

Image analysis assessment of fibrosis from liver biopsy of chronic hepatitis patients

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Background/aim

Liver fibrosis results from chronic inflammation of the hepatic parenchyma. Progressive accumulation of fibrous tissue eventually leads to cirrhosis and its complications. The severity of liver fibrosis defines the stage of chronic hepatitis and carries with it important clinical implications. Histological scoring systems such as Ishak provide descriptive evaluation of the liver tissue mainly in terms of architectural changes without measuring the amount of fibrosis. This study aimed to assess the theoretical advantage of image analysis morphometry for providing truly quantitative data with respect to the amount of fibrous tissue.

Materials and methods

A Lieca Qwin 500 image analyzer with a damaged area morphometry software was used applying the interactive method to measure the fibrous tissue area on the basis of different colors of hepatocytes and fibers following staining with Masson's trichrome stain. Forty-three patients (38 men and five women) were recruited into the study with a mean age of 45.5 years (range 15–58 years). Of them, 40 had chronic viral hepatitis and three had chronic nonviral hepatitis.

Results

Morphometric measurements of fibrosis obtained with the image analyzer were highly correlated with results obtained using the Ishak method. The correlation was found to be statistically significant using the χ^2 -test ($P < 0.0001$).

Conclusion

Quantitative image analysis for estimation of the percentage area of liver fibrosis is a simple and accurate method for evaluating fibrous tissue in patients with chronic hepatitis, aiding therapeutic approaches.

Keywords:

chronic hepatitis, image analysis, liver fibrosis, morphometry

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Introduction

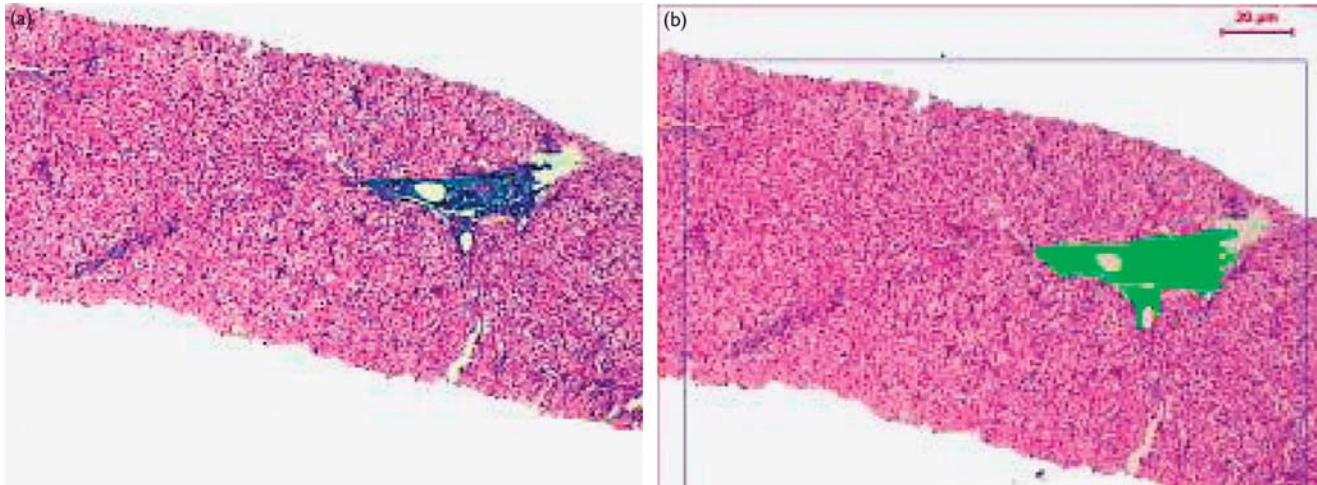
Liver fibrosis is the excessive accumulation of extra-cellular matrix (ECM) proteins, including collagen, which occurs in most types of chronic liver diseases [1]. Both fibrosis and cirrhosis are the consequences of a sustained wound-healing response to chronic liver injury [2]. The accumulation of ECM proteins distorts the hepatic architecture by forming a fibrous scar, and the subsequent development of nodules of regenerating hepatocytes defines cirrhosis. Cirrhosis produces hepatocellular dysfunction and increased intrahepatic resistance to blood flow, which result in hepatic insufficiency and portal hypertension, respectively [3].

The onset of liver fibrosis is usually insidious, and the related morbidity and mortality occur after the development of cirrhosis [1]. Major clinical complications of cirrhosis include ascites, renal failure, hepatic encephalopathy, and variceal bleeding. Patients with cirrhosis can remain free of major complications for several years. Decompensated cirrhosis is associated with short survival,

and liver transplantation is often indicated as the only effective therapy [4].

Fibrosis is a dynamic process; in healthy individuals, although there is no change in the structure of the ECM on histology, there are simultaneous catabolic and metabolic processes that reach equilibrium with each other. In the fibrotic state, there is excessive production of ECM, which outstrips the catabolism of ECM elements [5,6]. In advanced stages, the liver contains approximately six times more ECM than normal, including collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. Accumulation of ECM results from both increased synthesis and decreased degradation [1].

Chronic hepatitis is a morphological pattern seen most often in chronic viral hepatitis, as well as in autoimmune hepatitis, drug reactions, and some metabolic diseases. Chronic hepatitis, regardless of the cause, is characterized by several pathological changes that are present to a variable extent in each case. These include portal

Figure 1

Chronic hepatitis stage 1/6 by the Ishak scoring system (Masson's trichrome stain, $\times 50$). (a) The portal area showed expansion of fibrous tissue. (b) Binary image of the fibrous tissue in the portal area taken by an interactive method using an image analysis system with a damaged area morphometry software.

inflammation and sometimes lesions of bile ducts within the portal spaces, periportal injury and inflammation, several forms of degeneration and death by apoptosis of intra-acinar hepatocytes with an associated inflammatory response, and fibrosis that may involve only the portal and periportal areas or that may form septa [7].

It has been reported that Egypt has the highest prevalence of hepatitis C virus (HCV) worldwide, ranging from 6% to more than 40% of the population, with an average of 13.8% [8]. A previous parenteral therapy for schistosomiasis, which had inadequate sterilization techniques, has been implicated as the cause for this high prevalence rate [9].

The term 'image analysis' is reserved for a special discipline in pathology that aims to obtain diagnostically important information in an objective and reducible manner, by measuring and counting [10]. Quantitative analysis of histochemical and cytochemical components such as DNA, RNA, or the chromatin pattern on one hand (cytometry) and the quantitative analysis of geometric nonchemical cell and tissue components (morphometry) on the other developed somewhat independently [11–13].

The word morphometry means measurement of form; however, in 1969, Weibel, who was one of the main promoters of morphometry, defined the term as the quantitative description of structure. This structure may be macroscopic or microscope in size. The reasons for the increasing interest in application of morphometry in diagnostic pathology are its advantages in terms of objectivity, reproducibility, and the possibility of detecting minor differences or variations in a specimen that would otherwise escape subjective evaluation [14].

Quantitative measurement of the severity of liver fibrosis was achieved using computerized image analysis in Masson trichrome-stained sections. The results showed

a highly significant increase in the area of fibrosis in the case of rats treated with carbon tetrachloride [15].

Assessment of liver fibrosis is very important for the diagnostic and prognostic evaluation of chronic liver disease [16]. All histological scoring systems incorporate a categorical description of architectural changes, without reference to quantitative changes in liver collagen (fibrosis), as the stage of liver disease progresses or regresses [17].

Therefore, this study aimed to assess the theoretical advantage of morphometric image analysis in providing truly quantitative data with respect to the amount of fibrous tissue.

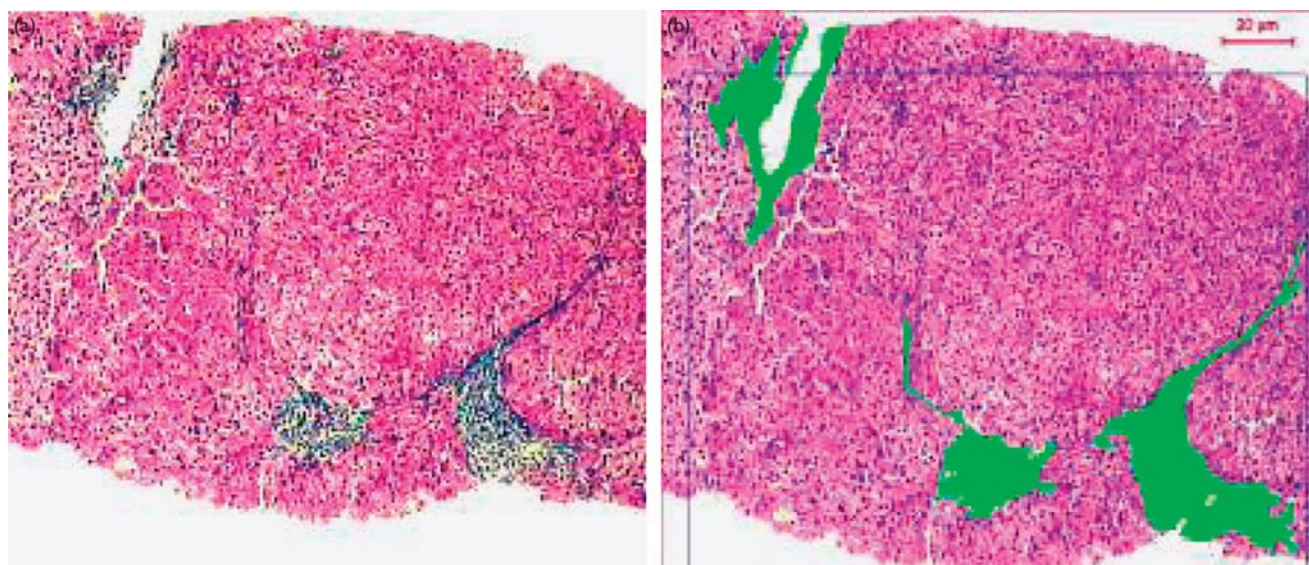
Materials and methods

A total of 63 stored, formalin-fixed, paraffin-embedded percutaneous liver biopsies from patients with chronic hepatitis were collected from multiple private laboratories. The paraffin blocks were serially sectioned at 5 μ m thickness. Thereafter, they were stained using routine hematoxylin–eosin and Masson's trichrome stain for assessment of fibrosis.

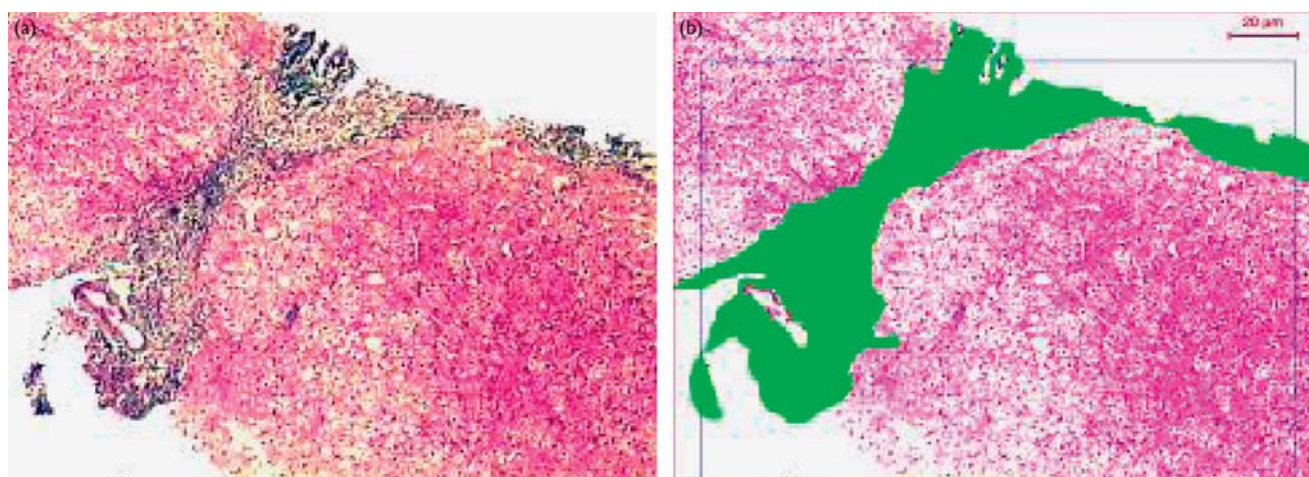
Twenty core biopsy specimens were excluded after applying the criteria for adequacy, which included a core length of 10 mm, in the absence of fibrous septa, and at least three portal tracts [18].

Each biopsy specimen was evaluated for the extent of fibrosis using the criteria of Ishak, which consist of seven descriptive criteria ranging from no fibrosis to cirrhosis, with corresponding scores assigned from 0 to 6 [19].

The percentage area of fibrosis for each liver biopsy specimen was calculated using the Lieca Qwin 500 Image

Figure 2

Chronic hepatitis stage 2/6 by the Ishak scoring system (Masson's trichrome stain, $\times 50$). (a) The portal area showed expansion of the fibrous tissue with focal septations. (b) Binary image of the fibrous tissue in the portal area taken by an interactive method using an image analysis system with a damaged area morphometry software.

Figure 3

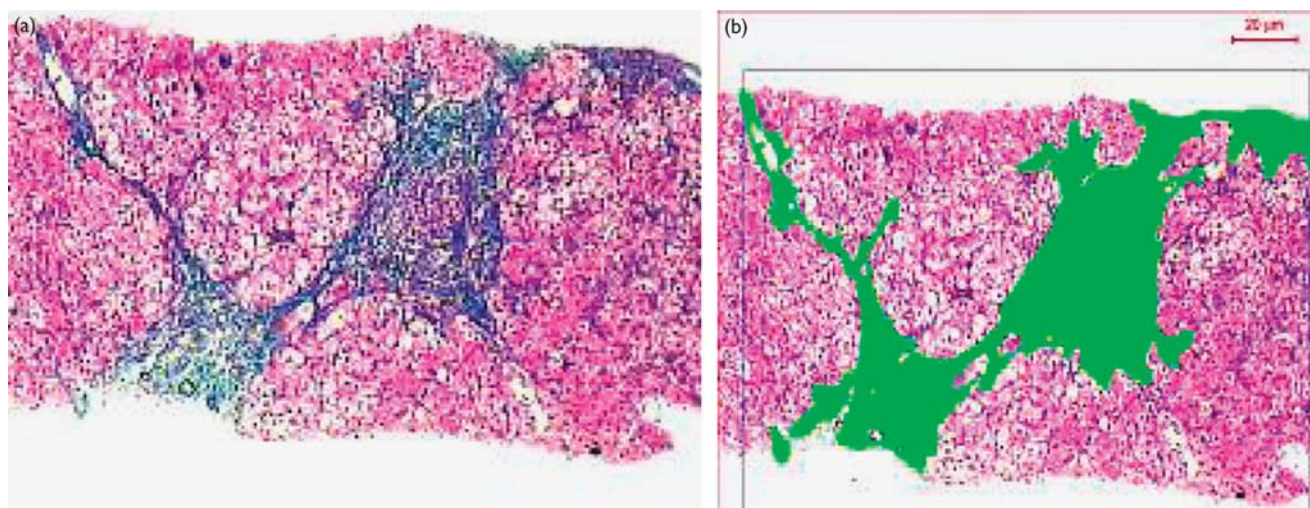
Chronic hepatitis stage 3/6 by the Ishak scoring system (Masson's trichrome stain, $\times 50$). (a) Fibrous expansion of portal areas with portal-portal bridging. (b) Binary image of the fibrous tissue in the portal area and portal-portal bridging taken by an interactive method using an image analysis system with a damaged area morphometry software.

Analyzer (Leica, Cambridge, England) in the Pathology Department, National Research Centre (Egypt). To capture the widest area of tissue, images of trichrome-stained sections were captured using a $\times 5$ magnification objective. The Masson's trichrome-stained section represented fibrosis as blue-stained and the parenchyma as red-stained regions. The total area of the section was the sum of the area of all microscopic fields, including parenchyma and fibrosis. Elements of the liver capsule were excluded from the computations. Fibrosis in the space of Disse was not evaluated [18].

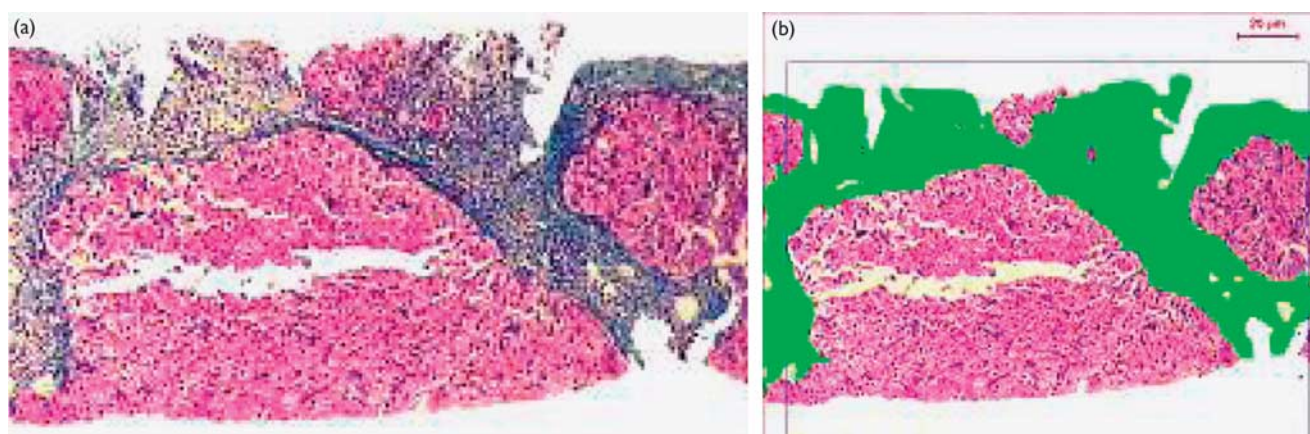
Statistical analysis

Data were statistically described in terms of frequencies (number of patients) and relative frequencies (%). The χ^2 -test was performed to compare the different study variables between the study groups. Yates's correction and Fisher's exact tests were used only when the expected frequency was found to be less than 5. A P value less than 0.0001 was considered statistically significant.

All statistical calculations were performed using Microsoft Excel version 7 (Microsoft Corporation, New York,

Figure 4

Chronic hepatitis stage 4/6 by the Ishak scoring system (Masson's trichrome stain, $\times 50$). (a) Fibrous expansion of portal areas with bridging (portal–portal and portal–central). (b) Binary image of the fibrous tissue in the portal area, portal–portal, and portal–central bridging taken by an interactive method using an image analysis system with a damaged area morphometry software.

Figure 5

Chronic hepatitis stage 5/6 by the Ishak scoring system (Masson's trichrome stain, $\times 50$). (a) Marked bridging fibrosis with an incomplete nodule. (b) Binary image of the fibrous tissue in the portal area and the bridging fibrosis taken by an interactive method using an image analysis system with a damaged area morphometry software.

New York, USA) and Statistical Package for the Social Science (SPSS Inc., Chicago, Illinois, USA).

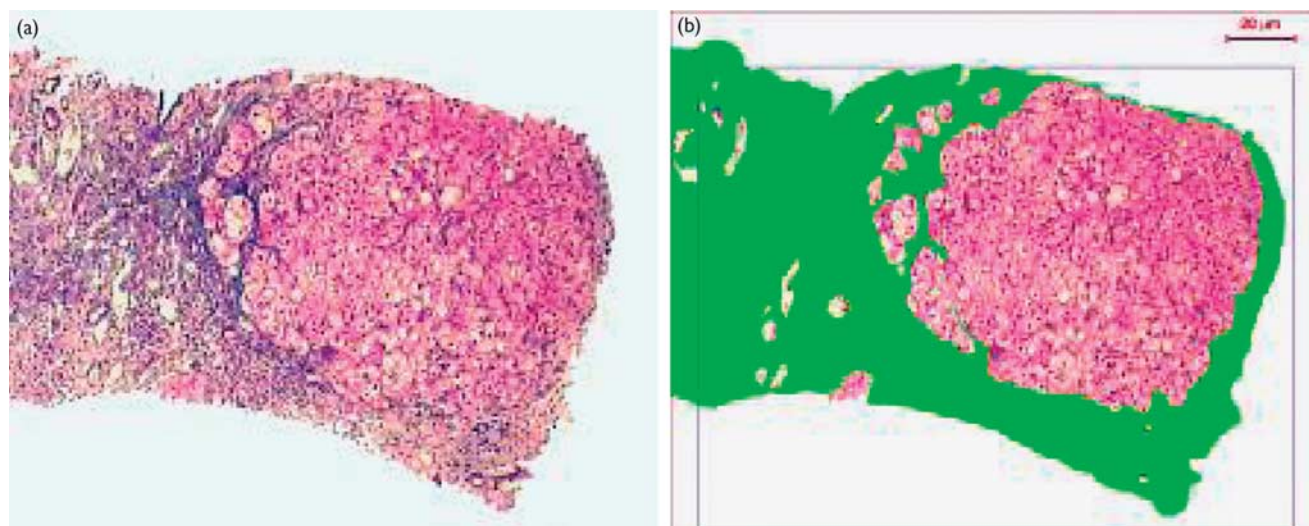
Results

This study included 43 patients with chronic hepatitis. According to the Ishak scoring system, of the 43 patients, 10 were in stage 1 fibrosis (23.26%) (Fig. 1), 15 were in stage 2 (34.88%) (Fig. 2), four were in stage 3 (9.3%) (Fig. 3), eight were in stage 4 (18.6%) (Fig. 4), four were in stage 5 (9.3%) (Fig. 5), and two were in stage 6 (2.33%) (Fig. 6, Graph 1).

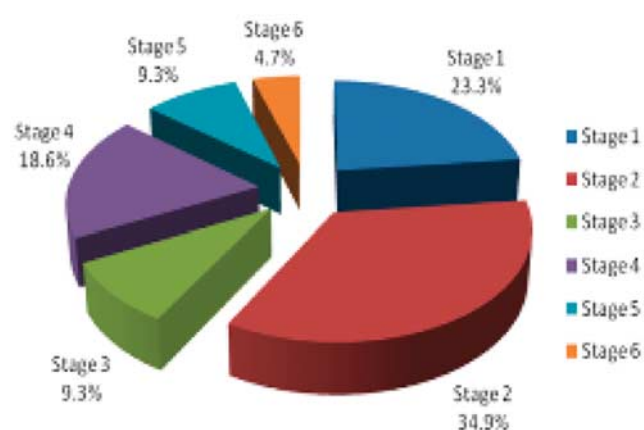
The mean age of the studied patients was 45.5, with two patients in the age group of 11–20 years, two in the age group of 21–30 years, six in the age group of 31–40 years, 14 in the age group of 41–50 years, and 19 in the age group of 51–60 years (Graph 2).

With respect to etiology, 40 patients had chronic viral (93%) and three had chronic nonviral hepatitis (7%) (Graph 3).

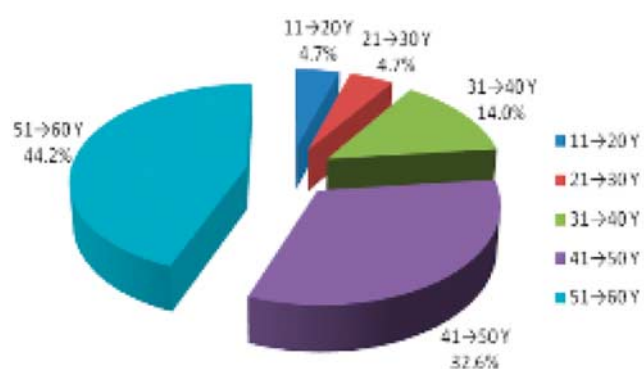
According to the sex distribution, 38 of the 43 patients (88.37%) were men, whereas the remaining five were women (11.63%) (Graph 4).

Figure 6

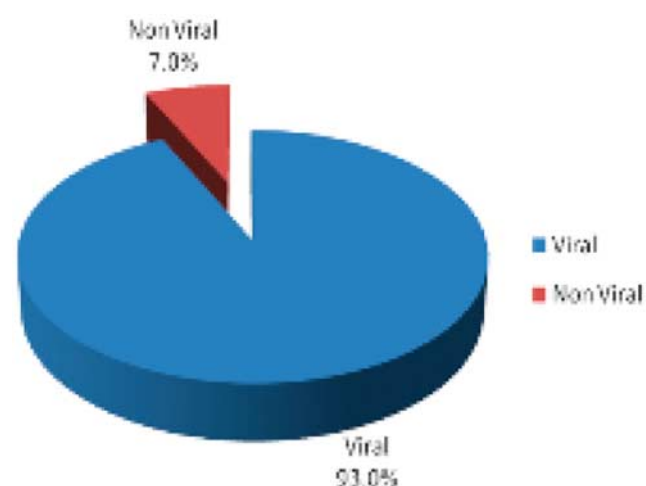
Chronic hepatitis stage 6/6 by the Ishak scoring system (Masson's trichrome stain, $\times 50$). (a) Marked bridging fibrosis, wide fibrous septa, and cirrhotic nodule formation. (b) Binary image of the wide fibrous septa taken by an interactive method using an image analysis system with a damaged area morphometry software.

Graph 1

Percentage of different stages of fibrosis showing the most common stage as stage 2 (34.9%) ($P=0.0060$).

Graph 2

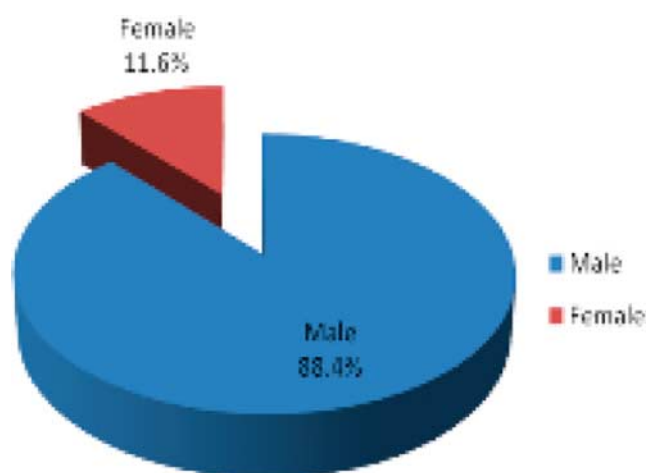
Age distribution in the studied patients showing that most of the patients were in the age group of 51–60 years (44.2%) ($P<0.0001$).

Graph 3

Percentage of chronic hepatitis etiology in the studied patients showing that most of the patients had viral etiology (93%) ($P<0.0001$).

By studying the relationship between fibrosis stage according to the Ishak scoring system and the morphometrically determined percentage areas of fibrosis, the fibrosis scores were assigned for each biopsy specimen according to the criteria of Ishak *et al.* [19]. The fibrosis percentage is the percentage of fibrous tissue in the core, as determined by digital image analysis of the trichrome-stained sections. Ten patients were classified as being of stage 1, with minimum percentage area of fibrosis 1.709, maximum percentage area 6.899, and mean percentage area 3.964; 15 patients were classified as being of stage 2, with minimum percentage area of fibrosis 3.31, maximum percentage area 7.123, and mean percentage area 5.273; four patients were classified as being of stage 3, with minimum percentage area of fibrosis 7.106, maximum

Graph 4

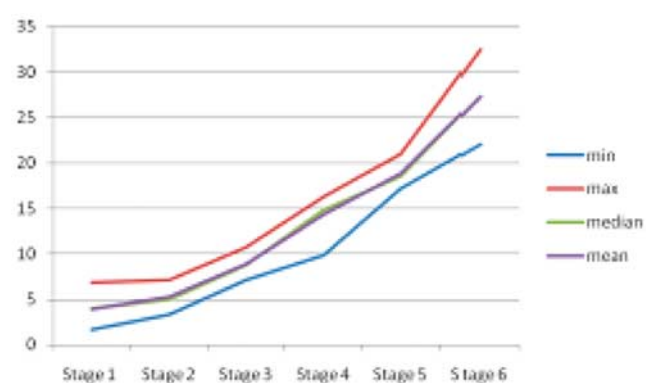


Sex distribution in the studied patients showing that there was a male predominance among chronic hepatitis patients (88.4%) ($P < 0.0001$).

Table 1 Ishak scoring system versus morphometric fibrosis area percentage

Stage of fibrosis	Number of patients	Morphometric fibrosis area percentage				
		Minimum	Maximum	Median	Mean	SD
Stage 1	10	1.71	6.89	4.01	3.96	1.39
Stage 2	15	3.31	7.12	4.97	5.27	1.23
Stage 3	4	7.11	10.79	8.81	8.88	1.51
Stage 4	8	9.86	16.29	14.88	14.37	2.09
Stage 5	4	17.17	20.98	18.55	18.81	1.71
Stage 6	2	22.06	32.49	27.28	27.28	7.38

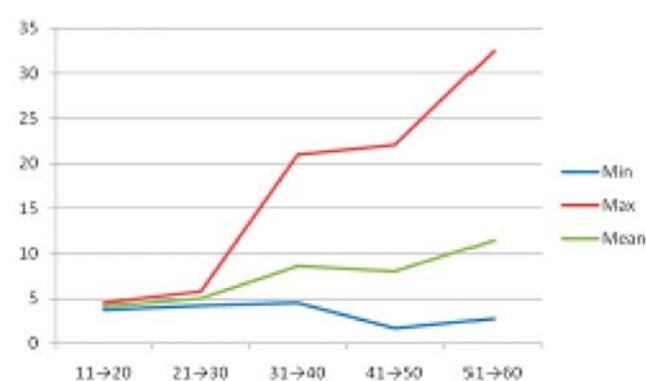
Graph 5



Correlation between liver biopsy morphometric fibrosis area percentage and Ishak fibrosis score, showing a positive correlation ($P < 0.0001$).

percentage area 10.798, and mean percentage area 8.881; eight patients were classified as being of stage 4, with minimum percentage area of fibrosis 9.858, maximum percentage area 16.298, and mean percentage area 14.883; four patients were classified as being of stage 5, with minimum percentage area of fibrosis 17.174, maximum percentage area 20.98, and mean percentage area 18.813; finally two patients were classified as being of stage 6, with minimum percentage area of fibrosis

Graph 6



Correlation between morphometrically determined percentage area of fibrosis in liver biopsy and age groups. The highest area percentage was in the age group of 51-60 years ($P < 0.0001$).

Table 2 Morphometric fibrosis area percentage in each age group

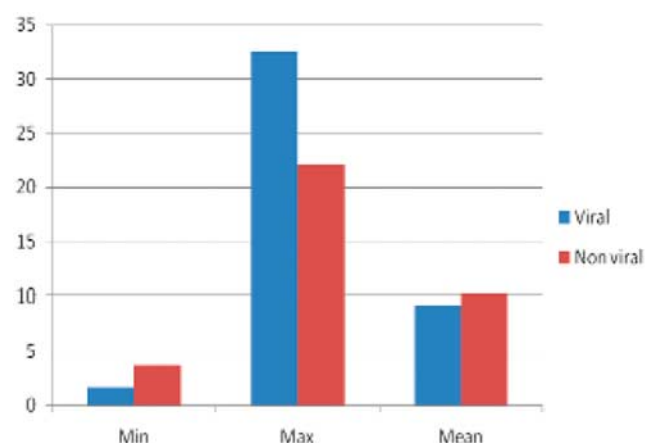
Age groups	Morphometric fibrosis area percentage		
	Minimum	Maximum	Mean
11-20	3.76	4.49	4.13
21-30	4.19	5.79	4.99
31-40	4.47	20.98	8.63
41-50	1.71	22.06	8.05
51-60	2.77	32.49	11.39

22.061, maximum percentage area 32.498, and mean percentage area 27.279. The correlation between the stage of fibrosis according to Ishak's scoring system and the morphometrically determined percentage area of fibrosis was statistically significant ($P < 0.0001$) (Table 1, Graph 5).

With regard to the relation between age and the morphometrically determined percentage area of fibrosis, in the age group of 11-20 years the minimum percentage area of fibrosis was 3.775, maximum percentage area was 4.489, and mean percentage area was 4.132; in the age group of 21-30 years, the minimum percentage area of fibrosis was 4.187, the maximum percentage area was 5.799, and mean percentage area was 4.993; in the age group of 31-40 years, the minimum percentage area of fibrosis was 4.467, the maximum percentage area was 20.98, and mean percentage area was 8.631; in the age group of 41-50 years, the minimum percentage area of fibrosis was 1.709, maximum percentage area was 22.061, and mean percentage area was 8.046; and in the age group of 51-60 years, the minimum percentage area of fibrosis was 2.774, maximum percentage area was 32.498, and mean percentage area was 11.385. The correlation between age and the morphometrically determined percentage area of fibrosis was statistically significant ($P < 0.0001$) (Graph 6).

Patients with chronic viral hepatitis showed the minimum percentage area of fibrosis to be 1.709, maximal percentage area 32.498, and mean percentage area

Graph 7



Distribution of morphometrically determined percentage area of fibrosis in liver biopsy for viral and nonviral hepatitis ($P < 0.0001$).

9.213, whereas patients with chronic no viral hepatitis showed the minimum percentage area of fibrosis to be 3.775, maximal percentage area 22.061, and mean percentage area 10.15. There was a statistically significant difference in the mean percentage area of fibrosis between chronic viral and nonviral hepatitis cases ($P < 0.0001$) (Table 2, Graph 7).

Discussion

Fibrosis and cirrhosis are the consequences of a sustained wound-healing response to chronic liver injury. Cirrhosis is the end result of many types of liver diseases and it consists of fibrosis and nodular regeneration [20].

Liver biopsy is an important part of the evaluation of patients with a variety of liver diseases. Besides establishing the diagnosis, the biopsy is often used to assess the severity of the disease in terms of both grade and stage. Stage in most chronic liver diseases relates to the degree of scarring, with the end stage being cirrhosis with its clinical complications; grade relates to the severity of the underlying disease process [21].

Chronic liver disease and cirrhosis are prevalent worldwide, regardless of race, age, or sex. However, there are marked geographical variations in terms of incidence and prevalence, largely depending on the prevalence of causative factors. Although virtually any chronic liver disease may progress to cirrhosis, the most common causes of liver fibrosis and cirrhosis globally are thought to be hepatitis B virus and HCV [20].

Egypt reports the highest prevalence of HCV worldwide, ranging from 6% to more than 40% of the population, with an average of 13.8% [8]. Hepatitis B virus prevalence varies from 0.1 up to 20% across the world, with high prevalence (10–20%) in South-East Asia, China, and sub-Saharan Africa [22].

In this study, we recruited 43 patients with a mean age of 45.5 years (range 15–58 years). This mean age was lower than that seen in the study by Lin *et al.* [23], who recruited 31 patients with a mean age of 41.6 years (range 24–66).

With respect to the age group, in this study the prevalence was highest in the age group of 51–60 years, whereas according to the survey by the National Center for Health Statistics in the USA (1999–2002), in which serum samples from 15 079 random participants were tested for HCV antibody and HCV RNA, the highest prevalence was seen in the age group of 40–49 years [24].

With respect to sex, our study included 43 patients, of whom 38 were men and five were women. The male predominance is similar to that seen in the study by Lin *et al.* [23], which included 31 patients of whom 25 were men and six were women. In another study conducted by the National Center for Health Statistics in the USA (1999–2002), in which serum samples from 15 079 random participants were tested for HCV antibody and HCV RNA, the prevalence of chronic hepatitis C and hence liver fibrosis was found to be highest among non-Hispanic black men [24].

The study showed predominance of viral etiology, with 40 patients having chronic viral hepatitis in contrast to three having chronic nonviral hepatitis. In the study by Lin *et al.* [23], 16 patients had chronic hepatitis B, 12 patients had chronic hepatitis C, and three patients were alcoholics, whereas in the study by Friedenber *et al.* [25] most of the patients had undergone a biopsy for the evaluation of hepatitis C; however, some patients with hepatitis B, autoimmune liver disease, and nonalcoholic steatohepatitis were also included.

The first widely applied scoring system for inflammation and fibrosis in chronic hepatitis was that of Knodell *et al.* [26]. Because this system failed to provide a sufficiently clear separation of fibrosis and inflammatory activity, Ishak *et al.* [19] proposed a modification of the Knodell system that attempted to address this and other deficiencies. It consisted of a seven-tier staging system for fibrosis, with values ranging from 0 (no fibrosis) to 6 (cirrhosis). Several other scoring systems are also currently in use. The METAVIR Cooperative Group uses a five-stage (F0, F1, F2, F3, and F4) system that has been validated systematically [27,28]. Other similar approaches for quantification of inflammation and fibrosis in liver biopsy specimens have been developed and include those of Scheuer *et al.* [29] and Pawlotsky [30] and the system convened by the International Association for the Study of the Liver [31].

In this study, to evaluate the degree of fibrosis, conventional hematoxylin and eosin and Masson's trichrome stains were used. The degree of fibrosis was recorded on the basis of the Ishak scoring system. We studied 43 patients and found no patients in stage 0, 10 in stage 1, 15 in stage 2, four in stage 3, eight in stage 4, four in stage 5, and two in stage 6, indicating that the most common stage was stage 2 and the least

common was stage 0, which was in agreement with the results of O'Brien *et al.* [32] who studied 230 patients, recording 10 patients in stage 0, 45 in stage 1, 57 in stage 2, 40 in stage 3, 22 in stage 4, 13 in stage 5, and 43 in stage 6, with the most common stage being stage 2 and the least common being stage 0.

Although numeric grades are used, histological scoring systems are qualitative. The different grades do not represent a continuous scale. Although valuable information on architectural changes associated with fibrosis has been obtained, it is relatively imprecise because of the small number of grades. Moreover, experienced pathologists are needed to perform the assessment, and interpretations may be subjective at times. An alternative way to assess fibrosis in liver tissue is by image and morphometric analyses. Morphometry is the evaluation of forms; in histopathology it describes the measurements obtained from two-dimensional sections. Their low intra-assay variability makes them suitable for quantitative morphological approaches [33].

Several reports in the literature have addressed the measurement of liver fibrosis by computerized image analysis to derive a ratio of fibrosis to the total area of the biopsy specimen and compare it with the variable ordinal histopathological scoring system. Chevallier *et al.* [34] compared the computerized fibrosis ratios with a detailed subjective scoring system (Knodell's system) that incorporated evaluation of central veins, pericellular fibrosis, portal tracts, and the number and width of septa in 200 patients with chronic liver disease due to multiple causes. Kage *et al.* [35] compared image analysis-derived fibrosis ratios using a four-stage fibrosis classification system. Pilette *et al.* [36] compared fibrosis ratios and subjective scores (METAVIR) in a series of 243 patients with chronic liver disease. Lin *et al.* [23] evaluated 16 patients with hepatitis B, 12 with hepatitis C, and three with alcoholic liver disease, using Knodell's fibrosis score, and compared them using the morphometric and colorimetric methods.

The present study showed an overall statistically significant positive correlation between fibrosis ratio and the ordinal score ($P < 0.0001$), which was in agreement with the results of other studies, using the Ishak scoring system. O'Brien *et al.* [32] evaluated 230 patients with hepatitis C and compared fibrosis ratios calculated using an image analysis program with Ishak scores. Hui *et al.* [33] validated a new image analysis system, Bioquant Nova Prime (Boston, USA), for estimating collagen content in liver biopsy samples from patients with chronic hepatitis B. The biopsies were stained with picrosirius red and the areas containing collagen were measured. The results were correlated with the laboratory parameters and Ishak-modified histological scores. Finally, Friedenberg *et al.* [25] evaluated 91 patients with chronic hepatitis and compared between fibrosis ratios calculated using an image analysis (FibroXact, School of Information Sciences and Technology, Philadelphia, USA) program and Ishak scores. All the studies reported a statistically significant correlation between fibrosis

assessed by image analysis and the respective classifications for fibrosis.

In conclusion, morphometric assessment of the percentage area of fibrosis is a simple, accurate, and reproducible method for evaluating fibrosis in liver biopsies from chronic hepatitis patients. There is a significant correlation between the morphometric image analysis of the percentage area of fibrosis and the Ishak staging system of fibrosis in liver biopsies from chronic hepatitis patients. Morphometric image analysis for the assessment of liver fibrosis provides quantitative results, proven by the overlap of the percentage area of fibrosis noticed between the successive Ishak stages. There is significant correlation between age and the mean morphometrically determined percentage area of fibrosis. There is a male predominance in chronic hepatitis, mostly in cases of chronic viral hepatitis.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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