ORIGINAL ARTICLE

Significance of YKL-40 in comparison to Fibroscan Elastography in Staging liver Fibrosis in Chronic Hepatitis C

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ABSTRACT

Background: Since ages, liver biopsy has been used to determine the estate of fibrosis since in all types of chronic liver disease to provide proper and timely management to the patients. Due to severe complications of liver biopsy, other tools are used to ascertain fibrosis including biomarkers and radiological devices. This study's objective is to correlatively measure the efficacy of direct serum marker YKL-40 with fibroscan liver and one of the non-invasive mathematical algorithm i.e. alanine aminotransferase and platelet ratio index in patients with hepatitis C with Child's Class A or B.
Methodology: This observational cross sectional analysis was managedat a private setup and hospital facilities providing tertiary care inKarachi during the duration of January 2019 till September 2019. Total 70 patients were incurred with the age distribution of 16-65 years of each sex with hepatitis C in Child's class A or B.
Result: Out of 70 patients, 57 were studied due to lack of follow up of the rest. There were 42 females. The calculated mean age among these patients was 43±13 years. There was a significant co-relation observed between YKL-40 and fibroscan liver with the p-value of 0.004. There was also significant co-relation found between YKL-40 and one the indirect mathematical calculated marker i.e Alanine aminotransferase and Platelet ratio index with a p value of 0.032.
Conclusion: YKL-40 can be used for the estimation of staging of liver fibrosis in comparison to fibroscan elastography, which is an operator dependent procedure with certain limitation. The single indirect mathematical calculated marker also shows significant result to determine stages of liver fibrosis.
Key Words: Chronic Hepatitis C, YKL-40, Fibroscan Elastography, Alanine aminotransferase Platelet Ratio Index

INTRODUCTION

Liver affected by fibrosis is a familiarand late manifested squeal of nearly all diversified categories of chronic liver disease. Early diagnosis of liver fibrosis might be of some help in the commencement of early treatment thus giving benefit to the patients by delaying complications and need for liver transplantation. Liver biopsy and histopathology is neverthelesshighest reliability test for diagnosis of fibrosis affecting liver, but because of its invasive nature leading to major complications like bleeding, it's not a very famous procedure amongst patients.[1]

There are certain non-invasive procedures available to ascertain the diagnosis of liver fibrosis. Among them fibroscan elastography is the commonest and widely used these days. Fibroscan is a non-invasive device which helps in measuring the stiffness of liverthrough a technique of transient elastography. It measures the degree of liver damage, surveildisease progression and guide prognosis for further management.[2]The pitfalls in this procedure are that it is operatordependent, cannot be performed in moderate to massive ascites, there are some limitations with increasing age and obesity.[3]

Because of invasive nature of liver biopsy, and few contraindications in performing fibroscan elastography, certain direct and indirect markers are used for grading of liver fibrosis in hepatic system. The direct indicatorsare straightwayplay their role in deposition and elimination of extracellular matrix generated by hepatocellular cells and various liver cells.[4] Serum levels of liver markers increase with increasing fibrosis, and decreases with treatment. The markers included are procollagen, hyaluronic acid, laminin, collagenases and their inhibitors; YKL-40, cytokines and proteomic markers.[5]YKL-40 is stoutlyexpressed in human hepatic system and joint interfacing cartilage. It is strongly elevated in various joint illnesses like rheumatoid arthritis, osteoarthritis, in synovial fluid and hepatic disease due to alcohol.[6]The function related to Physiology, although, is still not clear,contributes in tissue remodelling and extracellular matrix degradation.[7] However, the use of YKL-40 clinically in various chronic liver disease still needselaboration for the assessment of degree of liver fibrosis.

The indirect markers included are alanine aminotransferases, aspartate aminotransferases, alkaline phosphatase,glutamyl gamma transferase, and platelets. Among these indirect markers ratio of different markers are used to calculate fibrosis.Fibrosis-4 (FIB-4) Calculator, Alanine aminotransferase (AST) to Platelets Ratio Index (APRI), gamma glutymyl transferase to platelet ratio index (GAPRI)and Forn's index are in use for the assessment of fibrosis.[8]However ratio of the indirect markers are not strong enough for diagnosing liver fibrosis if used alone.

This study is conducted to compare one of the direct markers of liver fibrosis i.e. YKL-40, with fibroscan elastography and compare it with one of the non-invasive mathematical algorithmi.e. APRI forchronic hepatitis C infection patients.

METHODOLOGY

This observational cross-sectional analysis, involving comparative parameters in the study wasconducted at both

a private setup and hospital facilities in Karachi providing tertiary care to the patients,during the period of January 2019 to September 2019. Participants were enrolled only after taking verbal and signed consent. Prior permission from the hospital management was taken as well.

All individuals affected with chronic hepatitis C with child's class A or B, of either sex between the age group 16-65 were incurredin the study.

Patients with other etiologies of chronic (Hepatic) liver disease like hepatitis B, autoimmune hepatitis, Wilson's disease, and alcoholic liver disease were closeout from the study.

Patients with hepatic encephalopathy, massive ascites and active variceal bleeding were also closeout from study.

Patients with diagnosed or suspected hepatocellular carcinoma were also excluded.

Each patientwas subjected to have haemoglobin, Alanine amiotransferase, aspartate aminotransferase, Platelets, albumin, Prothrombin time, International normalized ratio (INR), YKL-40, fibroscan and ultrasound liver. The grading of fibrosis was done with the help of Metavir classification. Fibrosis graded as F0 to F4, <7.0 kPa was taken as normal.[9]Alanine amiotransferase to platelet ratio index (APRI) was calculated on all patients by using following formula:[10]

$$\text{APRI} = \frac{\text{AST Level (IU/L)} \;/\; \text{AST (Upper Limit of Normal (IU/L)}}{\text{Platelet Count } (10^9/\text{L})} \times 100$$

Ratio and result of this mathematical algorithm is contemplatedfor assessing the degree of fibrosis, and it may help in providing early treatment. To increase the significance of YKL-40, these easy calculations may enhance the degree of fibrosis and thus further helping in avoiding liver biopsy.

Statistical analysis was done on SPSS 23. Continuous variables were explained as mean ± SD and % for categorical variables. Comparison of all variables was done by using Pearson's x^2. All data was assimilatingby using variance analysis and significance of p value was considered as <0.05.

RESULT

Out of 70 patients inducted in the study, 13 were excluded because of lack of follow-up. Out of 57 patients, there were 42 females. The age range among these patients was 19-62 years with mean age among females was 40.80 ± 14.24, and in males it was 49.46 ±14.24.The mean haemoglobin among females was 9.70 ± 1.79, while in males it was 11.33± 2.34. The mean ALT and AST were 48.14 IU, and 56.10 IU respectively (table1). The mean YKL-40 among females was 267.06 ± 251.13, while in males it was 352.46 ± 439.75. The mean APRI among females and males were 1.39 ± .40 and 2.17 ± 2.61 respectively.

There is a significant co-relation seen by using Pearson's x^2 among YKL-40 and fibroscan elastography with a p-value of 0.004 (fig 1).The rest of the parameters including Hb, ALT, AST and Platelets were not significantly associated with either YKL-40 or fibroscan elastography (table2). There is also significant association was observed between APRI with YKL-40 and fibroscan with a p-value of 0.032 and 0.012 respectively (fig 2, 3).

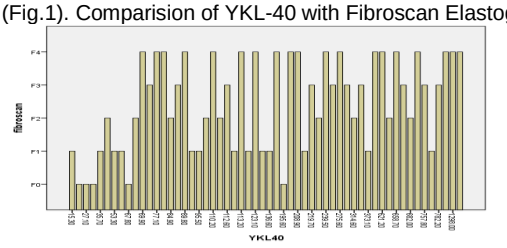
Table 1: Laboratory analysis

Variables	Mean	Standard deviation
Haemoglobin (gm/dl)	10.14	60.2 ±
ALT (IU/L)	48.15	25.05 ±
AST (IU/L)	56.11	38.56 ±
GGT (IU/L)	54.23	36.75 ±
Platelets (103/L)	144.86	92.38 ±
Albumin (mg/dl)	3	226.0 ±
INR	1.28	52.0 ±
YKL-40	289.54	87.903 ±

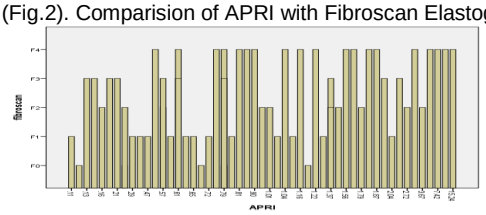
Table 2: Co-relation of YKL-40 and APRI with different parameters (p-value)

Variables	Fibroscan	Hb	ALT	AST	GGT	Platelets	Albumin	INR	APRI
YKL-40	0.004	0.019	0.849	0.33	0.505	0.178	0.054	0.8	0.032
APRI	0.012	0.571	0.012	0	0.133	0.007	0.209	0.124	

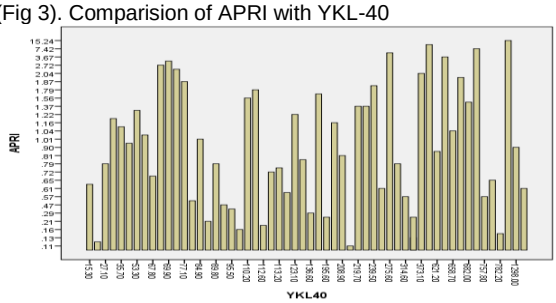
(Fig.1). Comparision of YKL-40 with Fibroscan Elastography



(Fig.2). Comparision of APRI with Fibroscan Elastography



(Fig 3). Comparision of APRI with YKL-40



DISCUSSION

For evaluating liver fibrosis, YKL-40, is one of the newer non-invasive biomarker which is becoming popular among researchers. In the following study this marker was found to

be beneficial for the patients as it is significantly co-related with liver fibrosis. The same inference was made in a research done in China.[11] Also the other non-invasive mathematical algorithm was substantially associated with liver fibrosis, when compared with fibroscan elastography.

Fibrosis of (hepatic organ) liver is a typical injure healing course in reaction to chronic injury to the liver occurring naturally.[12] The reasons of liver fibrosis is multifactorial including congenital, metabolic, inflammatory or toxins.[13] Diagnosing hepatic fibrosis is a decisive step for the appraisal of severe intensity of disease and management. This includes certain serum markers which directly co-related with progression of liver fibrosis. Among all these markers there are certain direct or indirect mathematical algorithm used by the researchers. YKL-40 is one of those non-invasive markers used widely these days in viral hepatitis and alcoholic hepatitis and has a significant association with liver fibrosis.[14]

The popularity of these non-invasive tools like fibroscan elastography and certain directly specific with categorically indirect markers for the assessment of degree of fibrosis of liver is increasing because of invasiveness of liver biopsy although gold standard among all test.[15] Fibroscan elastography is commonly use for the imposition of stage of severity of liver disease in almost all varieties of chronically affected liver (disease) such as chronic viral hepatitis due to Hep B and C, liver disease due to alcohol and also useful in fatty deposition of liver. It is a plain, easy, uncomplicated, convenient, method to assess hardness of liver which is non-intrusive and not invasive. There are certain limitations in fibroscan elastography like overestimation of fibrosis with active hepatitis, cholestasis, mass lesion or liver congestion. In addition to these there is also limited study in patients who have BMI >35Kg/m^2, having massive ascites or are in older age group.[3,16] Fibroscan result ranges from lower value of 2.5 kPa to higher value of 75 kPa.[17]Around 90-95% of healthy population has a score of <7.0kPa (median 5.3 kPa). Regardingunearthing of fibrotic cirrhosis in chronic hepatitis virus C, the value of threshold level is 14 kPa, while patients having reading >7kPa may have stiffness with the probability of 85%.[18,19] The fibroscan score of <7kPa, cannot completely exclude the chances of fibrosis. Thus itis better to interpret the result with the help of other markers as well which includes both direct and indirect markers.

Among all non-invasive biomarkers hyaluronic acid along with the identified type III procollagen-N-peptide (P III P), reading value levels are well measured in forbearing with chronic liver disease.[20] Hyaluronic acid, a glycosaminoglycan is synthesizes by mesenchymal cells and later debauched by hepatic sinusoidal cell. The levels of hyaluronic acid are directly correlated with advanced fibrosis.[21] While the levels of P III P, which is an extension peptide of collagen turnover,has a correlated standoutin histologically identifiable inflammation than fibrosis.[22]Because of these limitations, there is always a need of more reliable, accurate and easy to perform non-invasive biomarkers for the evaluation of liver fibrosis.

YKL-40is in use since early nineties for the spotting of liver fibrosis. It is a glycoprotein with a molecular weight of 39-kilodalton and stay to the biochemical group of chitinase like proteins, although lacking the true enzymatic-hydrolytic properties in terms of activity.[23]It is also named as , human cartilage glycoprotein 39, chitinase-3-like protein, chondrex and breast regression protein 39.[24] It expressed in variegated cell types including certain cancer cells, neutrophils, chondrocytes and endothelin cells.[25] Use of YKL-40 as a potential tagof fibrosis of liver and its validity is seen in various studies done before.[26]The levels of YKL-40 or CH313L1 are used to differentiate between early and advanced fibrosis when the level reading value>73.4 ng/mlis consideredto identify evolved fibrosis with the sensitivity levels of around 94% and estimated specificity closely up to the level of slightly more than 87%.[27]It can also be used to distinguish between mild stage of liver fibrosis to extensive stage with an applauding predictive value of 80%.[28] In this study the same result is observed and YKL-40 has a strong association with liver fibrosis as seen in a study done previously.[29,30] We have also compared both fibroscan and YKL-40, with APRI. APRI is an indirect mathematical algorithm marker for the assessment of liver fibrosis. All indirect markers are less invasive, easy to apply, less expensive and also helps in monitoring treatment. There are certain disadvantages though, as they are less reliable to be considered as organ specific and maycounterinfluence by untenable other different sites of inflammation. They are also not high on accuracy to discriminately identify intermediate steps. In the current study only APRI calculation was done. The estimated calculation of <0.5 has prediction of less fibrosis while levels >1.5 has prediction of significant fibrosis with the specificity of 91% but sensitivity of only 46%.[31] The correlation of APRI with YKL-40 and fibroscan is also significant enough like YKL-40 and fibroscan in the current study.

CONCLUSION

t is thus concluded that direct serum biomarkers like YKL-40 or CH313L1 can be used as an evaluative means instrumental for the staging of fibrosis in hepatitis C, but its efficacy much can be enhanced if coupled with fibroscan elastography and APRI. Previous studies have done showing less significance in calculating one indirect mathematical algorithm to incur a diagnosis of fibrosis although showing significant result in our study. As these markers are non-invasive and easy to perform in comparison to biopsy taken from liver, may be used judiciously in terms of evaluation of liver fibrosis and thus helping the commencement of early treatment.

REFERENCES

1. Bravo AA, Sheth SG, Chopra S. Liver biopsy. The New England Journal of Medicine. 2001; 344(7), 495-500
2. Voican CS, Louvent A, Trabut JB, et.al. Transient elastography alone and in combination with fibrotest for the diagnosis of hepatic fibrosis in alcoholic liver disease. Liver International. 2017; 37(11): 476-481
3. Castera L, FoucherJ,Bernard PH, et.al. Pitfalls of liver stiffness measurement: a 5 year prospective study of 13.369 examinations. Hepatology 2010; 51: 828-835
4. Wynn TA. Cellular and molecular mechanisms of fibrosis. Matrix as a modulator of hepatic fibrogenesis. Semin Liver Dis. Aug 2001; 21(3):351-372.
5. Afdhal NH, Nunes D. Noninvasive biochemical markers of liver fibrosis. J Gastrointestin Liv Dis. 2006;15: 149-159.

6. Johansen JS, Jansen HS, Price PA. A new biochemical marker for joint injury. Analysis of YKL-40 in serum and synovial fluid. Br J Rheumatol. 1993; 32: 949-955.
7. Peltomma R, Pailmela L, Harvey S, Helve T, et.al. Increased level of YKL-40 in sera from patients with early rheumatoid arthritis: a new marker for disease activity. Rheumatol Int. 2001; 20: 192-196.
8. Gressner OA, Weiskinchen R, Gressner AM. Biomarkers of hepatic fibrogenesis and genetic predisposition pending between fiction and reality. J Cell Mol Med. Sept 2007; 11(5):1031-1051.
9. Kemp W, Roberts S. FibroScan and transient elastography. Australian family physician. 2013 Jul; 42(7):468.
10. Wai CT, Greenson JK, Fontana RJ, et.al. A simple non-invasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. J Hepatology. Aug 2003; 38(2): 518-526.
11. Huang H, Wu T, Mao J, Fang Y, Zhang J, Wu L, Zheng S, Lin B, Pan H. CHI3L1 is a liver-enriched, noninvasive biomarker that can be used to stage and diagnose substantial hepatic fibrosis. Omics: a journal of integrative biology. 2015 Jun 1;19(6):339-45.
12. Ebrahimi H, Naderian M, Sohrabpour AA. New concepts on pathogenesis and diagnosis of liver fibrosis. A review article. Middle Easr Did. Dis. 2016; 8: 166-178.
13. Wen-Ce Z, Quan-Bao Z, Liang Q. Pathogenesis of liver cirrhosis. World J Gastroenterol. 2014; 20(23): 7312-7324.
14. Johansen JS, Møller S, Price PA, Bendtsen F, Junge J, Garbarsch C, Henriksen JH. Plasma YKL-40: a new potential marker of fibrosis in patients with alcoholic cirrhosis? Scandinavian journal of gastroenterology. 1997 Jan 1;32(6):582-90.
15. Braticevici CF, Papacocea R, Tribus L, Badarau A. Can We Replace Liver Biopsy with Non-Invasive Procedures?. Liver Biopsy. 2011 Sep 6:225-40.
16. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008; 48:835-847.
17. Afdhal NH. Fibroscan. GastroenterolHepatol. 2012 Sep; 8(9): 605-607.
18. Friedrich-Rust M, Ong MF, Martens S, et.al. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008; 134: 960-974.
19. Tsochatzis EA, Gurusamy KS, Nitaoula S, et.al. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. J Hepatol 2011; 54:650-659.
20. Guechot J, Poupon RE, Giral P, Balku B, et.al. Relationship between procollagen III aminoterminalpropeptide and hyaluronan serum levels and histological fibrosis in primary biliary cirrhosis and chronic viral hepatitis C. J Hepatol 1994; 20: 388-393.
21. Patel K, Lajoie A, Heaton S, Pianko S, et.al. Clinical use of hyaluronic acid as apredictor of fibrosis change in hepatitis C. J Gastroenterol Hepatol. 2003; 18: 253-257.
22. Nojgaard C, Johansen JS, Christensen E, Skovgaard LT, et.al. Serum levels of YKL-40 and PIIINP as prognostic markers in patients with alcoholic liver disease. J Hepatol. 2003; 39: 179-186.
23. Saitou Y, Shiraki K, Yamanaka Y, et.al. Noninvasive estimation of liver fibrosis and response to interferon therapy by a serum fibrogenesis marker, YKL-40, in patients with HCV-associated liver disease. World J Gastroenterol. Jan 2005; 11(4):476-481.
24. Hakala BE, White C, Recklist AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein famly. J Biol Chem. 1993; 268: 25803-25810.
25. Johansen JS. Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibrosis and cancer. Dan Med Bull. May 2006; 53(2): 172-209.
26. Tao H, Yang JJ, Shi KH, et.al. The significance of YKL-40 protein in liver fibrosis.Inflamm Res. 2014 Apr; 63(4):249-254.
27. Huang H, Wu T, Mao J, et.al. CHI3L1 Is a Liver-Enriches, non-invasive biomarker that can be used to stage and diagnose substantial hepatic fibrosis. OMICS A Journal of integrative Biology. 2015; 19(6): 339-345.
28. Tran A, Benzaken S, Saint-Paul MC, et.al. Chondrex (YKL-40), a potential new serum fibrosis marker in patients with alcoholic liver disease. Eur J GastroenterolHepatol. Sept 2000;12(9):989-993.
29. Yan L, Deng Y, Zhou J, Zhou H, Wag G. Serum YKL-40 as a biomarker for liver fibrosis in chronic hepatitis B patients with normal and mildly elevated ALT. P Infection. 2018 JUN; 46(3): 385-393.
30. Lin B, Wu S, Liu Y, Liu L, Mushtaq S. Chitinase-3-like protein 1 as a predictor for the progression or regression of liver fibrosis. Hepatoma Res. 2018; 4: 48.
31. Chou R, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection: a systematic review. Ann Intern Med. 2013; 158: 807-820