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# Diagnostic role of nuclear phosphoprotein c-Myc for staging liver fibrosis in chronic hepatitis C patients

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Assessment of liver fibrosis severity is essential in managing chronic hepatitis C (CHC) patients. This study aimed to evaluate the nuclear phosphoprotein c-Myc expression in different stages of liver fibrosis, and estimate its role to construct a simple noninvasive test for liver-fibrosis staging. A total of 148 CHC patients and 36 healthy individuals were enrolled in this study. Serum concentration of c-Myc was determined using ELISA. Multivariate discriminant analysis and area under receiver-operating characteristic curves (AUCs) were used for evaluated for diagnostic power of c-Myc. Interestingly, CHC patients was associated with a significantly higher c-Myc level (OD) than normal individuals (0.91±0.2 and 0.27±0.1; respectively). Moreover, the level of c-Myc was increased with progression of liver fibrosis stages where it was higher ($P < 0.0001$) in patients with significant fibrosis (1.1±0.4) than those with mild fibrosis (0.76±0.2) and in patients with advanced liver fibrosis (1.15±0.4) than those with non-advanced ones (0.81±0.3). c-Myc yielded an AUC of 0.75 for prediction of significant liver fibrosis with 80.2% sensitivity and 77.0% specificity. As well, it revealed a valuable power for identifying advanced liver fibrosis (AUC=0.77, sensitivity=77.0%, specificity=68.0%) and liver cirrhosis (AUC=0.83, sensitivity=75.0% and specificity= 72.0%). c-Myc can be served as a potential biomarker for liver fibrosis staging that may minimize the need for liver biopsy.

**Keywords: Fibrosis, Diagnosis, Biomarkers, c-Myc, chronic hepatitis c**

## INTRODUCTION

Globally, CHC constitutes a major health care challenge, affecting around 71 million people (WHO 2019). Liver fibrosis is the center of diagnosis and management of essentially all chronic liver diseases (Sebastiani et al., 2014). Consequently, in hepatitis C virus (HCV)-infected persons, knowing the hepatic fibrosis progression rate can help inform patients and providers in making treatment decisions (Butt et al., 2015). Nevertheless, liver biopsy that remains the most specific and sensitive way for liver fibrosis diagnosis and staging, is an invasive screening tool linked to many adverse events including high costs, errors in sampling and patient discomfort (Lambrecht et al., 2018, Morling and Guha 2016). Therefore, there is a constant stream of ingenious non-invasive hepatic fibrosis markers, which are validated for use in clinics (Chin et al., 2016); although these tools lack the sensitivity and specificity to detect small changes in the progression or regression of both early and late stages of fibrosis (Lambrecht et al., 2018).

On the other hand, *c-myc* is a well-characterized proto-oncogene that induces cellular transformation and modulates apoptosis

(Lin X. et al., 2017). C-Myc oncoproteins are involved in the development and progression of many human cancers (Dauch et al., 2016) including colorectal (Rochlitz et al. 1996), breast (Scorilas et al., 1999) and prostate (Jenkins et al., 1997) cancers in addition to hepatocellular carcinoma (HCC) (Holczbauer et al., 2013). It has been reported that c-Myc over-expression is present in up to 70% of viral and alcohol-related HCC (Lin Che-Pin et al., 2010).

In this study, we aimed to evaluate c-Myc level in different stages of liver fibrosis, and to assess the extent to which it could predict different stages of liver fibrosis.

## MATERIALS AND METHODS

### Subjects

Blood samples from 148 CHC patients were collected from Tropical Medicine Department, Mansoura University hospitals, Mansoura, Egypt. Chronically infected persons were anti-HCV positive and HCV-RNA positive. All patients were negative for anti-hepatitis A virus, hepatitis B virus surface antigen and anti-human immunodeficiency virus. In addition, blood samples from 36 healthy individuals, negative for anti-HCV antibodies, were included in the present study. This study was approved by the ethical guidelines of the Helsinki Declaration and an informed consent was obtained from all participants.

METAVIR system of liver fibrosis (F) for each patient was derived from the fibroscan: Cut-off values of fibroscan were < 7.1 kPa for F1; 7.1-8.8 5 kPa for F2; 9.5-9.6 kPa for F3 and 12.5-14.6 for F4 (Sandrin et al. 2003). The baseline distribution of fibrosis stages for the 148 patients with CHC was F1 (64/148; 43.2%), F2 (41/148; 27.7%), F3 (23/148; 15.6%) and F4 (20/148; 13.5%). Also, 148 CHC patients were classified according to significantly of liver fibrosis into two main groups: CHC patients with mild fibrosis (F1) (64/148; 43.2%), and those with significant fibrosis (F2-F4) (84/148; 56.8%). Moreover, CHC patients classified according to advancement of liver fibrosis into two main groups: CHC patients without advanced fibrosis (F1-F2) (105/148; 71.0%) and CHC patients with advanced fibrosis (F3-F4) (43/148; 29.0%). Exclusion criteria for the study were history of HCC, previous treatment, evidence of coexistent liver disease, and liver transplantation.

### Laboratory assays

All the subjects underwent laboratory investigation which include liver function tests using an automated biochemistry analyzer (A15, Biosystem, Spain) and a full blood count on an automated haematology analyzer (Sysmex Corporation, Kobe, Japan). Alpha-fetoprotein (AFP) levels was estimated by chemiluminescence using Immulite (1000) AFP kit (Diagnostic Products Corporation; Los Angeles, CA, USA). Serum concentration of c-Myc was determined using enzyme-linked immunosorbent assay according to the protocol of Attallah et al., that has been previously described in detail (Attallah et al., 2017).

### Statistical analysis

Statistical analyses were done using SPSS software v.17.0 (SPSS Inc., Chica go, IL) and GraphPad Prism package v.5.0. (GraphPad Software, San Diego, CA). Differences were assessed using Student *t*-test or $X^2$ test in case of continuous variables or categorical variables, respectively. Statistical significance was defined at the level of 0.05. The diagnostic efficiency was assessed using the area under the receiver operating characteristic (AUC) curves. The common indicators of diagnostic performance including specificity and sensitivity were calculated from a 2×2 contingency table.

## RESULTS

### Patient characteristics

The demographic data and laboratory blood markers of CHC patients are summarized in Table 1. As expected, the data showed that there were significant differences ($P < 0.05$) in distribution of all evaluated laboratory blood markers (ALT, AST, AST/ALT ratio, ALP, albumin, total bilirubin and platelets count) except AFP between patients with significant fibrosis and those with mild fibrosis. While there was no significant difference ($P > 0.05$) in levels of albumin, AFP and platelet count by comparing patients with advanced liver fibrosis and those with non-advanced liver fibrosis as well cirrhotic patients and non-cirrhosis patients.

### Quantification of c-Myc in sera of CHC patients

CHC patients was associated with a significantly high ($P < 0.0001$) c-Myc level (OD) than normal individuals (0.91±0.2 and 0.27±0.1; respectively).

**Table 1; Patients' characteristics**

| Variables | Mild F1 (n=64) | Significant F2-F4 (n=84) | *P* value | Non-advanced F1-F2 (n=105) | Advanced F3-F4 (n=43) | *P* value | Non-cirrhosis F1-F3 (n=128) | cirrhosis F4 (n=20) | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| **Mean age ± SD, years** | 41.5±8.3 | 44.5±6.5 | 0.285 | 42.9±7.7 | 45.4±5.9 | 0.767 | 42.6±7.4 | 47.3±7.3 | 0.065 |
| **Gender (male/female)** | 46/18 | 57/27 | 0.599 | 75/30 | 28/15 | 0.449 | 88/40 | 15/5 | 0.326 |
| **ALT (U/L)** | 26.5±5.5 | 75.2±31.2 | < 0.0001 | 60.9±23.2 | 78.6±36.2 | 0.020 | 61.2±12.7 | 71.2±19.4 | 0.035 |
| **AST (U/L)** | 27.3±6.2 | 55.6±30.2 | < 0.0001 | 48.6±20.5 | 61.7±35.1 | 0.030 | 50.4±12.6 | 63.4±18.7 | 0.042 |
| **AST/ALT ratio** | 0.8±0.1 | 1.3±0.3 | 0.009 | 0.9±0.1 | 1.3±0.2 | 0.036 | 0.9±0.03 | 1.1±0.01 | 0.050 |
| **ALP (U/L)** | 61.2±15.8 | 89.3±46.8 | 0.024 | 74.1±37.9 | 100.1±49.9 | 0.017 | 82.0±21.4 | 133.2±27.5 | 0.001 |
| **Albumin (g/dL)** | 4.51±0.42 | 4.04±0.40 | 0.043 | 4.31±0.29 | 4.18±0.45 | 0.626 | 4.2±0.04 | 4.3±0.07 | 0.963 |
| **Total bilirubin (mg/dL)** | 0.52±0.12 | 0.82±0.35 | < 0.0001 | 0.74±0.31 | 0.89±0.37 | 0.022 | 0.6±0.01 | 0.9±0.01 | 0.039 |
| **Platelet count x $0^9$/L** | 213.0±47.0 | 184.0±50.0 | 0.017 | 192.0±45.0 | 178.0±52.6 | 0.066 | 185.6±39.2 | 180.5±33.2 | 0.680 |
| **AFP (U/L)** | 4.2±0.9 | 9.8±2.5 | 0.020 | 5.9±0.3 | 9.3±1.7 | 0.083 | 6.8±1.1 | 10.5±3.2 | 0.126 |

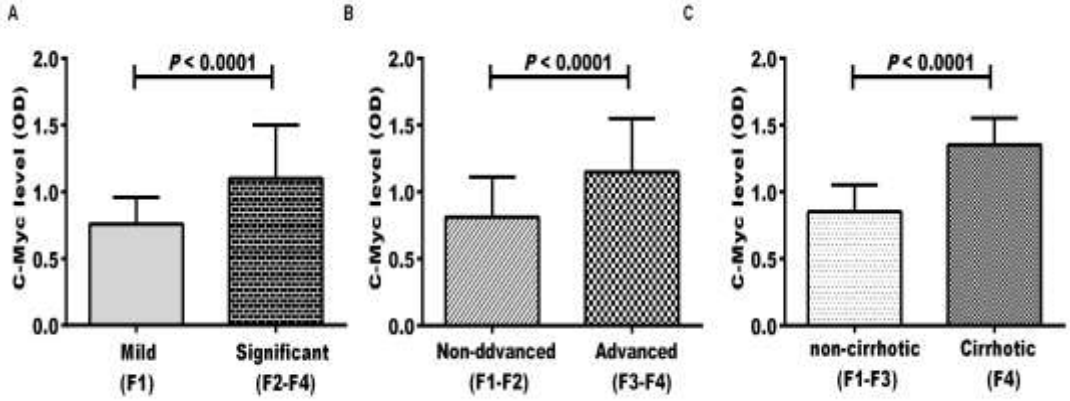


**Figure 1: Evaluation of c-Myc optical density (OD) level in (A) patients with mild liver fibrosis (F1) against those with significant liver fibrosis (F2–F4); (B) patients with advanced liver fibrosis (F3–F4) against those with non-advanced liver (F1–F2); (C) patients with non-cirrhotic liver fibrosis (F1–F3) against liver cirrhosis (F4) patients. *P* < 0.05 considered significant.**

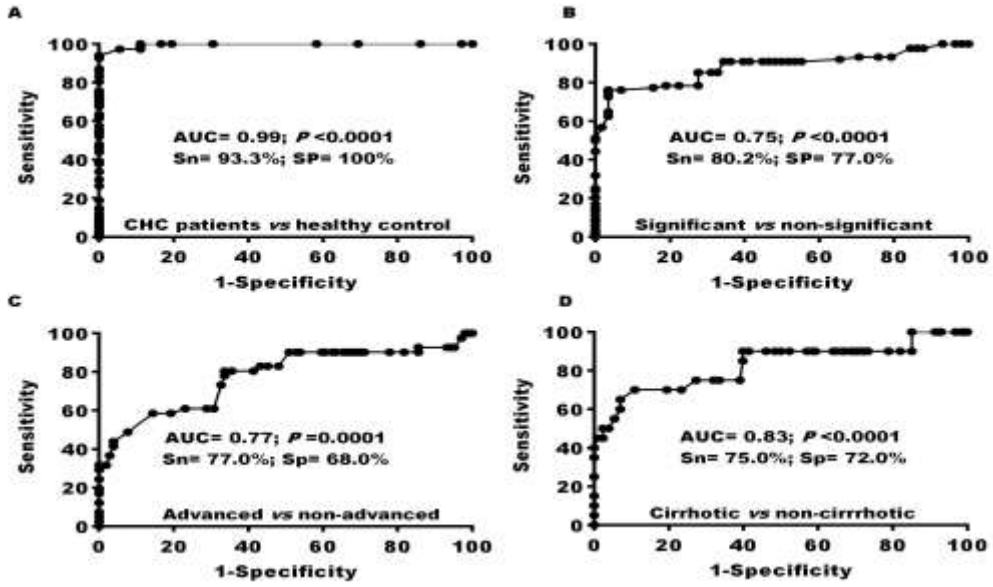


**Figure 2:Receiver operating characteristic (ROC) curves of c-Myc to discriminate (A) CHC patients from healthy control; (B) patients with significant liver fibrosis (F2–F4) from those without; (C) patients with advanced liver fibrosis (F3–F4) from those without; (D) liver cirrhotic (F4) patients from those with non-cirrhotic liver (F1–F3). *P* < 0.05 considered significant. AUC= area under curve, Sn= sensitivity, Sp= specificity.**

Moreover, the level of c-Myc was increased with progression of liver fibrosis stages where it was higher ($P < 0.0001$) in patients with significant fibrosis (1.1±0.4) than those with mild fibrosis (0.76± 0.2) and in patients with advanced liver fibrosis (1.15±0.4) than non-advanced (0.81± 0.3). Also, cirrhotic patient associated with high c-Myc level (1.35±0.2) than non-cirrhotic individuals (0.85±0.2), Figure 1.

**Diagnostic performance of c-Myc for fibrosis staging**

Additionally, c-Myc was further assessed for its diagnostic power and discriminative ability in CHC detection using ROC analysis. Absolute specificity (100%) was obtained to discriminate CHC patients from healthy control with an AUC of 0.99 and 93.3% sensitivity at a 1.2 cutoff, Figure 2A. Also, c-Myc yielded valuable power for the

prediction of significant liver fibrosis (AUC=0.75, 80.2% sensitivity, 77.0% specificity). As well, c-Myc provided a higher AUC of 0.77 and 0.83 with a high sensitivity (77.0% and 75.0%) and specificity (68.0% and 72.0%) for identifying advanced liver fibrosis (F3–F4) and liver cirrhosis respectively, Figure 2B-D.

## DISCUSSION

Chronic liver disease was associated with high expression of *c-myc* that could increase the susceptibility to liver cancer (Chan et al., 2004). This elevation of *c-myc* may be a result of epigenetic changes, like those relating to translation, transcription, methylation or degradation. Because oncogene activation of *c-myc* can increase proliferation, promote the cell cycle, and induce genetic instability, thereby accelerating tumor progression (Vafa et al. 2002, Yin et al., 1999). Herein, CHC patients was associated with a significantly high ($P < 0.0001$) c-Myc level and this level was increased with progression of liver fibrosis stages where it was higher ($P < 0.0001$) in patients with significant fibrosis (1.1±0.4) than those with mild fibrosis (0.76±0.2) and in patients with advanced liver fibrosis (1.15±0.4) than non-advanced (0.81±0.3). Also, cirrhotic patient associated with high c-Myc level (1.35±0.2) than non-cirrhotic individuals (0.85±0.2).

c-Myc involvement in progression of chronic liver disease has been emphasized in some studies (Chan et al. 2004). It was found that the expression of c-Myc protein in hepatocytes is present in the initial degrees of fibrosis, and its overexpression is frequently detected in patients with advanced liver fibrosis stages (de Almeida et al. 2014). During liver injury, hepatic stellate cells (HSCs) are the cells that primarily contribute to fibrogenesis (Ying et al., 2017). Platelet-derived growth factor subunit B (PDGF-B) is the most effective factor in stimulating HSCs differentiation, proliferation, and migration (Kocabayoglu et al., 2015). Additionally it promotes HSCs transformation into myofibroblast and collagen production and deposition (Czochra et al. 2006). It was found that modulation of c-Myc in hepatocytes, either because of inflammatory response to liver injury or to gene amplification, caused moderate hepatocyte apoptosis, increased proliferation of hepatocyte and abnormal PDGF-B expression (Zheng et al., 2017).

Based on ROC analysis c-Myc had a valuable diagnostic power and discriminative ability in CHC detection. Absolute specificity (100%) was obtained to discriminate CHC patients from healthy control with an AUC of 0.99 and 93.3% sensitivity. Also, c-Myc had superior diagnostic accuracies for identifying significant fibrosis (AUC=0.75, sensitivity=80.2% and specificity=77.0%), advanced fibrosis (AUC=0.77, sensitivity=77.0% and specificity=68.0%) and cirrhosis (AUC=0.83, sensitivity=75.0% and specificity=72.0%). These results exceeding those of amino-terminal peptide of type III procollagen (PIIIP) (AUC=0.74) and type IV collagen (AUC=0.74) to differentiate significant fibrosis (F2-F4) from mild stage of fibrosis (F0-F1). The sensitivity and specificity were 78% and 75% for PIIIP and 65% and 69% for type IV collagen, respectively (Saitou et al. 2005). Also, c-Myc diagnostic performance is similar to hyaluronic acid that has sensitivity and specificity of 75% and 81%, respectively for identifying significant fibrosis, but positive predictive value of hyaluronic acid has been reported as 61% (Gressner et al., 2007) and there is an overlap among stages and grades in liver fibrosis (Murawaki et al., 2001).

## CONCLUSION

In conclusion, measurement of serum c-Myc level represents a reliable test for assessing liver fibrosis degree that could be a valuable in predicting not only significant fibrosis but also advanced fibrosis and cirrhosis with high accuracy.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## ACKNOWLEGEMENT

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## AUTHOR CONTRIBUTIONS

Attallah AM, El-Far M and Omran MM were chief investigators who conceptualized and designed the study. Ahmed O. Gheith was investigator who collected data from the literature, collected samples and carried on with different experiments and techniques. Omran MM and Ahmed O. Gheith acquired data and performed all data and statistical analysis. Attallah AM, Omran MM and El-Far M interpreted data and wrote final manuscript. All authors read, reviewed and approved the final manuscript.

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