THE RELATION BETWEEN NON INVASIVE MARKERS AND USING THE FIBROTIC INDEX IN LIVER BIOPSY SPECIMEN, FROM HEPATITIS C PATIENTS, AT HOSPITAL ALOR STAR

By

DR. RAZUL MD NAZRI B. MD KASSIM

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TABLE OF CONTENTS

CHAPTER TWO: OBJECTIVES

CHAPTER THREE: METHOD

LISTS OF TABLES AND FIGURES

ABREVATION

ALP - alkaline phosphatase

ALT - alanine aminotransferase

AST - aspartate aminotransferase

AT- II – angiotensin II

GMT – gamma- glutamyltranferase

HDL – high density lipoprotein

HUKM – Hospital Universiti Kebangsaan Malaysia

K / DOQI – kidney disease quality outcome initiative

LDL – low density lipoprotein

PDGF – platelet derived growth factor

RAAS- renin- angiotensin – aldosterone system

VEGF – vascular endothelial growth factor

ROC – receiver operating curve

AUROC- area under receiver operating curve

ABSTRAK

Latar belakang

Pengambilan cebisan hati adalah kaedah terbaik di dalam rawatan pesakit Hepatitis C, terutamanya untuk pengelasan tahap fibrosis hati. Kami mengharapkan ujikaji ini memberikan maklumat perhubungan di antara paras serum biokimia didalam darah dengan tahap fibrosis hati.

Kaedah

Kami mengkaji pesakit Hepatitis C yang didiagnosis melalui, PCR dan darah mereka diambil untuk ujian biokimikal. Kami menganalisa 60 orang pesakit. Perbandingan fibrosis hati di buat dengan paras serum biokimikal yang mempunyai hubungan paling baik.Sebelas petunjuk biokimikal digunakan dan tahap fibrosis hati juga dikaji. Tahap fibrosis di bahagika kepada: S0= tiada fibrosis, S1= sedikit fibrosis pada traks portal, S2=fibrosis dikebanyakan traks portal dan fibrosis septa pendek, S3= fibrosis di kebanyakan traks portal dan sedikit fibrosis diantara portal, S4= fibrosis di kebanyakan traks portal dan semua portal mempunyai fibrosis dan sedikit fibrosis diantara portal dan sentral, S5= kebanyakan traks di antara portal dan sentral mempunyai fibrosis dan sedikit nodul,S6= cirrhosis sama ada sedikit atau spenuhnya. Analisa statistik dibuat dengan ` mann whitney U test dan lengkungan ROC.

Keputusan

Nilai mean ALT,GMT, aldosterone, HDL dan albumin dengan tahap fibrosis rendah dan tinggi adalah masing-masing adalah 82 ± 4 and 125 ± 31, 47.5 ± 31 and 81± 25, 116.6 ± 56 and 184 ± 43, 1.4 ± 0.3 and 0.9 ± 0.2 and, 42 ± 4 and 35 ± 3.2. Analisa kawasan bawah lengkungan ROC mempunyai ketepatan yang diguna pakai.

Kesimpulan

Kami mendapati serum biokimikal seperti tersebut diatas mempunyai hubungan yang boleh diguna pakai dengan tahap fibrosis hati.

ABSTRACT

Background

Liver biopsy is a mandatory procedure in the management of patients with hepatitis C virus infection, especially for staging fibrosis. We aimed, in our cross sectional study, to assess the relation of the serum biochemical markers for the diagnosis of clinically significant fibrosis(including early stages).

Method

We assessed liver biopsy patients with detectable hepatitis C by PCR, for eligibility and took their blood sample for the biochemical test. The analysis was done on 60 patients. We devised a fibrosis index that included the most informative markers (combined With age and sex). Eleven serum markers were assessed as well as fibrosis stages: S0= no fibrosis, S1=fibrous expansion of some portal tracts ± short fibrous septa, S2=fibrous expansion of most portal tracts ± short fibrous septa, S3= fibrous expansion of most portal areas with occasional portal to portal (PP) bridging, S4=fibrous expansion of portal areas with marked bridging (PP as well as portal-central (PC), S5= marked bridging (PP and or PC) with occasional nodules (incomplete cirrhosis), S6= cirrhosis, probable or definite. Statistical analysis was done using mann whitney U test and receiver operating characteristics (ROC) curves.

RESULTS

Mean value of ALT, GMT, aldosterone, HDL, and albumin with mild fibrosis and severe fibrosis, was 82 ± 4 and 125 ± 31, 47.5 ± 31 and 81± 25, 116.6 ± 56 and 184 ± 43, 1.4 ± 0.3 and 0.9 ± 0.2 and, 42 ± 4 and 35 ± 3.2, respectively with a significant difference between them was ($p < 0.05$). The area under the operating receiver characteristics showed significant accuracy for each biochemical parameters.

CONCLUSION

We found positive correlation between the ALT, GMT, aldosterone, albumin and fibrosis stages on liver biopsy in hepatitis C patients. Serum biochemical markers have significant correlation with stages of liver fibrosis.

1. INTRODUCTION

1.1 Background

Worldwide, viral hepatitis infection, including hepatitis B and Hepatitis C, represent the primary caused of liver fibrosis. If left untreated fibrosis can progress to cirrhosis, ultimately leading to liver failure and possibility of death. A major clinical problem facing medical community is how to best evaluate and manage the increasing numbers of patients infected with viral hepatitis especially Hepatitis C. According to the latest consensus conference liver biopsy is still recommended in most patients. Liver biopsy is considered as a gold standard technique because it confirm clinical diagnosis, assess the severity of necroinflammatory activities and fibrosis, evaluate possible concomitant disease process and guides therapeutic interventions. (Aturo A. Bravo et al, 2001)

However, liver biopsy is an invasive and expensive method, and can have the remote risks of life threatening complications. Moreover the accuracy of assessing liver fibrosis has also been questioned. (Aturo A. Bravo et al, 2001)

1.2 Limitation of Liver Biopsy

Limitation Due To Sampling Error

Regev A. et al (2002), in their systemic review have demonstrated sampling errors not only in patients, with liver disease with a high degree of intrahepatic heterogenecity, such as billiary cirrhosis but also in patient with alcoholic or Hepatitis C

induced fibrosis and inflammation. Furthermore the well accepted 4 stages of METAVIR score was used to stage fibrosis, about one third of the scores differed by at least one stage in the same patient when biopsy was taken from the right lobe compared with the left lobe. The similar results were obtained for grading of inflammation. (Regev A. et al, 2002)

1.2.1 Limitation Due To Complication and Contraindication

(a) Limitation Due To Complications

Liver biopsy is a safe procedure when it is performed by experienced operators. Froehlich et al (1993), noted that lower complication rate occur among physicians who performed more than 50 biopsies per year. Approximately 1 to 3 percent of patients required hospitalization for complication after liver biopsy. Sixty percent of the complications occur within 2 hours and 96 percent occur after 24 hours. Very rarely, there are possibility of intraperitoneal hemorrhage following the procedure and it usually becomes apparent within the first two to three hours after that. In addition the mortality rate after liver biopsy is approximately 1 in 10 000 to 1 in 12 000 and it is highest among those who have malignant lesions. (Aturo A. Bravo 2001)

(b) Limitation Due To Contraindication

In many of the cases, liver biopsy cannot be performed due to certain reasons which include:

i. Uncooperative patient

ii. History of unexplained bleeding

iii. Prothrombin time more than 3 to 5 seconds than control

iv. Platelet count less than 50 000 per mm^3

v. Use of non steroidal anti-inflammatory drugs within 7 to 10 days

vi. Suspected vascular tumors or echinococcal cyst in the liver

vii. Inability to get appropriate site for biopsy. (Aturo A. Bravo 2001)

As mentioned above, all these limitations of liver biopsy as well as the probability of inter-observer and intra-observer discrepancies in the assessment of liver histology have driven efforts for the development of accurate and reliable non- invasive means to assess the severity of liver lesion, and particularly of fibrosis. (Nikolaos V. et al 2005)

1.3 The Significant of Association Between Non Invasive Markers with Liver Fibrosis Score

There is an increasing evidence supporting pivotal role of non invasive blood markers such as ALT, ALP and hylurounic acid in predicting liver fibrosis score. The AST and ALT were elevated in advanced liver fibrosis and a study by Chun Tao W et al 2003 on AST and AST/ALT ratio showed that they were important predicted parameters of either significant fibrosis or cirrhosis. Hylurounic acid, an important component of extracellular matrix, appears to be a reliable marker for the noninvasive assessment of fibrosis. Studies showed serum hylurounic acid level were low in patients without liver disease, whereas the level were often high in patients with hepatic fibrosis (Sandeep M et al, 2006). In general fibrosis can be classified as wound- healing response to various chronic stimuli. It is characterized by deposition of extracellular matrix proteins which include glycoproteins, collagens and proteoglycans. Many studies have shown that, the hepatic stellate cell, is the primary cell- type in the liver responsible for excess collagen synthesis during hepatic fibrosis. The activation of the hepatic stellate cell leads to regulating cytokines signaling and cellular signaling pathways, these resulted in increase serum liver enzyme, and hepatic inflammation and there were study which found that activated hepatic stellate cell express both the renin- angiotensin and AT-II. This perhaps indicating that once angiotensin stimulated the activation of hepatic stellate cell and injury can be exacerbated by paracrine and autocrine signaling. Therefore by measuring the level of enzyme produced by the liver in response to activation of hepatic stellate cell, it will reflect the dynamic changes in the liver. (T. Shigeki, J.P Christopher and A.R Richard, 2005)

T. Poynard et al (2004), have used six serum biochemical markers which consisted of alpha 2-macroglobulin, haptoglobulin, gamma glutamyltransferase, total bilirubin, apolipoprotein A1 and alanine amino transferase and compared with the severity of the fibrosis stage and necroinflammatory grade in the liver. They found biochemical provides a more accurate (quantitative and reproducible) picture of fibrogenic and necrotic events occurring within the liver than liver biopsy.

Apart from the biomarkers mentioned above, there were studies conducted using other blood markers, such as JF. Yao et. al, (2003) whom had conducted animal studies to demonstrate the effect of aldosterone on hepatic stellate cell activation. They used rat hepatic stellate cell which were incubated with aldosterone or spironolactone, an aldosterone receptor antagonist at different concentration. They found the proliferation and collagen synthesis of hepatic stellate cell was enhanced by aldosterone and the effect was dose dependent. The higher the aldosterone dose, the more significant hepatic cell activation increased. In conclusion aldosterone can activate the hepatic stellate cell and subsequently lead to liver fibrosis.

Recently, S. Damien et al. (2006) recruited 138 patients and the aim of this study was to assess the reliability of previous study done by T. Ponyard et al. in 2004, whereby the study combined the quantitative results of six serum biochemical markers in predicting liver fibrosis and activity among Hepatitis C infected patients and in their study they have included patients with Hepatitis C induce systemic vasculitis. In addition other biochemical non-invasive liver fibrosis indexes in such Hepatitis C infected populations were examined. more importantly

the presence of systemic vasculitis or non septic serum inflammation in Hepatitis C infected patients does not modify the high accuracy of the biochemical non-invasive scoring indexes, in predicting significant liver fibrosis.

1.4 The Role of Liver Enzyme In Relation to The Liver Fibrosis or Inflammation

1.4.1 Alanine Aminotransferase

The enzyme is a sensitive indicator for liver damage therefore, it is a more reliable indicator compared to other liver enzymes. It is found primarily in the liver and significantly lesser amount are present in other body tissue. When liver parenchymal is damaged, alanine aminotransferase is released resulting in elevation in serum. Therefore, it is currently considered that serum ALT correlates with the degree of histological inflammation and necrosis of the liver cell. Furthermore, the higher the ALT level, the more rapid the development of cirrhosis and hepatocellular carcinoma. (Joseph A K and colleagues, 2005)

1.4.2 Aspartate Aminotransferase

The elevation of the aspartate aminotransferase is not always due to liver diseases, but if the elevations are more than four times of the upper limit, it is a significant indicator for cholestatic jaundice or infiltrative liver disease. Especially in the present of gamma-glutamyltransferase. This is because the enzyme rarely elevated in condition other than liver disease. (Daniel S P et al, 2001)

1.5 The Role of Biosynthetic Function of the Liver in Liver Fibrosis

1.5.1 Serum Albumin Level

Serum albumin is synthesized exclusively by hepatocytes. It has long half-life, approximately 15 to 20 days with 4% degradation per day. Therefore it can be used as chronicity markers of liver disease. In hepatitis albumin levels below 3 g/dL should raise the possibility of chronic liver disease. Hypoalbuminemia is more common in chronic liver disorders such as cirrhosis and usually reflects severe liver damage. (S.P Daniel et al, 2001)

1.5.2 Serum Coagulation Profile

With the exception of factor VIII, the blood clotting factors are made exclusively in the hepatocytes. Serum prothrombin time, which collectively measure factors II, V, VII and X, is useful to measure hepatic synthetic function and helpful in both the diagnosis and assessing the prognosis of liver disease.(G. Marc, J.H Hoofnagle, 2001)

1.5.3 High-Density Lipoprotein(HDL) and Low Density Lipoprotein(LDL)

Lipoprotein is complexes that transport lipid, to or from the tissue. All nucleated cell synthesized cholesterol but only hepatocytes can efficiently metabolize and excrete cholesterol from the body. Because the liver is the principal site of formation and clearance of lipoproteins, liver disease can profoundly affects plasma lipid level.(Daniel R J and Helen H H, 2001)

1.5.4 Serum Haptoglobulin

Haptoglobulin is an alpha globulin that is present in high concentration in the serum. It is synthesized in the liver and the synthesis is decreased in patient with hepatocellular and increased in inflammatory state.(Franklin B H, 2005)

1.6 The Role of Renin-angiotensin-aldosterone system (RAAS) in Liver Fibrosis

1.6.1 The Role of Angiotensin II (AT-II)

The RAAS is an important contributor to the regulation of the blood pressure, water and salt balance and tissue growth. The major effectors of the RAAS, which is the angiotensin II is a powerful vasoconstrictor that triggers the release of aldosterone. In addition to its role in the blood pressure regulation, angiotensin II has a variety of action that associated with cardiovascular and renal pathology. Angiotensin II has been shown to contribute to vascular remodeling by activating signal transduction pathways, that promote cell growth inflammation and fibrosis. Daniel D A (2006)

Recently Hitoshi Y et al 2006, had done a study on animal to look at the interaction between AT-II and VEGF in rat liver fibrosis development. They concluded that AT-II increase VEGFmRNA expression in the activated hepatic stellate cell in a time and is dose dependent manner. These results indicated that augmentation of the VEGF during liver fibrosis development was, at least partly, mediated by AT-II, and that the activated hepatic stellate cell was one of the VEGF-induced cells

1.6.2 Role of Serum Aldosterone

Cirrhosis is frequently associated with the increase levels of aldosterone in the plasma and the urine and it has role in the activation of the hepatic stellate cell. Rombouts et al(2000) has demonstrated aldosterone had direct pro-fibrogenic effect on the hepatic stellate cell. They have conducted animal study by exposing rat hepatic stellate cell culture to high, near-physiological or physiological concentration of hormone and they measured a few parameters that indicate the activation of the hepatic stellate cell. Their data showed at pharmacological and near physiological concentrations enhance the synthesis of procollagen product, which indicates the activation of hepatic stellate cell.

Caligiuri et al (2000) have shown that the incubation of the hepatic stellate cells with canrenone, an aldosterone antagonist, lead to the inhibition of a number of PDGF-induce changes and one of it is PDGF-induce hepatic stellate cell proliferation and collagen type 1 synthesis. This study provide evidence indicating that anti-aldosterone drugs may directly act as anti-fibrogenic drugs.

1.7 Progression of Liver Disease in Relation to Histopathology Finding in Liver Biopsy

1.7.1 Stages of Disease Progression

Like other liver disease, Hepatitis C progresses in stages. The usual progression is from inflammation to fibrosis to cirrhosis. Fibrosis is the important stage in the development of liver cirrhosis. The amount of fibrosis is the way of assessing how quickly the disease appears to be progressing. (Douglas D T, 2003)

Michiyasu Y and colleagues, 2000 showed that the stage of liver disease was almost linear according to the time, however, activity grade was not as linearly correlated as fibrosis stages. Thus, clinically relevant progression of chronic Hepatitis C would better estimated by the fibrosis stage than the grade of histological activity. They found that the median rate of fibrosis progression per year was 0.133 fibrosis per unit and three independent factors were associated with an increase rate of fibrosis which are:

i. age older than 40 years old

ii. daily alcohol consumption of 50 g or more

iii. male sex

iv. immune deficiency or immunosuppressive therapy.

1.7.2 Scoring and Grading Liver Biopsies

Liver biopsy is a test in which small pieces of liver tissue are removed and examined under the microscope. The three main things that will be looked for are inflammation, fibrosis and cirrhosis. The biopsy report may also reveal other histological and pathological findings such as the presence of lymphoid nodule, damage to the small bile ducts, and/or the presence of fat. (Douglas D T, 2003)

The amount of the injury to the liver, will be term as inflammatory grade and fibrotic stage. There are three different methods used for scoring liver biopsy. The three scoring and grading systems for liver biopsies are the Original HAI (Histology Activity Index), the Modified HAI and the Metavir. Each of the scoring systems based upon visual assessment of portal and periportal fibrosis.

1.7.2(a) Original Histological Activity Index (HAI)

The HAI scoring system range from 0 to 22 and fibrosis is staged as 0.1. 3 and 4. This discontinuous scale was developed to allow for clear separation of mild from extensive fibrosis which has important prognostic value. The HAI system is simple and has been widely used. However, the intra-and inter-observer reproducibility of the HAI is not very good and the distinction between stages 1 and 3 may be difficult. In addition, its discontinuous scale complicates statistical analysis in clinical trials. (Douglas D T, 2003)

1.7.2(b) Modified HAI score

The modification of the HAI scoring system was proposed by Ishak et al, 1995. This system is more sensitive in assessing fibrosis. Fibrosis stage is scored continuously from 0 to 6, which permits a better assessment or the effect of therapy on fibrosis. Ishak score's is better validated and gives a more accurate assessment of fibrosis.(Douglas D T, 2003)

1.7.2(c) Metavir scoring system

This system is simple. Fibrosis stages are scored continuously from 0 to 4. This System has been carefully validated in large group of patients with chronic Hepatitis C and has shown good intra- and inter- observer reproducibility. Important limitations of these scoring systems should be emphasized. Hepatic fibrosis may not be homogenous throughout the liver and liver specimen obtained by needle biopsy may not accurately reflect the overall average degree of fibrosis. The reliability of the assessment of fibrosis stage increase with the size of the liver sample. In most studies, a minimum of 10 mm is required. Regardless of biopsy length, however, fibrosis may be underestimated and cirrhosis missed in some patients.(Douglas D T, 2003)