37.5 ± 19.2	36.4 ± 17.2	
Weight (kg)	LS	87.8 ± 17.8	81.7 ± 19.3	0.013
	CON	84.8 ± 21.3	86.1 ± 14.9	
BMI (kg/m^2)	LS	37.3 ± 15.4	26.6 ± 9.3	0.032
	CON	38.8 ± 17.5	37.4 ± 14.8	
Waist circumference (cm)	LS	107.2 ± 22.6	98.7 ± 28.4	0.017
	CON	107.3 ± 15.6	112.6 ± 19.4	
ALT (IU/L)	LS	59.5 ± 20.4	36.6 ± 16.9	0.002
	CON	58.6 ± 18.2	62.3 ± 20.3	
AST (IU/L)	LS	57.9 ± 25.7	52.2 ± 13.5	0.726
	CON	58.8 ± 14.7	58.3 ± 11.9	
GGT (IU/L)	LS	68.7 ± 18.8	57.4 ± 10.3	0.059
	CON	68.8 ± 11.7	69.1 ± 18.2	
Cholesterol (mmol/L)	LS	5.8 ± 0.8	4.3 ± 1.6	0.027
	CON	5.9 ± 1.0	5.9 ± 2.1	
Triglycerides (mmol/L)	LS	2.3 ± 0.5	2.1 ± 0.5	0.859
	CON	2.3 ± 0.6	2.8 ± 0.7	
Fasting glucose (mmol/L)	LS	7.4 ± 1.4	5.8 ± 1.4	0.615
	CON	7.3 ± 1.7	7.1 ± 2.3	
Fasting Insulin (μIU/mL)	LS	16.9 ± 4.2	12.4 ± 8.6	0.072
	CON	17.8 ± 8.9	21.5 ± 5.7	
HOMA-IR	LS	2.5 ± 0.6	0.96 ± 0.5	0.041
	CON	2.4 ± 0.7	2.87 ± 0.6	
L/S ratio	LS	0.8 ± 0.4	1.0 ± 0.2	0.214
	CON	0.7 ± 0.3	0.8 ± 0.3	
VFA, cm^2	LS	145.9 ± 37.6	124.3 ± 37.3	0.035
	CON	148.5 ± 39.2	164.5 ± 48.8	
Metabolic syndrome (%)	LS	366 (54.3)	112 (16.6)	0.017
	CON	178 (53.6)	142 (42.8)	

[1]*P* value compares the mean difference between the baseline and post intervention changes in the variables between life intervention and control groups. Data as percentage or mean ± SE. Derived from stratified analysis of variance for repeated measures, Huynh-Feldt correction used as appropriate. BMI: Body mass index; ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: γ-glutamyltransferase; HOMA-IR: Homeostasis model assessment of insulin resistance; VFA: Visceral fat area; L/S: Liver-spleen; CON: Control group; LS: Lifestyle intervention group.

AST and GGT between two groups (both $P > 0.05$).

After 12 mo, ALT decreased significantly in the LS group from 59.5 ± 20.4 U/L to 36.6 ± 16.9U/L but not in the CON group from 58.6 ± 18.2 U/L to 62.3 ± 20.3U/L ($P = 0.002$). There was no obvious difference in AST and GGT in the two groups (both $P > 0.05$).

Metabolic syndrome change

In the LS group, after 6 mo the metabolic syndrome rate changed from 371 (53.4%) initially to 142 (20.4%); however, in the CON group, the metabolic syndrome rate changed from 182 (53.2%) to 159 (46.8%). There was a greater difference between the LS and CON groups ($P = 0.026$).

After 12 mo, the metabolic syndrome rate was initially 366 (54.3%) to 112 (16.6%) in the LS group and in the CON group, the rate was from 178 (53.6%) to 142 (42.8%). There was a greater difference between the LS and CON groups ($P = 0.017$).

HOMA-IR and other factors

After 6 and 12 mo, there was no obvious difference in cholesterol, triglycerides, fasting glucose, fasting insulin and L/S ratio in the LS and CON groups (all $P > 0.05$). However, after 12 mo, the HOMA-IR score decreased obviously ($P = 0.041$) although after 6 mo lifestyle intervention there was no obvious change of HOMA-IR. This result also appeared in the VFA after 6 and 12 mo interventions. After 12 mo, the VFA decreased obviously ($P = 0.035$), but after 6 mo lifestyle intervention, there was no obvious decrease of VFA.

DISCUSSION

Chengyang is a district of Qingdao in Shandong province. It has an area of 553.2 km^2 and had around 737 200 inhabitants in the 2011 6th national census. In the last ten years, Chengyang's economy has developed by leaps and bounds; improved living standards and modern lives result in its residents having a sedentary lifestyle and being overweight. Pang *et al* performed a citywide nutrition survey in Qingdao and the result showed that fat energy accounted for 35.3% of the total and animal fat accounted for 35.0% of dietary fat[16]. Tian and her colleague investigated 4078 residents in Qingdao and found the prevalence rate of central obesity (with the increasing of waist circumference) was 57.2%[17].

Although the prevalence and manifestations of NAFLD in inhabitants of Chengyang-one major district of Qingdao have not been fully investigated, NAFLD accompanied with obesity is being observed in our clinic with increasing frequency. Research indicates that obesity may be the most significant single risk factor for the development of NAFLD in adults worldwide[7]. Some strategies to modulate this burden of NAFLD are likely to have benefits beyond attenuating liver disease to the broader realm of obesity-related cardiometabolic risk reduction. Tock *et al*[7,18] hypothesized that a 7% to 10% weight reduction through intensive lifestyle intervention would lead to improvements in biochemical and histological features of NAFLD. A multitude of weight loss methods have been performed, including low-calorie diets and medicine therapies, but a well-designed structured lifestyle intervention performs well for longer-term weight loss maintenance[18].

Because there is still no broadly approved pharmacological therapy for NAFLD now, we planned this study, the first large longitudinal study in obese NAFLD patients in Chengyang District of Qingdao. We found that intensive lifestyle intervention could improve biochemical features of NAFLD. Both the 6 and 12 mo lifestyle modification interventions in our study resulted in clinically relevant improvements in body weight, BMI, waist circumference, liver function (ALT levels) and metabolic syndrome incidence. However, to achieve alleviation such as insulin resistance (HOMA-IR) and visceral fat accumulation, our patients needed a 12 mo lifestyle intervention.

During the 6 and 12 mo period of study, the patients continued the regimen in collaboration with physicians, hygienists, dietitians and nurses. For physical activity, we

emphasized limiting hours of television viewing every week. One slogan we encouraged our patients with was "You burn more calories sleeping than watching TV" [19]. For diet, we strictly limited the amount of carbohydrates according to local eating customs of Chengyang District. Our study also showed that lifestyle intervention after 6 mo had similar results as after 12 mo, except insulin resistance (HOMA-IR) and visceral fat accumulation. NAFLD patients could achieve an improvement in HOMA-IR and visceral fat accumulation after a 12 mo lifestyle intervention. According to the second hit hypothesis, insulin resistance plays a key role in a later stage of liver diseases: insulin is involved in switching from NAFLD to non-alcoholic steatohepatitis[20]. Early intervention in patients with fatty liver can revert the systemic phenotype associated with insulin resistance[21].

It seems that for patients with metabolic syndrome, a 6 mo lifestyle intervention was not enough and at least 12 mo should been applied; longer-term intervention perhaps could achieve a better therapeutic effect. It was very encouraging that a 6 mo lifestyle intervention had some effect but a 12 mo lifestyle intervention had a better effect for Chengyang NAFLD patients.

The Japanese Community Health Promotion and Nutrition Section of the Health Sciences Council has put forward a slogan: "Firstly, physical activity and exercise. Secondly, diet and complete smoking cessation. Lastly, medication". More emphasis was placed on policies for physical activities and exercise[22-25]. Our study result is consistent with this view. More lifestyle intervention should be performed in obese NAFLD patients in Chengyang District. Lifestyle intervention should be the first method in the treatment of NAFLD patients in Chengyang District of Qingdao.

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COMMENTS

Background

Present modern lifestyle results in more obesity in Chinese citizens, Nonalcoholic fatty liver (NAFLD) being one of the most common. NAFLD often accompanies obesity, dyslipidemias, hypertension, insulin resistance and the metabolic syndrome. Lifestyle intervention would lead to improvements of obesity as well as NASH. It is helpful to guide people with NAFLD to make lifestyle modifications.

Research frontiers

Recent studies showed that weight reduction achieved through lifestyle interventions could improve liver histology in NASH and a 7% to 10% weight reduction was recommended. Although there were some weight loss and lifestyle modification studies for NASH in China, quantified measures such as weight reduction and waist circumference change had not been put forward.

Innovations and breakthroughs

The 6 and 12 mo life modification intervention based on physical activity, nutrition and behavior therapy is effective in improving NAFLD in Chengyang District of Qingdao. This intervention offers a practical approach for treating a large number of NAFLD patients in this urbanizing district.

Applications

The study results suggest that suitable life modification intervention is a potential therapeutic way that could be used to improve and prevent a large number of NAFLD patients in Chengyang District of Qingdao.

Terminology

Life modification intervention is based on physical activity, nutrition and behavior therapy. It includes a diet tailored to the individual's requirements and increased physical exercise.

Peer review

This is a good descriptive study in which the authors showed that lifestyle intervention is effective in improving NAFLD in both 6 and 12 mo. This intervention offers a practical approach for treating a large number of NAFLD patients in Chengyang District of Qingdao.

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CASE REPORT

Herbal hepatoxicity from Chinese skullcap: A case report

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Abstract

The use of herbal supplements has increased considerably over the last decade. We report a case of an elderly woman who began taking Move Free Advanced for arthritis, which in addition to glucosamine and chondroitin, contained two herbal ingredients, Chinese skullcap and Black Catechu. Our patient presented with significant cholestasis and hepatitis which significantly improved after discontinuation of the supplement. Since neither the patient nor the treating physician recognized this supplement as a potential hepatotoxin, she resumed taking the supplement and again suffered from considerable hepatotoxicity. Liver biopsy at that time was consistent with acute drug induced liver injury. She, once again, recovered after discontinuation of the supplement. Review of the literature confirms that Chinese skullcap has been implicated as a possible hepatotoxic agent which was demonstrated in this case.

INTRODUCTION

The use of complementary and alternative medicine has increased fivefold over the past 10 years, with approximately 42% of Americans taking some form of herbal medication[1]. Herbal supplements causing drug induced liver injury is also increasingly being recognized. Chinese skullcap (*Scutellaria baicalensis*) belongs to the mint family and has been studied as a possible anti-inflammatory agent[2]. This herbal supplement is currently available in the United States as Move Free Advanced® which is an over the counter arthritis remedy comprised of glucosamine, chondroitin, Chinese skullcap and black catechu. Recently, reports of hepatotoxicity have been described after taking this supplement[3]. We report a case of an elderly woman who experienced biopsy proven Chinese skullcap induced hepatotoxicity that reoccurred with re-challenge.

CASE REPORT

A 78-year old caucasian woman initially presented to her

primary care physician with acute painless jaundice. She was otherwise asymptomatic. Past medical history was significant for osteoarthritis and hyperlipidemia. Past surgical history included a cholecystectomy. She was not on any prescription medications, but took a multivitamin daily and three weeks prior had started Move Free Advanced®, a glucosamine/chondroitin supplement with a recommended dose of one tablet twice a day. She denied alcohol use and had no risk factors for viral hepatitis. Initial studies revealed a serum bilirubin of 7.2 mg/dL (normal value 0.1-1.2), aspartate aminotransferase (AST) 1053 U/L(normal value 15-56), alanine aminotransferase (ALT) 1626 U/L(normal value 15-59), alkaline phosphatase (ALP) 354 U/L (normal value 33-131) and Gamma-glutamyl transpeptidase (GGT) 599 U/L (normal value 11-63) . Hepatitis A, B and C serologic studies were negative. She was hospitalized at an outside hospital briefly for two days for observation during which time she was not given any medications. Upon discharge home, she remained off all medications and two weeks later her bilirubin had decreased to 2.3 mg/dL, AST 415, U/L ALT 678 U/L and ALP 279 U/L.

Her osteoarthritis worsened and after consulting her physician she was told that glucosamine and chondroitin were not hepatotoxic and thus probably not the cause of her hepatitis, so she restarted the Move Free Advanced supplement. Within 2 wk, she noted jaundice again and her laboratory studies revealed: bilirubin 4.7 mg/dL, AST 1177 U/L, ALT 1206 U/L, and Alkaline phosphatase 286 U/L. An abdominal/pelvis computed tomography (CT) was performed and was unrevealing. A percutaneous liver biopsy was performed. Upon referral to the University of Chicago Medical Center the following laboratory tests were all normal: ANA, p-ANCA, smooth muscle antibody, serum electrophoresis and iron studies. The bottle of Move Free Advanced revealed that in addition to glucosamine and chondroitin, it contained two herbal ingredients, Chinese skullcap and Black Catechu. The relative concentrations of Chinese skullcap and Black Catechu are not known since the herbal component is listed on the package as "Uniflex Proprietary Extract - 250 mg". Review of the liver biopsy showed portal tracts containing mild, predominately mononuclear cell infiltrates, with many eosinophils. There was also significant lobular inflammatory cell infiltrates, including eosinophils. Numerous acidophil bodies were seen and scattered ballooned hepatocytes were also present. No fibrosis was seen and iron staining was negative (Figure 1). This was consistent with acute drug induced hepatitis. She was instructed to discontinue her Move Free Advanced supplement. Four weeks after stopping, her bilirubin normalized to 0.9 mg/dL, AST 80 U/L, ALT 120 U/L, ALP126 and GGT 142.

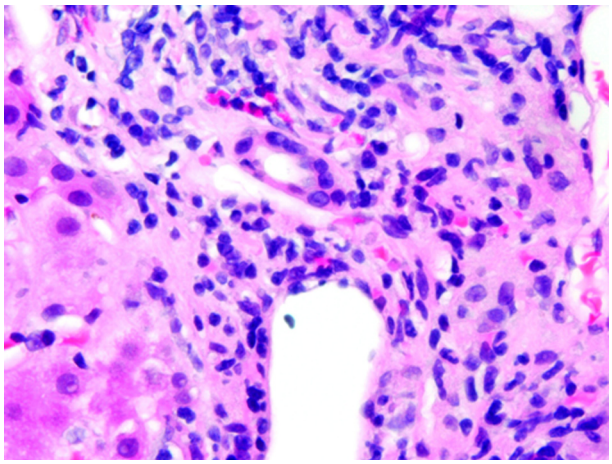

Figure 1 Liver biopsy consistent with acute drug induced hepatitis.

DISCUSSION

The diagnosis of herbal drug induced hepatotoxicity is one of exclusion and requires taking a detailed medication history. Several scoring systems exist to standardize the evaluation of drug induced toxicity, such as the Council for International Organizations of Medical Sciences scale, and can be useful in diagnosing herbal medication induced hepatotoxicity[4]. Rechallenging remains the gold standard to determine causality but is not clinically recommended.

In this case, the patient developed an acute hepatitis clinical picture that improved following discontinuation, and then returned when she re-challenged herself with the glucosamine/condroitin supplement. Also, the liver biopsy supports the impression of drug-induced hepatitis. Although there are no documented cases of hepatotoxicity for gluocosamine, chondroitin or black catechu, Chinese skullcap has been implicated as a hepatotoxin in the past[3].

Chinese skullcap (*Scutellaria baicalensis*) is a plant that belongs to the Mint family and is also known as huang qin, baikal and scutellaria. It is related to American Skullcap (*Scutellaria lateirflora)* and even though they are not the same, both are often referred to as Skullcap or scutellaria making differentiation difficult. They are both commonly used as a relaxant. Recently the combination of Chinese skullcap and Black Catechu has been studied for possible anti-inflammatory properties[2].

One of the early documented cases of Chinese skullcap hepatotoxicity described a 49-year old woman who developed acute hepatitis that resolved but returned after she rechallenged herself to the an herbal preparation that contained mistletoe, skullcap, kelp and motherwort[5]. At that time, mistletoe was the only herb known to contain a potential hepatotoxin, so it was suggested as the causative agent. However, later evaluation of the mixture suggested mistletoe was probably not an ingredient, and therefore raised the possibility that one of the other ingredients was the hepatotoxin[6]. Four cases of acute hepatitis have been reported in women taking the herbal preparations *Kalms* and *Neurelax*, both of which contained skullcap and *Valerian*[7]. Fulminant hepatic failure occurred in a 28 year old man with multiple sclerosis who was taking zinc, skullcap and pau d' arco[8]. Finally, in a recently

published case report, 2 elderly women were found to have hepatotoxicity after taking the same product as our patient, Move Free Advanced[3]. Both of these patients had improvement in liver tests after discontinuation of the supplement. A limitation of this case report was that neither of the patients had a re-challenge of the supplement and no liver biopsy specimens were available, as was demonstrated in this case.

Treatment of herbal induced hepatoxicity involves withdrawal of the offending agent. More advanced cases of liver failure require supportive care and can be fatal or lead to liver transplantation.

In our case, herbal hepatotoxicity from Skullcap was most likely the causative agent of liver injury based upon liver histology and re-challenge. This case highlights the importance of considering herbal supplement induced hepatotoxicity in the differential of any patient who presents with acute hepatitis. This case also highlights the importance of checking manufacturers' labels when drug induced hepatitis is suspected because herbal additives are not always evident on first glance.

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CASE REPORT

Acute autoimmune hepatitis mimicking metastatic liver disease: A case report

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Abstract

We report progressive painless jaundice in a 39 year old female with a suspicion of metastatic liver disease on ultrasound and computed tomography scan of the abdomen. Although the most frequent liver lesions are liver metastasis because of dual blood supply of the liver and the impact of hormones, the index case proved to have acute autoimmune hepatitis (AIH) after liver biopsy was undertaken. AIH, an unresolving inflammation of liver, occurs predominantly among females worldwide. It may present acutely and even fulminant hepatitis has been described. The index case had a dramatic response to steroid treatment with total recovery and complete resolution of liver lesions. She is clinically fine and has been regularly attending our clinic for the last year. To our knowledge from a Medline search, this is the first report where AIH was seen to mimic metastatic liver disease.

Key words: Metastatic liver disease; Auto immune hepatitis; Jaundice; Liver biopsy

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INTRODUCTION

Focal liver lesions are more often discovered with widespread use of diagnostic imaging modalities. Despite tremendous advancements in the field of radiology, radiological features are not definite. Difficulties may occur in differentiating atypical hemangiomas and focal nodular hyperplasia. Even differentiation of granulomatous lesions of the liver from primary benign or malignant lesions is also difficult. Liver biopsy becomes an indispensable tool to identify these lesions in a given case. The index case, with multiple liver lesions on radiography, proved to have acute auto immune hepatitis on liver biopsy. The etiopathogenesis of acute auto immune hepatitis (AIH) is unknown and is a diagnosis of exclusion. Multiple factors that trigger autoimmune phenomenon include infectious agents, drugs and toxins[1]. There can be a long time lag between exposure of a triggering agent and the disease[1] and, in time, steroid treatment determines the prognosis. Any treatment delay may result in irreversible liver cell damage.

CASE REPORT

A 39 year old female presented to our clinic at King Fahad Medical City, Riyadh with a history of progressive painless jaundice of 2 wk duration without any viral prodrome or offending drug intake. The patient is a non al-

coholic and denied a history of drug abuse. On examination she was conscious, oriented and icteric. Her vitals were stable. Her physical examination was unremarkable. Laboratory tests showed normal hemogram but erythrocyte sedimentation rate was raised at 52 mm/H (normal, 0-20 mm/H). She had an elevated INR 2.7 s (*N* = 0.9-1.2 s). Her liver function tests revealed predominantly conjugated hyper bilirubinemia [Bilirubin 439 umol/L (*N* = 0.17.1 μmol/L)] with elevated liver enzymes (aspartate aminotransferase: 818 U/L and alanine aminotransferase: 514 U/L). Serum alkaline phosphatase levels were also elevated at 327 IU/L (*N* = 50-136 U/L). An ultrasound examination (Figure 1) showed a heterogeneous echo pattern of the liver with infiltrative hyper echoic areas, suggesting liver metastasis. Common bile duct was normal and no intra or extra hepatic biliary ductal dilatation was noted. The *intra* hepatic veins and portal vein was normal. The gall bladder was also normal. An abdominal computed tomography (Figure 2) showed diffuse enlargement of the liver with multiple hypo dense lesions with no vascular invasion in both lobes of liver. No visceral mass lesion was seen in the computed tomography (CT) scan. Tumor markers CA 19-9 levels were elevated at 114.8 KU/L (*N* = 0-37 KU/L) and AFP levels were also elevated [15.5 ug/L (*N* = 0-10 μg/L)]. However, CEA and CA-125 levels were within normal range. She underwent upper and lower endoscopy, which were within normal limits. Mammography revealed no abnormality. Her ANA, ASMA and ALKM levels were normal and viral markers (HBsAg, Anti-HCV, IgM HEV, IgM HAV, IgM EBV and HSV) were negative. Her celiac profile and HIV serology were also negative. Further evaluation revealed normal serum ceruloplasmin levels and normal iron studies. However, she had elevated immunoglobin levels; IgG 16.2 g/L (*N* = 0.7-16 g/L) and IgM M 2.6g/L (*N* = 0.40-2.3 g/L). Thyroid function tests were suggestive of hypothyroidism [TSH 17.93 mIU/L (*N* = 0.27-4.2 mL/L), T3 4.40 pmol/L (*N* = 0.3.1-6.8 pmol/L)]. She was started on Tab. Thyroxin 50 μg once daily and supportive treatment. She underwent liver biopsy after transfusion of 4 units of fresh frozen plasma. The liver biopsy revealed extensive hepatocellular necrosis with cholestasis. There was no evidence of granuloma or malignancy. Keeping in mind hyper gammaglobulinemia and the absence of malignancy, autoimmune hepatitis was considered as a diagnosis of exclusion and an AIH score of 17 was calculated. She was started on prednisolone (40 mg tablets) once daily. Liver cell function tests were monitored. She showed progressive improvement in her liver cell functions and her coagulation parameters improved over a period of 6 wk. Repeat ultrasound examination after a period of 9 mo (Figure 3) showed a normal hepatobiliary system and clearance of previous lesions. The patient has been followed up at our clinic for the last year, with normal liver function tests.

DISCUSSION

The index case had diffuse liver lesions on radiology (ultrasound and CT scan abdomen) and various disorders giving rise to such a radiological picture include lymphoma, metastasis from carcinoma, breast, lung or melanoma. Her tumor markers were elevated; hence, it was prudent to rule out an internal malignancy. However, no malignancy was found on evaluation and liver biopsy was undertaken to evaluate the liver lesions. Liver biopsy revealed the absence of any malignancy and instead bridging necrosis was seen. Keeping in mind hyper gamma globulinemia and a negative etiological workup, she was managed as acute auto immune hepatitis and started on steroids (1 mg/kg). She responded to this treatment and there was an improvement in her liver function tests. All her liver lesions disappeared on ultrasound (Figure 3) after 9 mo. Liver histology proved to be a key investigation in the index case. The histological patterns can be variable in acute auto immune hepatitis. It may resemble acute viral hepatitis or drug induced hepatitis[2,3], even centrilobular or perivenular patterns resembling acute toxic injury have been described[4]. It is very important to differentiate drug induced liver injury from AIH on

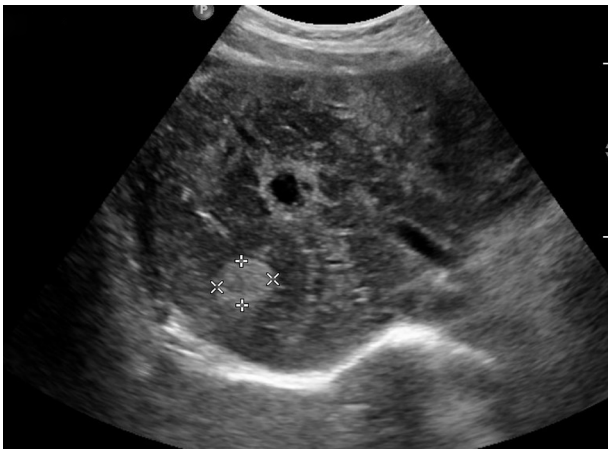

Figure 1 Ultrasound liver showing multiple hypo echoic lesions in liver.

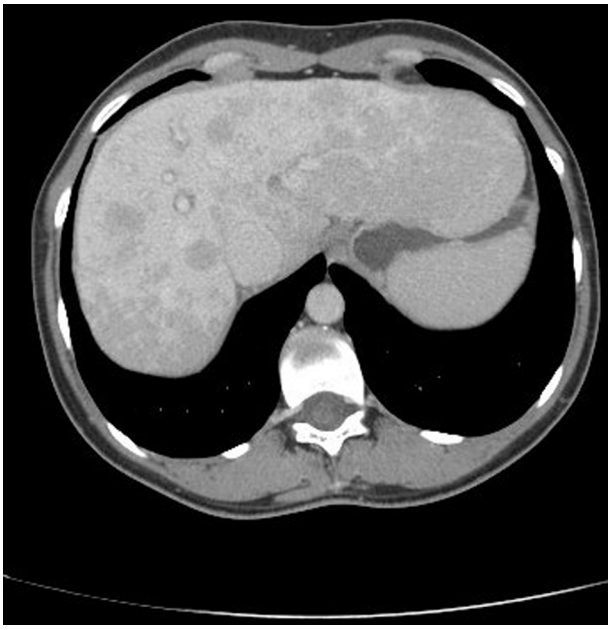

Figure 2 Computed tomography scan abdomen showing multiple hypodense liver lesions suspicious of liver metastasis.

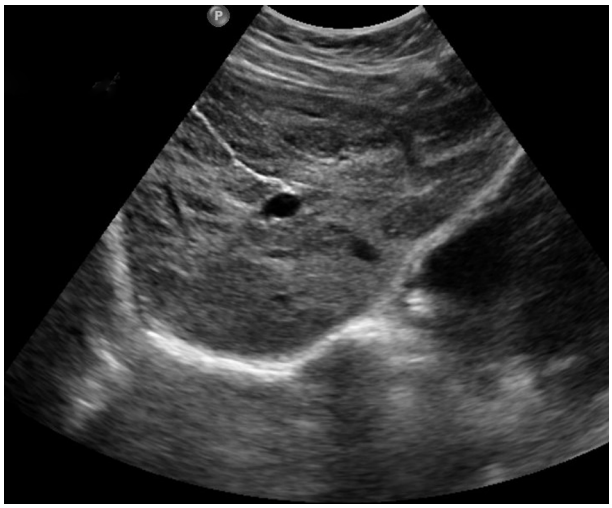

Figure 3 Ultrasound liver (after 9 mo) complete resolutions of earlier liver lesions.

histology as the treatment modalities are diagonal. Suzuki *et al*[5] compared Ishak scores, prominent inflammatory cell types in 35 cases of drug induced liver injury with 28 cases of AIH. The authors observed that interface hepatitis, focal necrosis and portal inflammation were more severe in AIH and also Ishak inflammation scores were more severe in AIH than in drug induced liver injury. In contrast, drug induced liver injury was characterized predominantly by hepatocellular cholestasis and portal neutrophils. The authors in this study were of the opinion that the pattern of injury could be used to differentiate drug induced liver injury from AIH.

Auto immune hepatitis presenting as acute hepatitis is known to occur in 25% of patients[6]. The AIH scoring system based on clinical and histological features proposed by international AIH group[7] is widely used to manage AIH. However, acute onset AIH is one of the conditions which may not fulfill these criteria[8], warranting an alert clinical attitude. The clinical suspicion of acute AIH needs to be high as the disease has an excellent response to steroids and, in time, treatment can prevent fatality, which otherwise can occur to the tone of 40% over a six month period in an untreated case[9]. The classical auto antibodies ANA and ASMA are usually supportive in making the diagnosis but ANA negativity has been described up to 28% of AIH and ASMA can be negative up to 56% of patients[10], as in the index case. Fujwara *et al*[8] observed that immunoglobulin levels correlated with increased disease activity on histology and in their series of 39 patients of auto immune hepatitis, 7 (39%) patients had normal IgG levels. Immunoglobulin levels in the index case were elevated and she had extensive hepatic necrosis on histology. There are no morphological features that are pathognomonic of acute AIH, but the characteristic histological picture is that of an interface hepatitis with predominantly lympho plasmacytic necroinflammatory infiltrates, with or without lobular involvement and bridging necrosis, often with the formation of liver cell rosettes[7]. There are only a few reports on the histological features of acute-onset AIH. Lefkowitch *et al*[11] first reported AIH cases presenting histologically as acute hepatitis. Data from Japan showed features of acute hepatitis occurring in 5.6% of patients on histological examination[12].

In conclusion, it may be said that the present case brings to the fore the role of liver biopsy in suspected metastatic liver disease when investigations do not reveal any primary source. Furthermore, despite negative auto antibodies, the possibility of acute AIH must be borne in mind while evaluating acute hepatitis of unknown etiology as the response to steroids is excellent and life saving.

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Events Calendar 2012

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San Francisco, CA, United States

January 20-21, 2012
AGA Clinical Congress of Gastroenterology and Hepatology: Practice, Evidence and Quality in 2012
Miami, FL, United States

January 27-28, 2012
28th Annual Meeting of the German Association for the Study of the Liver
Hamburg, Germany

January 30-31, 2012
5th International Conference on the Management of Patients with Viral Hepatitis
Paris, France

February 8-10, 2012
Stockholm Liver Week 2012
Stockholm, Sweden

February 16-19, 2012
22nd Conference of the Asian Pacific Association for the Study of the Liver
Taipei, Taiwan, China

March 16 -17, 2012
Hepatitis Single Topic Conference
Atlanta, GA, United States

March 16-17, 2012
ESGE - Workshop on Advanced Endoscopy with Live Demonstrations
Vienna, Austria

March 31-April 1, 2012
27th Annual New Treatments in Chronic Liver Disease
San Diego, CA, United States

April 18-22, 2012
The International Liver Congress by EASL
Barcelona, Spain

April 27-28, 2012
The European Society for Paediatric Gastroenterology, Hepatology and Nutrition
Stockholm, Sweden

May 16-19, 2012
International Liver Transplant Society 18th Annual International Congress 2012
San Francisco, CA, United States

May 19-22, 2012
Digestive Disease Week 2012
San Diego, CA, United States

June 22-23, 2012
EASL Monothematic Conference: Vascular Liver Diseases
Tallin, Estonia

July 1-5, 2012
10th World Congress of the International Hepato-Pancreato-Biliary Association 2012
Paris, France

September 5-8, 2012
International Congress of Pediatric Hepatology, Gastroenterology and Nutrition
Sharm El-Sheikh, Egypt

September 7-9, 2012
Viral Hepatitis Congress 2012
Macclesfield, United Kingdom

September 7-9, 2012
The Viral Hepatitis Congress
Frankfurt, Germany

September 14-16, 2012
The International Liver Cancer Association's 6th Annual Conference
Berlin, Germany

September 20-22, 2012
Prague Hepatology Meeting 2012
Prague, Czech Republic

September 20-22, 2012
1st World Congress on Controversies in the Management of Viral Hepatitis
Prague, Czech Republic

October 18-20, 2012
2nd World Congress on Controversies in the Management of Viral Hepatitis
Berlin, Germany

November 9-13, 2012
AASLD - The Liver Meeting 2012
Boston, MA, United States

November 9-13, 2012
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INSTRUCTIONS TO AUTHORS

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Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles, rapid communication and case reports, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1948-5182/g_info_list.htm.

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Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as ^{e}P

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Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

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Format

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Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
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