*British Journal of Medicine & Medical Research*
*12(4): 1-13, 2016, Article no.BJMMR.22003*
*ISSN: 2231-0614, NLM ID: 101570965*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Evaluation of the Diagnostic Value of Serum Cytokeratin-8 as a Marker of Liver Injury in Chronic Hepatitis C Patients

**Maaly M. Mabrouk[1*], Medhat A. Ghazy[2] and Mohamed A. Elrashidy[3]**

*[1]Department of Clinical Pathology, Faculty of Medicine, Tanta University, Tanta, Egypt.*
*[2]Department of Internal Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt.*
*[3]Department of Pathology, Faculty of Medicine, Tanta University, Tanta, Egypt.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author MMM designed the study, wrote the protocol and performed the laboratory investigation. Author MAG managed the literature searches, analyses of the study and wrote the first draft of the manuscript. Author MAE performed pathological examinations of biopsy. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/BJMMR/2016/22003
Editor(s):
(1) Roberto Manfredi, Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy.
Reviewers:
(1) Giovanni Tarantino, Federico II University Medical School of Naples, Italy.
(2) Consolato Sergi, University of Alberta, Canada.
(3) Ndomou Mathieu, University of Douala, Cameroon.
Complete Peer review History: http://sciencedomain.org/review-history/12163

***Original Research Article***

***Received 13th September 2015***
***Accepted 6th October 2015***
***Published 9th November 2015***

## ABSTRACT

**Background:** Hepatitis C virus (HCV) is considered the most health problematic in Egypt. Its severity ranges from mild illness to serious complications as cirrhosis and hepatocellular carcinoma. Keratins emerge as markers of liver injury beside significant contributors to liver disease pathogenesis.
**Aims:** We analyzed the cytokeratin-8 serum levels and blood mRNA expression in chronic hepatitis C patients to evaluate serum CK8 role as a marker of liver injury.
**Patients and Methods:** This study included 100 Egyptian patients with liver disease. They were 82 patients with chronic hepatitis C and 12 patients with hepatitis C-induced cirrhotic changes. Fifteen healthy controls were also included in the study. All studied subjects underwent a clinical assessment and complete laboratory evaluation. For patients groups a conventional abdominal ultrasonography and guided liver biopsy were performed with histopathological examination to

**Corresponding author: E-mail: maalymabrouk@yahoo.com;*

assess the grade of inflammation and stage of fibrosis according to the Metavir scoring. The levels of CK-8 serum and blood mRNA expression were measured.
**Results:** Serum CK-8 levels and mRNA expression were increased in HCV and cirrhotic patients compared to control group (*P* <0.001*). Serum CK-8 levels were positively correlated with Metavir score in patients groups (r=0.714, *P* <0.001) (r=0.447, *P* <0.001) and in selected patients with inconclusive FIB4 index (values in between 1.45-3.25) (r=0.291, *P* =0.048) (r= 0.486, *P* <0.001).
**Conclusions:** Serum CK 8 levels were positively correlated with Metavir score and FIB4 index. They may be useful for monitoring disease activity in chronic HCV patients especially with inconclusive FIB4 index.

*Keywords: Chronic hepatitis C; Cytokeratin 8.*

## 1. INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a significant public health problem in Egypt since there is no vaccine available, the response to usual treatment is limited. Globally, Egypt is believed to reach the highest HCV prevalence in the world [1]. HCV estimated prevalence in Egypt is 14.7% among general population in the year 2008 [2].

Liver responses to injury by fibrosis and ultimately it can give rise to liver cirrhosis which may lead to critical complications such as liver failure, hepatocellular carcinoma or life-threatening esophageal and gastric varices. Liver cirrhosis and cancer represent the global causes of death [3-4].

Staging HCV infection is primarily depending on degree of histologic fibrosis in a liver biopsy sample. However, the procedure is uncomfortable, painful, and costly. It carries a small risk for complication [5]. There is a trend to gain a benefit from utilizing an index based on serum fibrosis markers as the FIB-4 index [6-8]

Mammalian cytoskeleton is mainly composed of microtubules, actin and intermediate filaments [9,10]. Keratins are the largest subfamily of cytoplasmic intermediate filaments contains more than 50 functional genes, which are expressed mostly in epithelial cells and skin appendages [11,12].

Keratins can be classified according to their tissue expression model into simple-epithelial keratins and keratins expressed in stratified epithelia and skin appendages [11]. Keratins include type-I keratin polypeptides (K9-K20) and type-II keratins 1-8 (K1- K8). Epithelial cells express at least one type-I and one type-II unique keratins which are both required to form obligate noncovalent heteropolymers [13,14].

Exclusively, adult hepatocytes express K8 and K18 proteins (by KRT8/KRT18 genes), whereas hepatobiliary ductal cells express variable levels of K7, K19 and K20 [14].

Thus, hepatocytes are mainly sensitive to variation of keratin architecture and keratins appear as suitable markers of liver injury in addition to significant contributors to liver disease pathogenesis [12,15].

In this study, we investigated cytokeratin-8 serum levels and blood mRNA expression in patients with chronic hepatitis C to evaluate serum CK8 role as a marker of liver injury.

## 2. PATIENTS AND METHODS

### 2.1 Study Design

This study was conducted on 100 patients with chronic HCV-related liver disease referred to the Internal Medicine Department. In addition, 15 normal volunteers with no clinical evidence suggestive of chronic liver disease were included as controls. The control population had normal liver biochemical profiles and was negative for anti-HCV antibodies. The duration of the study was 1 year.

The study was approved by local Investigational Review Board. The study design followed the Institutional Committee for the Protection of Human Subjects adopted by the 18th World Medical Assembly, Helsinki, Finland. Written informed consents were obtained from all participants in the study and confirmed by the IRB.

The patient inclusion criteria for this study included the following:

Adult (23–58 years old), either gender with chronic HCV-related liver disease. Diagnosis was

based on the presence of anti-HCV antibodies and HCV RNA in serum for at least 6 months in addition to the histopathologic evidence of chronic hepatitis or cirrhosis. Patients were excluded from the study if they were positive for HBsAg, had autoimmune or metabolic liver diseases, hyperlipidaemia, decompensated liver disease, currently or previously receiving IFN-therapy or their platelets count less than $100x10^3/cm^3$.

All studied subjects received a clinical assessment and complete laboratory evaluation including fasting blood sugar level, complete blood count, liver biochemical profile, prothrombin time and activity , lipid profile, renal function tests, anti-nuclear antibodies (ANA), alpha fetoprotein (AFP). The hepatitis seromarkers HBsAg for HBV and anti-HCV were tested using ELISA (Abbott laboratories). The amount of HCV RNA in serum samples was quantified using a realtime PCR Step One instrument and software (Applied Biosystems).

For patients groups a conventional abdominal ultrasonography and guided liver biopsy were performed with histopathological examination to assess the grade of inflammation and stage of fibrosis according to the Metavir scoring system [16] and to exclude any combined liver insults. Serum samples at time of biopsy were used for serological analysis.

Liver biopsy requires using 16 gauge semi-automated biopsy needles. Liver specimens of 15 mm in length with minimal 4 portal tracts were fixed in 10% neutral formalin, then processed and embedded in paraffin. Sections were stained with haematoxylin and eosin, and Masson trichrome stains for detection of fibrosis.

Metavir scoring system demonstrated different stages of fibrosis (F0-F4) and grades of necro-inflammatory changes (A0-A3) [16]. The histopathological examination of all the liver biopsies was performed by a single hepatology expert pathologist.

#### 2.1.1 Calculated score

The FIB-4 index was calculated using Sterling's formula (7) as:

Age (y) × AST (IU/L) /platelet count ($\times 10^9$/L) $\times\sqrt{}$ALT (IU/L))

With regard to disease progression bias, the time interval between the determination of the FIB-4 index and liver biopsy was no longer than 7 days.

The patients were classified into the following groups:

Group (I): Patients with chronic hepatitis C (n = 82). Their ages ranged between 23–60 years old (41.4±9.2), they were 57 males and 25 females.

Group (II): Patients with histopathologic evidence of liver cirrhosis (HCV related) (n = 18). Their ages ranged between 25–56 years old **(**44.7±10.1), they were 11males and 7 females.

Group (III): 30 normal individuals served as the control group. Their ages ranged between 30–54 years old (43.2±7.4), they were 18 males and 12 females.

### 2.2 Measurement of Serum Cytokeratin-8 & Genetic analysis of Cytokeratin- 8 mRNA Expression

Serum levels of CK-8 were assessed using a quantitative measurement in serum using Sandwich ELISA(antibodies-online Inc. USA, Catalog no: ABIN817227).

#### 2.2.1 Genetic analysis of cytokeratin- 8 mRNA expression

Two ml blood in EDTA tubes obtained from peripheral blood of all subjects and RNA was extracted from samples using QIAamp RNA Blood Mini (QIAGEN GmbH, Hilden, Germany). All the RNA samples had an optical density 260/280 ratio ranging from 1.81 to 1.875, which confirms the good quality of the RNA. RNA samples with an optical density 260/280 ratio of less than 1.8 were excluded from the study.

The cDNA was reverse transcribed in a 10 µl mixture containing 6 µl of total RNA, 0.5 µl random primers (Promega, Madison, WI), 2 µl of reverse transcriptase buffer (Life Technology, Gaithersburg, MD), 1 µl of deoxyribonucleosid triphosphate (dNTP) mixtures (10 Mm), and 0.5 µl of AMV reverse transcriptase (5 U/µl). The mixture was incubated at 37℃ for 10 min, 52℃ for 45 min, 95℃ for 5 min, and in ice bath for 5 min. The synthesized cDNA samples were stored at – 20℃ until use .

The cDNA is used as a template to amplify the studied gene and it normalized using glyceraldehydes-3-phosphate dehydrogenase as housekeeping gene. The assay identification numbers of target and housekeeping genes are as followed: cytokeratin 8 (Assay ID # Hs01595539_g1) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene (Assay ID # Hs99999905_m1) using the primers and Probes assays listed in TaqMan Gene expression assays from Applied Biosystems.

DNA amplification of the cytokeratin 8 gene was carried out by real time PCR using the real time PCR Step One instrument and software (Applied Biosystems). This included an initial denaturation step for 10 min at 95℃, followed by 40 cycles of annealing and elongation for 15 seconds at 95℃ and 1 min at 60℃, respectively. Instrument raw data (fluorescence) for all the samples were converted into threshold cycles (Ct) using SDS 1.2 software (Applied Biosystems). Ct values were then imported onto an Excel worksheet for relative quantification (RQ).

For RQ calculation, the geometrical mean of GAPDH was used as a normalization factor.

Calculation of the results was performed by application of the comparative Ct method for RQ, $2^{-\Delta\Delta Ct}$

$$\Delta Ct;_{unknown} = Ct_{target} - Ct_{reference}$$

The difference in threshold cycles for target and reference genes is as follows:

$$\Delta\Delta Ct = Ct_{unknown} - Ct_{calibrator}$$

Where, $\Delta Ct,_{calibrator}$ is the mean value for a healthy control human.

$$RQ = 2^{-\Delta\Delta Ct}$$

The fold change, defined as the ratio between the averaged normalized expression levels of targets in neoplastic and corresponding non-neoplastic samples, was calculated. Normalized RQ values were log 2 transformed for statistical analysis.

### 2.3 Statistical Analysis

Statistical presentation and analysis of data in the present study was carried out; continuous data were expressed as mean±SD, whereas categorical data were expressed as number and percentage. Categorical data were compared using the $X^2$ test. Continuous data between two groups were compared using Student's t-test. Different parameters were compared using Pearson's correlation test. Statistical significance was defined as a P-value of less than 0.05. Analyses were carried out using the SPSS program, version 17 (SPSS Inc., Chicago, Illinois, USA) and the GraphPad Prism software (Graph-Pad Prism Software Inc., San Diego, California, USA).

## 3. RESULTS

The present study involved 82 patients with chronic hepatitis C and 18 patients with HCV-related liver cirrhosis.

The laboratory data of studied groups and histo-pathological features of the patients groups were illustrated in Table 1, Figs. 1-2.

There was an increase in serum CK8 levels in HCV and cirrhotic patients compared to control group (*P* <0.001*). There was also an increase in serum CK8 levels in cirrhotic patients compared to HCV patients (*P* <0.001*). There was no difference in viremia degree between the two patients groups ((*P* >0.05) (Table 1).

The relative expression levels of keratin 8 mRNA were found to be significantly upregulated, up to 7-fold in HCV patients, 10 fold in liver cirrhotic patients compared with normal controls. The cytokeratin 8 normalized mRNA levels in HCV and liver cirrhotic patients compared with normal controls were 7.5 (0.9–8.7), 10.5 (2.9–11.7) respectively, whereas in healthy controls it was 1.21 (0.5–4.1). Increased CK 8 mRNA expression was observed in 57 out of 82 HCV and 16 out of 18 cirrhotic patients, however there was no significant expression difference in comparison between HCV and cirrhotic groups Table 1.

There was a significant increased CK 8 mRNA expression with increased serum CK8 levels in both HCV and cirrhotic patients (P <0.001) Table 2.

There were positive significant correlations of serum CK8 levels with ALT, AST and FIB4 index, while there were negative significant correlations with platelet count and prothrombin activity (*P* < 0.05) Table 3.

There were positive significant correlations of serum CK8 levels with grade of inflammation

(r=0.714, *P* <0.001) and stage of fibrosis (r=0.447, *P* <0.001) in patients groups (Fig. 3).

There were positive significant correlations of serum CK8 levels with grade of inflammation (r=0.291, *P* =0.048) and stage of fibrosis (r= 0.486, *P* <0.001) in patients with inconclusive FIB4 index (values between1.45-3.25) (Fig. 4).

In ROC curve analysis serum CK8 gave significant data either when its levels compared between the patients groups or in different stages of fibrosis as following: when serum Ck > 6.9 (ng/mL), sensitivity, specificity positive predictive value, negative predictive value and AUC were 66.7, 96.3, 97.0,93.2, 87.2 respectively Table 4, Fig. 5.

**Table 1. The laboratory data and histopathological features of the studied groups**

| Variables | Chronic HCV (82) GI Mean ± SD | LC (18) GII Mean ± SD | Control group (30) GIII Mean ± SD | *P* Value |
|---|---|---|---|---|
| **Platelets (x10$^3$/cm$^3$)** | 188.3±41.5**[a] | 121.5±10.4* * | 317.1±59.1 | 0.007* |
| **Prothrombin activity (%)** | 89.6±8.2[a] | 78.2±4.0 ** | 91.1±21.4 | <0.001* |
| **Total bilirubin (mg/dL)** | 0.8±0.1 | 0.78±0.2 | 0.75±0.1 | >0.05 |
| **Serum ALT (U/L)** | 86.4±38.3** [a] | 8.5±22.0 ** | 20.2±3.3 | <0.001* |
| **Serum AST (U/L)** | 90.6±41.9**[a] | 65.2±23.2* * | 20.8±6.2 | <0.001* |
| **Serum ALP (U/L)** | 189.5±56.1 | 207.2±57.0** | 156.9±33.6 | 0.029* |
| **Albumin (gm/dL)** | 4.2±0.26 **[a] | 3.7±0.2** | 4.9±0.2 | <0.001* |
| **Serum AFP (ng/mL)** | 10.5± 3.8** [a] | 21.0±3.6 ** | 4.9±1.4 | <0.001* |
| **FIB4 index** | 1.75±0.67**[a] | 4.5±0.7** | 0.7±0.2 | <0.001* |
| **Serum CK8 (ng/mL)** | 4.1±1.4**[a] | 7.4±2.3 * | 0.4±0.1 | <0.001* |
| **HCV RNA level** | | | | |
| Low viraemia (up to $10^5$) | 35(42.68%)[b] | 7(38.89%) | 0 | |
| Moderate viraemia ($10^5$-$10^6$) | 38(46.34%)[b] | 8(44.44%) | 0 | >0.05 |
| High viraemia (>$10^6$) | 9(10.98%)[b] | 3(16.67%) | 0 | |
| **Ck8 Gene expression** | | | | |
| Low expression | 25(30.49%)[b] | 2(11.11%) | 26(86.7%) | |
| High expression | 57(69.51%)**[b] | 16(88.89%)** | 4 (13.3%) | <0.001* |
| **Grade of inflammation** | | | | |
| A0 | 10(12.2%) | 0(0%) | | |
| A1 | 29(35.37%) | 12(66.67%) | | |
| A2 | 34(41.46%) | 6(33.33%) | | |
| A3 | 9(10.98%) | 0(0%) | | |
| **Stage of fibrosis** | | | | |
| F0 | 6(7.32%) | 0(0%) | | |
| F1 | 40(48.78%) | 0(0%) | | |
| F2 | 30(36.59%) | 0(0%) | | |
| F3 | 6(7.32) | 0(0%) | | |
| F4 | 0(0%) | 18(100%) | | |

*ALT; alanine aminotransferase enzyme, AST; aspartate aminotransferase enzyme, ALP; alkaline phosphatase enzyme,AFP; alpha feto protein . **P** =0.05, ** **P** < 0.01 Relative to the control group, [a] **P** Value < 0.01 relative to the LC (liver cirrhosis) group, [b] **P** Value >0.05 relative to the LC group*

**Table 2. Relation of CK8 mRNA expression to serum CK8 (ng/mL) in patients groups**

| | CK8 mRNA expression | | | | | | T-Test | |
|---|---|---|---|---|---|---|---|---|
| | Low expression (n=27) | | | High expression(n=73) | | | | |
| | Mean | ± | SD | Mean | ± | SD | t | P-value |
| **CK8 (ng/mL)** | 2.7 | ± | 0.7 | 5.4 | ± | 1.9 | -6.8 | <0.001* |

* ***P*** *= 0.05*

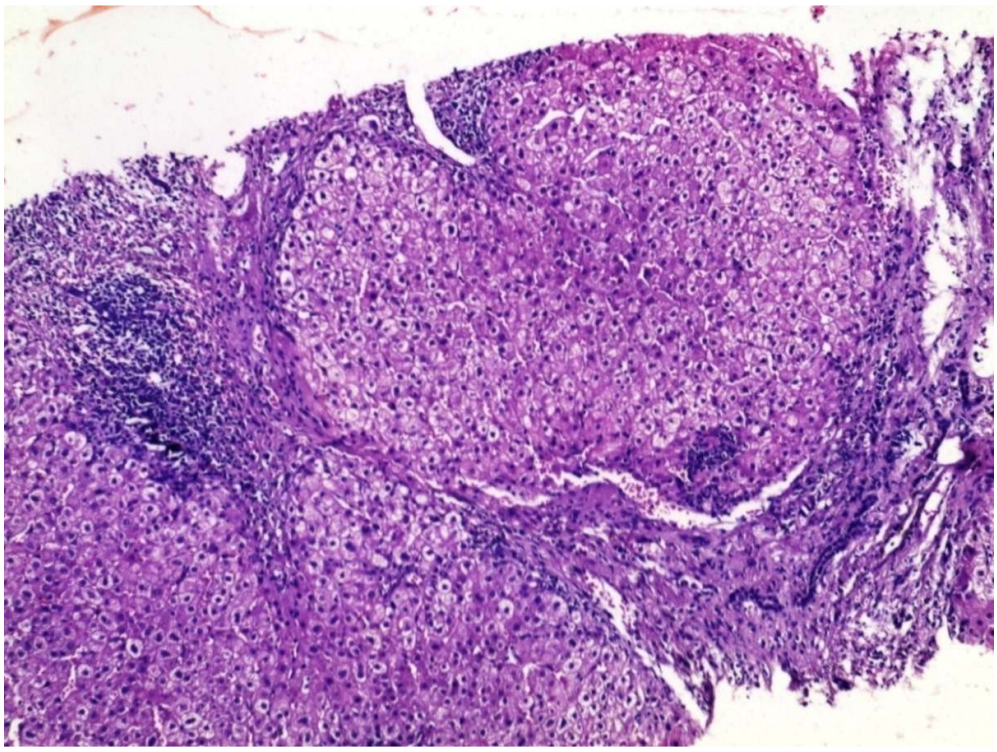

**Fig. 1. Cirrhotic heptic nodules (F4) as a progressive chronic hepatitis C (H&E100)**

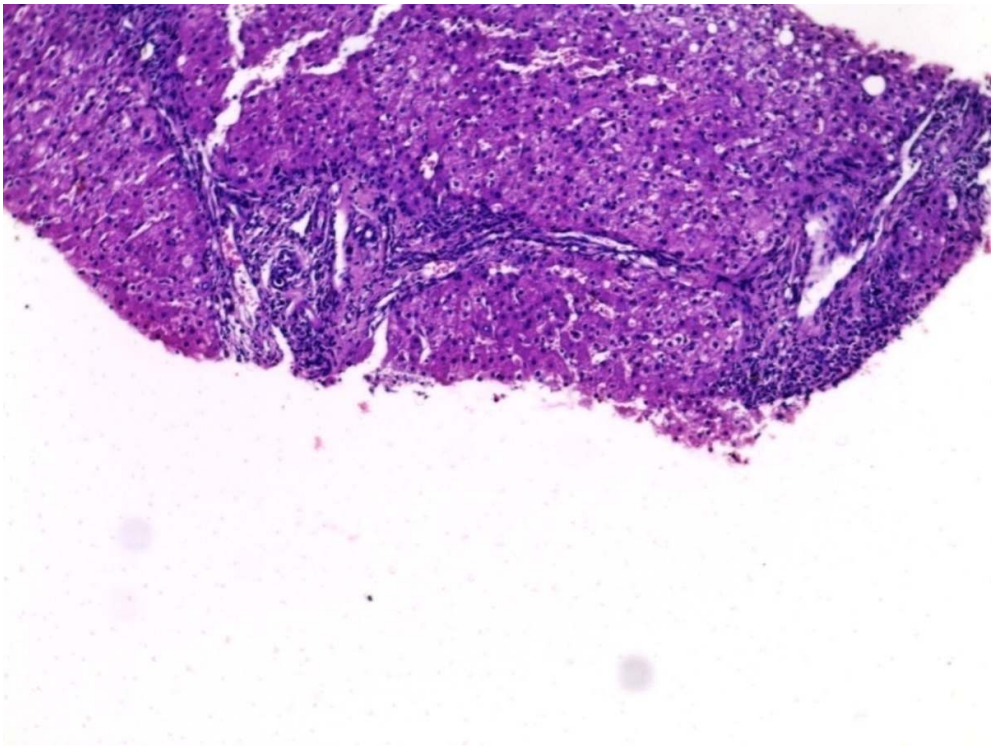

**Fig. 2. Picture of chronic active hepatitis c with bridging fibrosis with piece meal necrosis A2F2 (H&E100)**

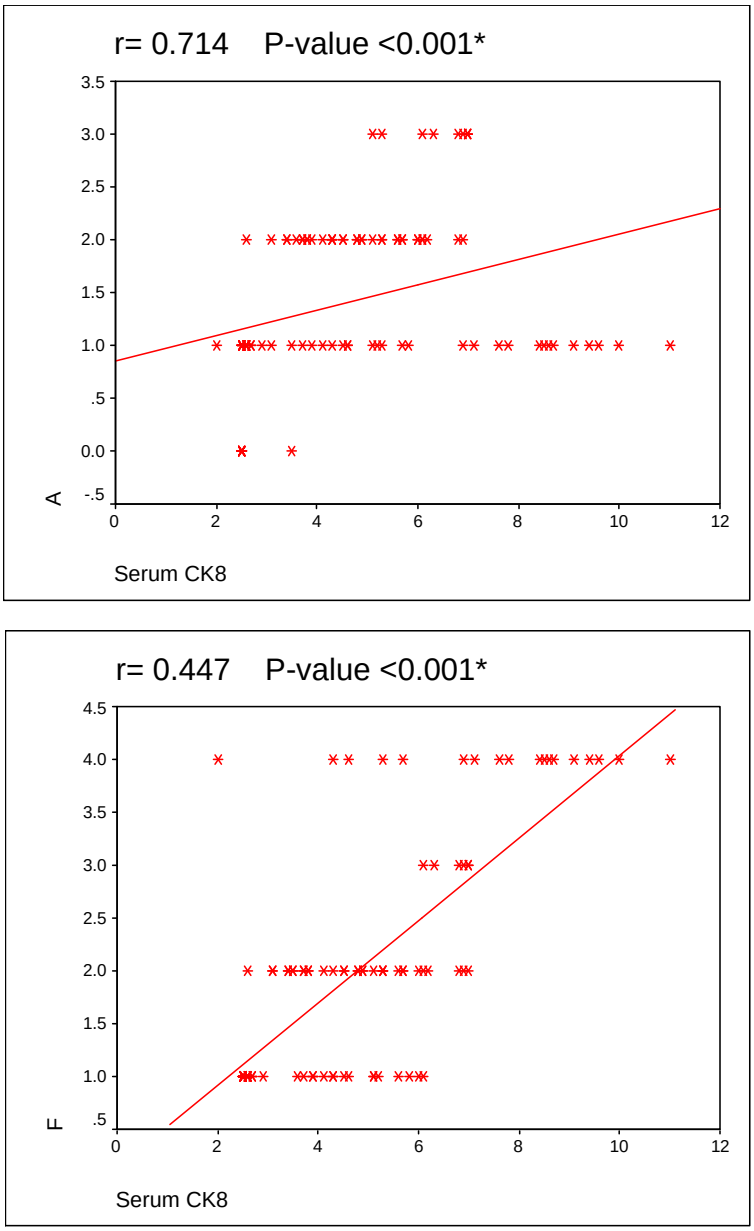


**Fig. 3. Positive correlations between serum CK8 (ng/mL) with grade of inflammation and stage of fibrosis in patients groups**

*A; grade of inflammation, F; stage of fibrosis*

Serum Ck > 2.9 (ng/mL), sensitivity, specificity, positive predictive value, negative predictive value and AUC were 96.4,63.6,77.1,93.3, 86.2 respectively (Table 5, Fig. 6).

## 4. DISCUSSION

Chronic hepatitis C virus infection is a major health problem worldwide especially in Egypt (2). The liver's response to injury by fibrosis. In the end this process can give rise to liver cirrhosis, which may result in the life threatening complications including portal hypertension, liver failure and hepatocellular carcinoma [17].

However, liver biopsy remains the gold standard for assessing presence, type and stage of liver fibrosis and for description of necroinflammation; it is a risky invasive procedure. Therefore, it cannot be carried out in many cases [18].

Mallory-Denk bodies (MDBs), are hepatocyte cytoplasmic inclusions present in several chronic liver diseases, including chronic hepatitis C (CHC) [12,19]. Keratin polypeptides 8 and 18 (K8/K18) are the main components of MDBs and expected to play a key cytoprotective function in the liver [12]. Keratin genes KRT8, KRT18 and KRT19 have been connected with increased liability to end-stage liver disease [20,21] and increased fibrosis in CHC [22].

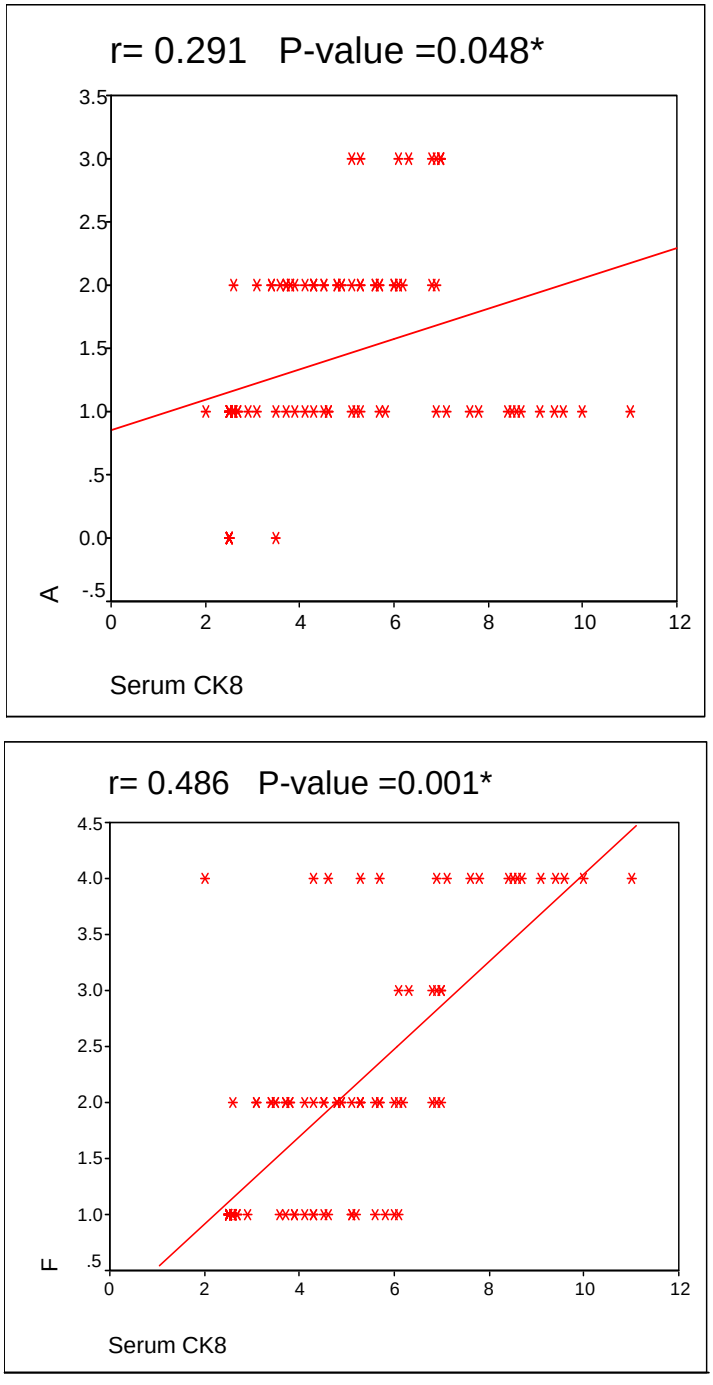


**Fig. 4. Positive correlations between serum CK8 (ng/mL) with grade of inflammation and stage of fibrosis in patients with inconclusive FIB4 index (FIB4 values between 1.45-3.25)**

*A; grade of inflammation, F; stage of fibrosis*

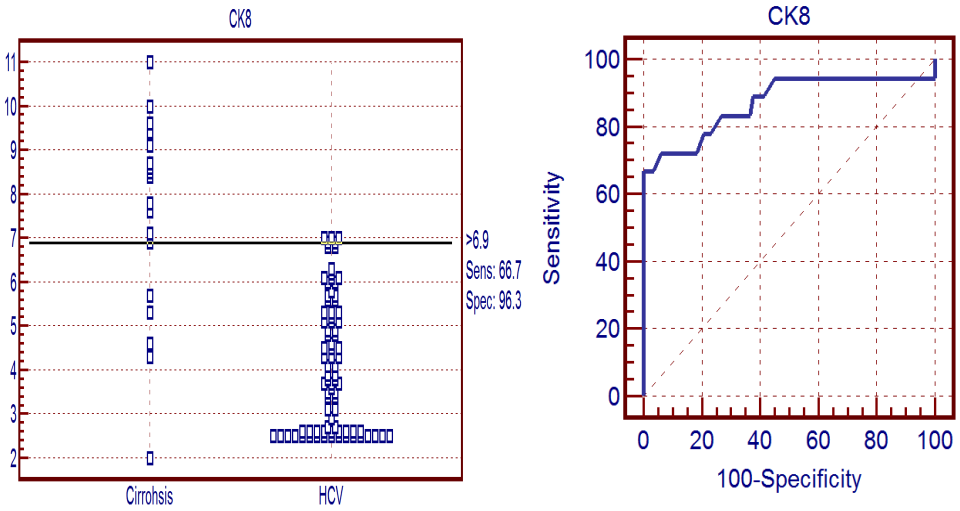


**Fig. 5. ROC curve of serum CK8 in patients groups**

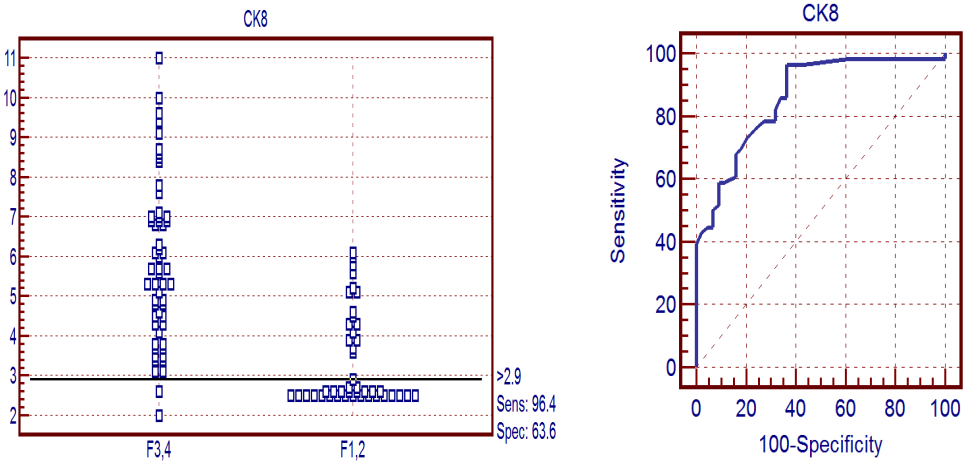


**Fig. 6. ROC Curve of serum CK8 in different degree of fibrosis**

**Table 3. Correlations between serum CK8 (ng/mL) and different parameters in patients groups**

| Correlations | | |
|---|---|---|
| | **Serum CK8(ng/mL)** | |
| | **r** | **P-value** |
| Age (years) | 0.14 | 0.15 |
| Platelet count (x10$^3$/cm$^3$) | -0.19 | 0.04* |
| Prothrombin activity( %) | -0.31 | 0.002* |
| Total bilirubin (mg/dL) | 0.18 | 0.06 |
| ALT( U/L) | 0.313 | 0.002* |
| AST( U/L) | 0.41 | <0.001* |
| ALP( U/L) | 0.03 | 0.69 |
| Albumin(gm/dL) | -0.14 | 0.11 |
| Serum AFP( ng/mL) | 0.18 | 0.07 |
| FIB4 index | 0.84 | <0.001* |

*ALT; alanine aminotransferase enzyme, AST; aspartate aminotransferase enzyme, ALP; alkaline phosphatase enzyme, AFP; alpha feto protein. * **P** =0.05*

**Table 4. ROC Curve of serum CK8 in different degree of fibrosis**

| Cutoff | Sens. | Spec. | PPV | NPV | Accuracy |
|---|---|---|---|---|---|
| >6.9 | 66.7 | 96.3 | 97.0 | 93.2 | 87.20 |

**Table 5. ROC curve of serum CK8 in different degree of fibrosis**

| Cutoff | Sens. | Spec. | PPV | NPV | AUC |
|---|---|---|---|---|---|
| > 2.9 (ng/mL) | 96.4 | 63.6 | 77.1 | 93.3 | 86.2 |

Keratin polypeptide 8 (K8) linked noncovalently with its partners K18 and/or K19 to form the intermediate filament cytoskeleton of hepatocytes and other simple-type epithelial cells. Normally, the total amount of cellular CK8 is present at a stable level. The function of CK8/18 in the liver is protection from mechanical and non- mechanical stress which can lead to cell death. Stress can affect cytokeratins expression levels, expression profiles and posttranslational modification [12].

Our study was performed to examine the cytokeratin 8 serum levels and blood expression in patients with chronic HCV infection and evaluate its role as a marker for early liver injury by correlating serum CK8 level with severity of hepatic inflammation, fibrosis and FIB4 index.

Holmberg et al. [24] observed in their large observational cohort that FIB-4is a significant index to clinicians because it is simplify to calculate and easily available from hospital or clinic laboratories during usual patient care. Therefore, it would be helpful to screen patients who need biopsy and therapy.

Vallet-Pichard et al. [7] identified for values outside 1.45-3.25, the FIB-4 index is a simple, accurate and inexpensive method for assessing liver fibrosis.

However, FIB4 index reflect alterations in hepatic functions rather than in extracellular matrix (ECM) metabolism. Many HCV studies have identified normal transaminase levels in about 25%–30% of chronic HCV patients; there may be a potential advantage in assessing marker of direct liver injury that does not include transaminases [25].

In the present study, patients with chronic HCV had higher serum CK-8 levels than healthy controls. The highest level was observed in patients with cirrhosis.

This study also revealed increased expression of CK8 in both liver disease groups than the control group. There was also an increased expression in cirrhotic patients than chronic HCV patients however it did not reach a significant value.

This was in agreement with Ku et al. [15] who observed that there was an increased in expression of CK8/18 in response to liver injury by 3-fold, which may be a factor of the essential cytoprotection provided by CK8 and CK18 in the liver.

Their cytoprotection presented by an important role in mechanical stability of hepatocytes, and a target through toxic stress by induction of apoptosis/necrosis. That was observed by Tarantino et al. [26] who found an increase in level of tissue polypeptide-specific antigen, a serological mirror of keratin 18 in non-alcoholic steatohepatitis patients (NASH) compared to either pure fatty liver patients (FL) or healthy volunteers. They also observed that this marker is a better than alanine aminotransferase activity, ultrasonography or the combination of both parameters in differentiating NASH from FL.

Toivola et al. [27] found that in chronic HCV infection, CK8 phosphorylation is a dependable marker for liver disease progression (hyperphosphorylation) or regression (relative hypophosphorylation or return to basal phosphorylation). Furthermore, Strnad et al. [23] found that a number of CK8 gene variants are increased in patients with chronic HCV infection.

Sun et al. [28] found in their study that CK8 mRNA expression increased in HCV infected cell culture compared to other culture and suggested that HCV up-regulates CK8 expression in HCV cell culture cells, and that CK8 expression is significantly associated with HCV.

This study observed a significant increased CK 8 mRNA expression with increased serum CK8 level in both HCV and cirrhotic patients.

Our study also found significant positive correlations between serum CK-8 levels and ALT, AST, FIB4, grade of inflammation and stage of fibrosis in both HCV and cirrhotic patients.

Furthermore, the present study also identified significant positive correlations between serum CK-8 levels and both grade of inflammation and stage of fibrosis in patients had FIB4 index values inside 1.45-3.25.

This study identified increased serum CK8 levels with increased degree of inflammatory activity. These finding encourage a central role for apoptosis in disease pathogenesis, and this finding was similar to the results reported by Bantel et al. [29] and Fuentes-Gonzalez et al. [30]. They explained infection with HCV by inflammatory liver damage and a long viral persistence related with an increased risk of rising hepatocellular carcinoma. Apoptosis initiation upon HCV infection may seriously result in liver damage, while inhibition of apoptosis may contribute to HCV persistence and development of hepatocellular carcinoma.

Strnad et al. [23] revealed that there was an association between CK8/18 variants with development of liver fibrosis in chronic hepatitis C patients.

Wang et al [31] and Lee et al. [32] in their study explained that CK8/18-deficient animals had a marked susceptibility to tumor necrosis factor (TNF)-induced cell death and Fas-induced apoptosis. As CK8 and CK18 are in resistance to TNF family receptors- and Fas-induced apoptosis.

Xun et al. [33] reported that CK8 was participate in cytoprotective role against HCV infection and modulate the cellular response to proapoptotic signals to resist apoptosis and fibrosis. CK8 could be used as a marker to predict liver disease severity, treatment response and fibrosis.

## 5. CONCLUSION

In conclusion, the serum levels of CK-8 were observed to be equivalent to its blood expression. As a result, serum CK-8 levels could be used as a marker for disease progression and activity in HCV patients instead of the invasive tool current ‘‘gold standard’’ biopsy especially in patients with inconclusive FIB4index (values inside 1.45-3.25).

## ETHICAL APPROVAL

The authors have obtained all necessary ethical approval from suitable Institutional or State or National or International Committee. This study is not against the public interest, or that the release of information is allowed by legislation.

## ACKNOWLEDGEMENTS

The authors would like to thank the staff members of the Internal Medicine, Clinical Pathology and Pathology Departments for their help. The authors declare that they did not receive any funds during preparation of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Aly Ohn ES, Anwar W, Sallam I. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet. 2000; 355(9207):887–91.
2. Mohamoud YA, Mumtaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. BMC Infectious Diseases. 2013;13:288.
3. Bosetti C, Levi F, Zatonski WA, Negri E, LaVecchia C. Worldwide mortality from cirrhosis: An update to 2002. J Hepatol. 2007;46:827-39.
4. Murray K, Finn L, Taylor S, Seidel K, Larson A. Liver histology and alanine aminotransferase levels in children and adults with chronic hepatitis C infection. J Pediatr Gastroenterol Nutr. 2005;41:634-8.
5. Piccinino F, Sagnelli E, Pasquale G, Giusti G. Complications following percutaneous liver biopsy. A multicenter retrospective study on 68,276 biopsies. J Hepatol. 1986; 2:165–73.
6. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006;43:1317–25.

7. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: An inexpensive and accurate marker of fibrosis in HCV infection. Comparison with Liver Biopsy and Fibrotest. Hepatology. 2007;46:32–6.
8. Adler M, Gulbis B, Moreno C, Evrard S, Verset G, Golstein P, Frotscher B, Nagy N, Thiry P. The predictive value of FIB-4 versus Fibrotest, APRI, Fibroindex and Forn Index to non-invasively estimate fibrosis in hepatitis C and nonhepatitis C liver diseases. Hepatology. 2008;47:762–3.
9. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. The cytoskeleton. In: Anderson M, Granum S, editors. Molecular biology of the cell. 5 th ed. New York, NY Garland Science. 2008;965–1047.
10. Molnar A, Haybaeck J, Lackner C, Strnad P. The cytoskeleton in nonalcoholic steatohepatitis: 100 years old but still youthful. Expert Rev Gastroenterol Hepatol. 2011;5:167–77.
11. Coulombe PA, Kerns ML, Fuchs E. Epidermolysis bullosa simplex: A paradigm for disorders of tissue fragility. J Clin Invest. 2009;119:1784–93.
12. Omary MB, Ku NO, Strnad P, Hanada S. Towards unraveling the complexity of 'simple' epithelial keratins in human disease. J Clin Invest. 2009;119:1794–805.
13. Omary MB, Coulombe PA, McLean WH. Intermediate filament proteins and their associated diseases. N Engl J Med. 2004;351:2087-100.
14. Omary MB, Ku NO, Toivola DM. Keratins: Guardians of the liver. Hepatology. 2002; 35:251-7.
15. Ku NO, Strnad P, Zhong BH, Tao GZ, Omary MB. Keratins let liver live: mutations predispose to liver disease and cross linking generates Mallory-Denk bodies. Hepatology. 2007;46:1639–49.
16. Bedossa P, Poynard T, the French METAVIR Cooperative Study Group. An algorithm for grading activity in chronic hepatitis C. Hepatology. 1996;24:289-93.
17. Strickland GT, Elhefni H, Salman T, Waked I, Abdel-Hamid M, Mikhail NN, Esmat G, Fix A. Role of hepatitis C infection in chronic liver disease in Egypt. Am J Trop Med Hyg. 2002;67(4):436–42.
18. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med. 2001;344:495-500.
19. Zatloukal K, French SW, Stumptner C, Strnad P, Harada M, Toivola DM, Cadrin M, Omary MB. From Mallory to Mallory-Denk bodies: What, how and why? Exp Cell Res. 2007;313:2033–49.
20. Omary MB, Ku N, Strnad P, Hanada S. Toward unraveling the complexity of simple epithelial keratins in human disease. J Clin Invest. 2009;119:1794–805.
21. Ku NO, Gish R, Wright TL, Omary MB. Keratin 8 mutations in patients with cryptogenic liver disease. N Engl J Med. 2001;344:1580–87.
22. Ku NO, Lim JK, Krams SM, Esquivel CO, Keeffe EB, Wright TL, Parry DA, Omary MB. Keratins as susceptibility genes for end-stage liver disease. Gastroenterology. 2005;129:885–93.
23. Strnad P, Lienau TC, Tao GZ, Lazzeroni LC, Stickel F, Schuppan D, Omary MB. Keratin variants associate with progression of fibrosis during chronic hepatitis C infection. Hepatology. 2006;43:1354–63.
24. Holmberg SD, Lu M, Rupp LB, Lamerato LE, Moorman AC, Vijayadeva V, Boscarino JA, Henkle EM, Gordon SC. Chronic Hepatitis Cohort Study (CHeCS) Investigators. Chronic Hepatitis Cohort Study (CHeCS) Investigators**.** Noninvasive serum fibrosis markers for screening and staging chronic hepatitis C virus patients in a large US cohort. Clin Infect Dis. 2013; 57(2):240-6.
25. Valva P, Casciato P, Diaz Carrasco JM, Gadano A, Galdame O, Galoppo MC, Mullen E, De Matteo E, Preciado MV. The role of serum biomarkers in predicting fibrosis progression in pediatric and adult hepatitis C virus chronic infection. PLoS ONE. 2011;6(8):e23218.
26. Tarantino G, Conca P, Coppola A, Vecchione R, Di Minno G.Serum concentrations of the tissue polypeptide specific antigen in patients suffering from non-alcoholic steatohepatitis. Eur J Clin Invest. 2007;37(1):48-53.
27. Toivola DM, Ku NO, Resurreccion EZ, Nelson DR, Wright TL, Omary MB. Keratin 8 and 18 hyperphosphorylation is a marker of progression of human liver disease. Hepatology. 2004;40:459-66.
28. Sun MZ, Dang SS, Wang WJ, Jia XL, Zhai S, Zhang X, Li M, Li YP, Xun M. Cytokeratin 8 is increased in hepatitis C

virus cells and its ectopic expression induces apoptosis of SMMC7721 cells. World J Gastroenterol. 2013;19(37):6178-87.
29. Bantel, H, Schulze-Osthoff K. Apoptosis in hepatitis C virus infection. Cell Death Differ. 2003;10(Suppl 1):S48-58.
30. Fuentes-González AM, Contreras-Paredes A, Manzo-Merino J, Lizano M. The modulation of apoptosis by oncogenic viruses. Virol J. 2013;10:182.
31. Wang Y, He QY, Tsao SW, Cheung YH, Wong A, Chiu JF. Cytokeratin 8 silencing in human nasopharyngeal carcinoma cells leads to cisplatin sensitization. Cancer Lett. 2008;265(2):188-96.
32. Lee J, Jang KH, Kim H, Lim Y, Kim S, Yoon HN, Chung IK, Roth J, Ku NO. Predisposition to apoptosis in keratin 8-null liver is related to inactivation of NF-kappaB and SAPKs but not decreased c-Flip. Biol Open. 2013;2(7):695-702.
33. Xun M, Wang H, Li B, He H, He Q, Chu Y. Cytokeratin 8 was over-expressed in cells harboring in vitro-transcribed full length hepatitis C virus 1b RNA, but down-expressed in HCV patients' serum. Clini Med Research. 2014;3(3):80-6.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://sciencedomain.org/review-history/12163*