University Name

Master of (degree)

Course number

A NONINVASIVE METHOD TRANSIENT ELASTOGRAPHY THAT IDENTIFIES INITIAL STAGE LIVER FIBROSIS & CIRHOSIS IN PATIENTS WITH HEPATITIS B

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ABSTRACT

Background

Many non-invasive techniques have been performed on the patients with HBV related fibrosis. The most appropriate results have been obtained using Transient Elastography (FibroScan) by measuring the liver stiffness. The accurate diagnosis of fibrosis related to HBV is essential for prognostication and treatment decisions. Non-invasive alternatives such as FibroScan have been developed due to the limitations of liver biopsy. Our main objective is to perform the Transient Elastography (FibroScan) to find out the presence or absence of fibrosis and whether or not it can stage fibrosis in individuals with HBV.

Methodology

A retrospective study is applied on the various stages of Fibrosis in HBV patients using non-invasive method FibroScan. The METAVIR scoring system is used to determine the level of inflammation and fibrosis of these patients through histopathological assessment. The grade in the system shows the activity or severity of inflammation, whereas the stage is the level of fibrosis or scarring. This scoring system consists of a regularly measured and readily available laboratory data to differentiate the HBV patients with and without advanced fibrosis. We took 145 patients with positive hepatitis B and examined them to check the test accuracy for various stages of fibrosis. The pathologists pre-examined each of these samples in the lab. At the end of the evaluation, five liver samples were categorized as F0, F1, F2, F3 and F4. Here, F0 refers to no-fibrosis, F1 refers to initial fibrosis without septa, F2 refers to fibrosis with septa, F3 refers to advanced fibrosis, and F4 is severe fibrosis with cirrhosis (C1). We enrolled 145 patients who are already diagnosed with HBV, and Elastography (FibroScan) was performed during the first visit.

Results

Chronic HBV-infected patients with minor fibrosis and cirrhosis were diagnosed correctly by using existing laboratory tools at initial stages. Routine demographic, clinical and laboratory parameters were analyzed to predict the existence or absence of advanced fibrosis. Independent indicators of liver fibrosis include platelet count, age, AST, ALT values and albumin. 80% of the patients were not affected by the disease, which means F0 was 80%. Yet some patients had fibrosis at different stages F1, F2, F3, F4 and C1. The FibroScan assessment revealed that out of 145 patients, only 11% patients were at stage F1 whereas the diagnostic value for F2, F3, F4, and C1 are $\leq$ 3% with F2=3%, F3 =1%, F4=2%, and C1=2%.

Conclusion

We have examined the performance of non-invasive tests such as FibroScan in diagnosing various stages of fibrosis. FibroScan is an effective method to detect HBV-related fibrosis and cirrhosis. Transient Elastography ensures accurate distinction between various stages of fibrosis in Hepatitis B patients. The results show that the proposed method can be very useful for the

automatic identification and classification of liver fibrosis, which will eventually allow clinicians to perform accurate diagnosis.

Keywords: Diagnosis; Hepatitis B; Liver Fibrosis

INTRODUCTION

Chronic HBV is a primary reason of liver diseases and deaths worldwide. While a portion of 250 million individuals who are chronically infected with HBV, are unlikely to undergo serious harm (require therapy) or develop complications of the severe liver diseases including hepatocellular carcinoma (HCC) and decompensated cirrhosis without intervention.[1] HBV is primarily centered in the liver where a cycle of hepatocyte damage and tissue repair results due to the contact of viral proteins with the immune system of the human body.[2] In this repair, repeated accumulation of the extracellular matrix leads to the growth of severe liver fibrosis over time. The increase in advanced fibrosis may be fast, slow, or intermittent which depends on the state of disease and the level of active liver injury and inflammation. Formal evaluation of liver fibrosis is important for the diagnosis of the disease and for assessing the urgency and response to the treatment. The main indicator of the result is the intensity of the liver disease. Cirrhosis results in lower survival rate and enhanced occurrence of HCC.[3] Patients without cirrhosis have a survival rate of 97% to 63%, whereas cirrhosis patients have survival rates of 55% and 25% respectively.[4] The conventional blood test such as alanine aminotransferase (ALT) is helpful in measuring disease activity. Fibrosis assessment via liver biopsy or dedicated non-invasive test can help in the assessment of liver disease in these patients.

Based on the complicated natural history of HBV infection, patients need a professional evaluation to be able to correctly stage the disease, understand viral serology, biochemistry, and start the monitoring or required treatment.[5] Non-invasive markers are helpful supplements for demonstrating the treatment results without repetitive liver biopsies.[6] In recent years, a number of non-invasive tests have been introduced with the goal of reducing the necessity for liver

biopsy and enhancing clinical practice.[7] The identification of liver fibrosis using TE-FibroScan is an important factor in the management of disease and prognosis for an individual with HBV.[1]

TE is one of the best non-invasive techniques and its purpose is to diagnose the HBV related fibrosis by measuring the stiffness of liver.[5] It can be carried out effectively in the outpatient's clinic or at the bedside with instant results. Limitations to this method include only 5% of cases with failure (no value) which were mostly recorded in patients with excessive thoracic fat. FibroScan is preferred for the early evaluation of liver fibrosis in formerly untreated chronic HBV patients. FibroScan has been accepted worldwide as the tool to analyze and evaluate mild and severe fibrosis (cirrhosis) in HBV/HCV patients, HIV-HCV co-infections and reoccurrence of hepatitis C following liver transplantation. FibroScan is the most appropriate method for early fibrosis diagnosis and portal hypertension assessment, which can also be predictive. Studies are needed using FibroScan to check two types of patients i.e. patients who are taking treatment and the other who don't. It also requires monitoring of the patients who are at risk of liver disease. Although FibroScan is a useful technique that measures the stiffness of the liver, the values of FibroScan must be interpreted on the basis of clinical, biological and morphological data. This review emphasizes on the widely used non-invasive fibrosis test, analyzing its diagnosis accuracy and applicability to patient treatment.

Chronic Hepatitis Grading & Staging

Both staging and grading are frequently used in chronic viral hepatitis. In general, the creation of scoring systems to be used in liver diseases has exceeded the clinical utility demonstration. The grade of disease reflects the rapid progress towards the end stage of the disease. While the stage of disease measures the degree to which it has advanced in its natural history, leading to the organ failure or death of the patient. The initial stages have lower level of

fibrosis or cirrhosis, whereas the end-stage in majority of chronic liver diseases is cirrhosis with clinical decompensation. The grade of the disease is the degree of infection and hepatocellular injury that is supposed to result in fibrosis in chronic necroinflammatory diseases (HBV, HCV & HDV) and autoimmune hepatitis. The grade represents the existing liver disease intensity with characteristics that alter according to the type of injury and its pattern. Ideally, grade and stage both must predict the diagnosis and direct the therapeutic treatment intervention, but in most of the chronic liver diseases the evidence is relatively minor. Moreover, therapeutic treatment can modify the natural history in unspecified manner. However, it is often asked from the pathologists to allocate the stage as well as grade in the process of fibrosis assessment to find out a patient's result.

Currently, different methods are used to demonstrate the stage and grade of chronic hepatitis. Simple staging and grading systems, such as Batts-Ludwig, Metavir, and the IASL systems, are used to determine the extent of inflammation and fibrosis of chronic hepatic patients. For the characteristic of interest, many staging and grading systems utilize three to four categories of increasing intensity in liver diseases. Therefore, for chronic hepatitis, a simple grading system like Metavir scoring system is used which can classify the disease process as mild, moderate, or marked. However there is a range of severity within these categories so that this system can provide more details. Several pathologists see it as a matter of course by adding the categories into three grade system, such as "mild-to-moderate" along with "moderate-to-marked".[8]

METAVIR System for activity grading in chronic HBV

To deal with the chronic hepatitis B and C, METAVIR scoring system is widely used for the diagnosis of necro-inflammation and fibrosis. In this system, the grade of activity is only

determined by the lobular necrosis and the interface hepatitis. The level of piecemeal and lobular necrosis has been separately calculated in many existing classifications, and their score has then been added, which indicates that both structures have equal weightage in the definition of activity. In this scoring system, the definition of activity added both lobular necrosis and piecemeal necrosis, however the values are different. It is justified by the fact that piecemeal necrosis is the major factor which discriminates against grade activity compared to lobular necrosis.

The first criterion (piecemeal necrosis) score is described as:

0: Missing

1: Focal modification of periportal plate in a few portal tracts

2: disperse periportal plate modification in a few portal tracts

3: Diffused modification in each portal tract of periportal plate

The next criterion (Focal lobular necrosis) score is described as:

0: below one necro-inflammatory foci per lobule

1: at least one necro-inflammatory foci per lobule

2: multiple necro-inflammatory foci per lobule or bridging necrosis.

The score for fibrosis is defined as:

F0: no fibrosis

F1: portal fibrosis with no septa

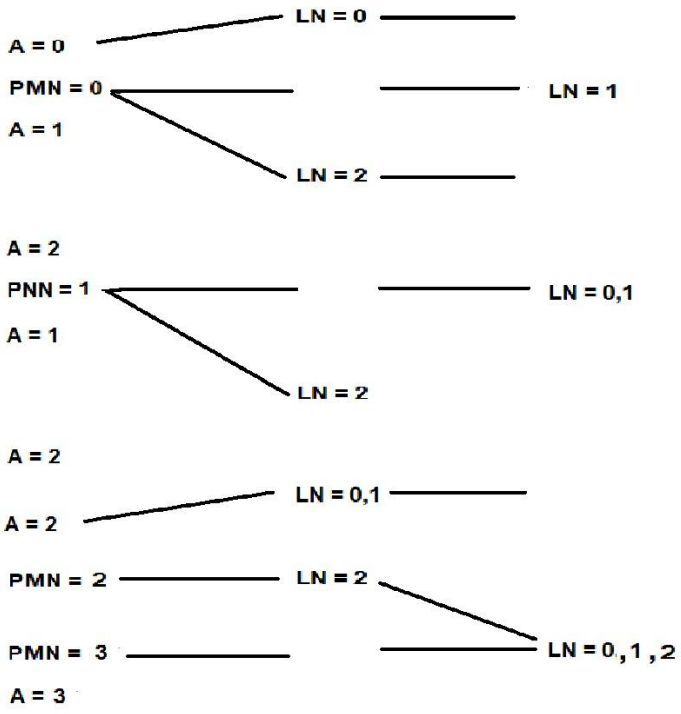
F2: portal fibrosis with rare septa

F3: various septa with no cirrhosis

F4: cirrhosis.

The advantage of using METAVIR scoring system is reproducibility, simplicity, and application to large proportion of biopsies. This score consists of two-number and two-letter coding system in the following figure: F=fibrosis and A=histological.[9]

Figure 1. METAVIR Scoring System & Fibrosis



stages

PMN: piecemeal necrosis; 0: none, 1: mild, 2: moderate, 3: severe. LN: Lobular necrosis; 0: no or mild, 1: moderate, 2: severe. A: histological activity; 0: none, 1: mild, 2: moderate, 3: severe.

Fibrosis Scoring	
Score	Description
0	No fibrosis
1	Portal fibrosis without septa
2	Portal fibrosis with rare septa
3	Numerous septa without cirrhosis
4	Cirrhosis

Modified and required (20)

Transient Elastography (FibroScan)

There are special end-to-end non-invasive techniques that are often used to assess liver fibrosis. Transient Elastography is among the world's most commonly used methods for quantifying liver fibrosis, even in CHC and HBV. There is a transducer installed in the axis of a vibrator at the end of the probe which helps in directing 50MHz pressure waves on the specified liver tissue. As a result, painless vibrations spread a shear wave which represents the velocity of the wave being returned to the ultrasound. The shear wave is followed, monitored, and shown in kilopascals by means of a device, thus assessing the liver fibrosis. TE is a quick bedside test which is very simple to perform, with instant clinical follow-up. In fact, TE is the liver stiffness (LS) measurement that depends on the level of fibrosis; it does not measure liver fibrosis directly.

Until now, this method has proven effective in evaluating fibrosis and diagnosing cirrhosis complexities like portal hypertension.[10] Thus, a number of measurements are essential for correct assessment. Even so, the results of assessment are proved to be challenging due to some limitations such as narrow intercostal space may increase false results, the patient have to hold the breath during the process for minimum errors, and the fasting conditions are essential as well. Another important factor is that one cannot perform FibroScan on the patients of ascites. A meta-analysis correlated the FibroScan values with the METAVIR score on the patients of HBV.

F2 has a value of 7.9 kPa, and F4 was related to value 11.7 kPa with a sensitivity of 0.859 and 0.929 respectively.[11] However, some researches reflect that the outcomes in HBV might be influenced by aminotransferases flares confronted in affected patients.[12] The European-Association for the study of liver in management of viral hepatitis approved this method for its accuracy, non-invasiveness, and ability of differentiating between different stages of fibrosis .[13]

The 27 studies meta-analysis in 2015 involved 4386 HBV patients in which high diagnostic precision was demonstrated by TE to assess liver fibrosis. The summary of sensitivity was 0.81, 0.82, and 0.86, the Specificity values were 0.82, 0.87, and 0.87, and the relevant AUROC values were 0.88, 0.91, 0.93 where fibrosis stages were F ≥ 2, F≥ 3, and F= 4, respectively. [14] Significant fibrosis (≥F2) cut-off values ranged from 5.8 – 8.8 kPa, fibrosis with septa (≥F3) ranged from 7.0 – 13.5 kPa and the cirrhosis (F4) ranged from 9.0 – 16.9 kPa.[15-19] TE has been shown to be effective for fibrosis evaluation in the latest research involving 263 HBV patients having the ALT levels < 2x Upper Limit of Normal (ULN), specifically ones diagnosed with significant fibrosis. The value of liver stiffness was observed to be substantially associated with liver fibrosis as well as necro-inflammatory activity, an option to explain TE measurments.[20] It is proposed by few authors that cut-off values for TE should include ALT levels which change with the swelling and redness in HBV. In case of patients with negative HBeAg, TE might be helpful to indicate the need for biopsy treatment.[21] In HBV patients, TE has been demonstrated as a prognosticator of liver-oriented results. Another analysis of 381 HBV patients (that were beginning the treatment) found growing accumulated incidence rates of HCC correlated with increased LS values in three graded groups including LS < 8, LS = 8-13, and LS > 13kPa, and LS was an autonomous HCC growth indicator instead of histological staging.[22]

Limitations for transient elastography include steatosis of liver stiffness values and the conflicting impacts of inflammation activity. A machine, an operator training and costs associated with it are needed for TE. TE was initially verified for HCV instead of HBV, with lesser cut-off values to identify fibrosis in HBV. While valid to diagnose cirrhosis, TE is facing decreased accuracy at lower fibrosis levels, same as in blood-based biomarkers, yet TE is still extremely useful addition to the clinical toolkit.

AST/ALT ratio (AAR)

There are various categories of non-invasive markers used to predict the intensity of HBV related fibrosis. Indirect markers use regular laboratory tests such as liver synthetic makers, transaminases or other readily available indicators which are relevant to the stage of liver diseases like red cell distribution width or platelet levels. Sensitive indications of liver cell damage include ALT, AST and serum aminotransferases. ALT refers to Alanine aminotransferase, and AST means Aspartate aminotransferase. In various etiologies of liver diseases, AAR is commonly used as a prognosticator of cirrhosis and the ratio of AST/ALT in ordinary subjects is around 0.8. In some cases, this ratio varies in many ways that a certain diagnosis can be suggested. This ratio may be effective as a non-invasive measure of cirrhosis and fibrosis in patients having chronic hepatitis B.[23] Williams et al in 1988[24] published a study in which out of a hundred HBV patients, the mean ratio of AST/ALT was calculated 0.59 for non cirrhosis patients and 1.02 for patients with cirrhosis. However, Eminler and colleagues[25] discovered that AAR performed less than other non-invasive blood algorithms when assessing the stage of fibrosis in 237 patients with HBV. Likewise, the potential of AAR to identify the advanced fibrosis (F2-F4) was limited in 319 HBV patients in an US group. [26]

PATIENTS AND METHODS

Patients

This retrospective study was performed from MAY 2016 to MARCH 2020. Patients have been examined at the Department of Radiology in Rehman Medical Institute. A total of 145 patients were registered to the study in which out of 145 patients, 110 were male and only 35 were female. These patients were previously diagnosed with chronic hepatitis B. Therefore, transient elastography (FibroScan) test was carried out to study the diagnosis of fibrosis stages in these patients. 123 patients were examined on their initial visit whereas 22 patients were on follow-up visits. The clinical follow-up has provided physicians with information for the next diagnostic measures. Statistical and laboratory data of patients were gathered at the time of first outpatient visit or initial hospital admission. The rest of details were collected from the electrical medical records. Chronic HBV have been detected with the evaluation of hepatitis B surface antigen (HBsAg) for over six months. Patients who suffered from the liver illness such as hepatitis A, C, E infection, previous nucleoside treatment, HCC, fatty liver disease, previous interferon therapy, ultrasonographic or clinical evidence of cirrhosis, and deficient liver tissue for the staging of fibrosis were all excluded from this study.

Table 1. Gender

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Female	35	24.1	24.1	24.1
	Male	110	75.9	75.9	100.0
	Total	145	100.0	100.0	

Table 2. Types Of Visits

Visits	Frequency	Percent	Valid Percent	Cumulative Percent

Follow-up	22	15.2	15.2	15.2
Initial Visit	123	84.8	84.8	100.0
Total	145	100.0	100.0	

Laboratory Examination

Every patient was subjected to complete clinical history along with laboratory and clinical examination which includes liver function test such as serum albumin, bilirubin, and liver enzymes, INR, alpha-fetoprotein, HCV RNA via polymerase chain reaction, hepatitis seromarkers for HBV (HBs AG) using ELISA method, prothrombin time and concentration, and complete blood count (CBC).[27] Abdominal ultrasonography was performed on every patient to diagnose the cirrhosis in the liver; presence of ascites, splenic size and portal vein diameter then FibroScan was performed to measure the liver stiffness

Liver Stiffness Measurement

Various measurements were carried out on the patients right liver lobe, lying on the back with the full abduction of right arm. The transducer tip has been coated with the coupling gel and placed between the rib bones onto the skin at right liver lobe. With the help of ultrasound, the operator found a part of the liver of at least 6cm thickness without huge vascular structures. After locating the measurement area, the operator pushed the button on probe to begin the procurement. The depth of measurement ranged from 25mm to 65mm under the skin surface. The software automatically rejected those measurements, which resulted in incorrect follow up of the vibration propagation or incorrect vibration shape. Out of total acquisitions, every patient underwent up to 10 successful measurements and the success rate has been estimated as a proportion of the amount of successful acquisitions relative to their sum, which showed the results in kilopascals (kPa). We kept the median value of successful measures to show the liver stiffness. The liver

stiffness measurement was considered reliable, only if it had at least a 30% success rate and at least five successful measurements. The entire test took less than five minutes.[28]

Statistical and data analysis

The major objective of the analysis was to find out the existence or absence of fibrosis (stages 1-4) through a combination of simple and clinical relevant variables. Statistical and Data analysis was conducted to evaluate relevant differences based on statistics using commercially software package (SPSS) version 17.[27] Measurement units are described as mean ± standard error of mean of data. The mean deviation of FibroScan measurements was calculated for all fibrosis stages. The TE diagnostic value was calculated by histopathological fibrosis staging (Metavir score) to diagnose all four stages of fibrosis. Here, F0 refers to no-fibrosis, F1 refers to initial fibrosis without septa, F2 refers to fibrosis with septa, F3 refers to advanced fibrosis, and F4 is severe fibrosis with cirrhosis (C1). The METAVIR scoring system was derived from individual factors for determining the level of fibrosis (scarring) in hepatitis B patients.

RESULTS

Our research included 145 patients of chronic hepatitis B out of which 110 were males and 35 were females. The mean age of the patients was 40.4 (17-70 years). The following table shows the laboratory details. The mean values for AST and ALT in our studies were 40.3 ± 7.53, and 76.95 ± 10.96, respectively. The patients were classified according to the METAVIR scoring system into F0 (n = 116), F1 (n =16), F2 (n =5), F3 (n =2) and F4/C1 (n =3/3). The mean stiffness of liver was 4.5 ± 0.2kPa for patients with F0 (range: 1.5-6.5), 7.1 ± 0.107kPa for patients with F1 (range: 6.5-7.5), 8.2 ± 0.30kPa for patients with F2 (range: 7.5-9.5), 11.5 ± 0.5kPa for patients with F3 (range: 9.5-12.5), 13.8 ± 0.61kPa for patients with F4 (range: 12.5-15.5), and 20.8 ± 0.45kPa for patients with C1 (range: 15.5 & above).

Table 3. The result of FibroScan of the included patients:

Fibrosis stages	Frequency	Percent	Cumulative Percent	Mean deviation	Standard Deviation
C1	3	2.1	2.1	1.8	0.78
F0	116	80.0	82.1	0.92	1.14
F1	16	11.0	93.1	0.21	0.26
F2	5	3.4	96.6	0.58	0.7
F3	2	1.4	97.9	0.5	0.70
F4	3	2.1	100.0	0.8	1.05
Total	145	100.0			

Table 4. Laboratory Findings Of Included Patients

Test	No of patients	Test Results (mean ±SEM)	Std. Deviation	Minimum	Maximum
AST (U/L)	5	40.3 ± 7.53	13.05	30	55
ALT(U/L)	135	76.95 ± 10.96	89	12	541

INR	6	0.99± 0.015	0.026	0.96	1.01
Platelet Count	20	24320.4± 24056.6	86737.5	1.18	313000

DISCUSSION

The findings of the current retrospective study in a group of patient with chronic liver disease have shown that Transient-Elastography is an effective method for diagnosing fibrosis and its intensity. Drawback of hepatic biopsy requires the creation of non-invasive methods like the Transient Elastography which can be used as a diagnostic tool to find out the liver fibrosis. It can also assist in preventing from severe fibrosis in the individuals with liver disease HBV. Followed by the liver biopsy, hepatic fibrosis should be detected with transient elastography. Moreover, its cut-off values of liver stiffness for various stages of liver fibrosis should be determined before carrying out the examination. The duration between the two tests should not be more than three months as it is mostly a valid time interval for the individuals with less severe fibrosis. Evaluators of the result should not be aware of the provided treatment. [27]

For the patients having chronic HBV, Hepatic-fibrosis is a reversible scarring reaction which eventually results in cirrhosis and nodule formation. The correct assessment of fibrosis is necessary to guide management and predict diagnosis. Histological assessment of the liver biopsy stays the gold standard for the quantification of fibrosis. Lately, there is a growing interest in the use of the non-invasive methods to provide more regular sampling and to prevent the occurrence of percutaneous biopsy. Several researchers have shown correlation between progression of fibrosis and activity grade to cirrhosis. The evaluation of fibrous scarring for the

staging of liver disease has always been a key function of liver biopsy analysis. However, it is recently considered that fibrosis is an irreversible process, and the changes-with-time are relatively slow. Thus, there are few stages for most of the grading and staging systems and as a result they are extremely inadequate to describe changes in fibrosis.

In our current study, we have tried to figure out the role of FibroScan in prognosis and staging of liver fibrosis in chronic HBV patients. In chronic HBV patients, numerous factors have been tested to evaluate the liver-fibrosis in these patients. Our research consisted of different clinical, laboratory, and demographic parameters to analyze the liver fibrosis in patients of HBeAg positive chronic HBV with ALT and AST values. Our research included 145 patients that were serologically proved to have chronic HBV and were selected for the FibroScan to identify patients with fibrosis. There were a few participants with various intensities of liver fibrosis. We categorized our patients on the basis of METAVIR scoring system bind to the outcomes of Transient Elastography into F0:116 patients, F1:16 patients, F2:5 patients, F3:2 patients, F4:3 patients, and C1:3 patients. Thus, out of 145 individuals 116 had no fibrosis which means 80% of patients were not affected by fibrosis at all (F0 = 80%). Only 16 patients were diagnosed with initial fibrosis without septa (F1 = 11%) and 5 patients with few septa (F2 = 3%). The remaining 8 people had severe fibrosis out of which 2 patients had advanced fibrosis (F3 = 1%), 3 were at sever fibrosis stage with no cirrhosis (F4 = 2%) and 3 had cirrhosis (C1=2%. Therefore, total number of cirrhotic patients in our research was too small.

Conclusion

FibroScan – Transient Elastography is a secure, rapid, and non-invasive method to obtain measurement of liver stiffness as it may be effective for staging and grading of liver fibrosis

along with results. Liver stiffness measurement as a result of Transient Elastography has a good interconnection with METAVIR score for liver fibrosis. This non-invasive evaluation technique can be used for the diagnosis of liver-fibrosis and monitoring the growth and regression of fibrosis in patients with Chronic HBV. The choice of the test is based on the cost, reliability, accuracy, availability of these tests and individual patient factors. The job of liver biopsy is becoming less renowned due to the continuous growth of noninvasive fibrosis tests, demonstrating the better prognosis performance for cirrhosis, and prediction for diagnosis of liver-oriented results.

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