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Cytokeratin 18 as marker for non-invasive diagnosis of acute liver diseases

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Abstract

Background: Assessment of CK-18 cell death biomarkers allows for the early detection of liver damage in acute liver diseases. Our aim of work is to prove that CK-18 cell death biomarkers in serum enables early detection of liver damage in acute liver diseases. **Patients and methods**: a cross sectional study that conducted. It included 40 child with acute liver disease and compared to 25 age and sex matched childen as a control group who presented at outpatient clinics and inpatient departments in Beni-Suef university hospital. All patients underwent complete history taking, complete physical examination and investigations including Alanine Aminotransferase, Aspartate Transaminas, Albumin, CBC, CRP, Urea ,creatinine and Serum CK -18 measurement using ELISA kit. **Results:** CK18 levels were significantly higher in liver disease patients than controls. There was significantly difference between the studied groups regarding their CK18 level. There was insignificant

correlation between CK18 and labs in Acute Hepatitis. There was a significant positive correlation between CK18 and INR in fulminant hepatitis. When ROC curve was plotted, CK18 was found to have a significant role in diagnosing of liver disease as Acute Hepatitis and Fulminant Hepatitis with reasonable sensitivity, specificity, PPV, NPV. **Conclusion and recommendations:** CK18 may help in diagnosis of liver diseases in children. Further multicenter studies on a large number of populations with serial measurements of CK18 levels are recommended to further evaluate the role of CK18 in the diagnosis of liver disease in children and to detect the prognostic value of CK18 regarding mortality.

1. Introduction :

Unfortunately, acute are suspected in any child who will present with jaundice, oedema, ascites, and/or hepatic encephalopathy. [1]**.** In acute liver diseases serum bilirubin, albumin and prothrombin time (PT) are the markers of worsening of the hepatic disease. Increased bilirubin and PT and decreased albumin are bad prognostic signs [2]. Cytokeratin-18 (CK18) is an essential hepatic intermediate filament protein. After apoptosis of injured hepatic cells, caspase cleaved CK18 fragments pass to the blood [3].

Evaluation of CK-18 cell death biomarkers helps early determination of hepatic destruction in acute liver diseases. This is even meaningful when transaminases are within the normal range. Determination of CK-18 biomarker may give an idea about the disease activity & severity as well [4]

Aim of the work

Our aim of work is to evaluate the role of serum CK-18 in liver diseases in children and its prognostic value.

2. Patients and Methods:

It is a cross sectional study was done in the duration from May 2022 to February 2023 .The children included in this study were 40 child suffering ALD and compared to 25 age and gender matched

children as a control group who presented at outpatient clinics and inpatient departments in Beni-Suef university hospital.

2.1 Inclusion criteria:

All Children aged between 1-15 y were included .

All children suspected to have acute liver disease.

2.2 exclusion criteria:

Children aged < 1 y and > 15 y.

Children with chronic liver diseases.

2.3 All participants were subjected

1. Full history taking;

Detailed history, past medical, Symptoms related to gastrointestinal disorders

, Respiratory problems or Neurologic symptoms .

2. Radiological investigations:

a) Trans-abdominal ultrasound for all participants

3. Laboratory test: Alanine Aminotransferase , AST Albumin CBC,CRP,Urea, Cr and **Serum CK18** assessment via ELISA kit based on the following 5 ml of venous blood samples was taken via venipuncture with complete aseptic method from the cases as well as controls.The blood was left to clot via leaving it undisturbed at RT .

The clot removed through centrifugation at 3000 rpm for 5 min. The kit uses a double-Ab sandwich ELISA to determine the level of Human CK18 in samples. Addition of CK18 to monoclonal Ab Enzyme well, then it's precoated with Human CK18 monoclonal Ab, incubation; thereafter , addition of CK-18 Abs labeled with biotin, and combined with Streptavidin-HRP in order to produce immune complex; followed by incubating and washing again to eliminate the uncombined enzyme. Thereafter, Chromogen Solution A, B, were added, the color of the liquid changed to the bluish, And at the influence of acid, the color ultimately became yellowish. The chroma of color and the concentrations of CK18 of sample were positively correlated.

Ethical Considerations:

1- Approval of the managers of the hospital in which the study was carried out was taken.(Approval No:FMBSUREC/08052020/Gomaa)
2- Any non-expected risks that appeared during the course of the study were explained to the participants
3- We had an informed written consent from all cases included..
4- Privacy and confidentiality of data was ensured.

Statistical analysis:

The data we collected were tabulated, coded and analyzed via SPSS for Windows, version 23. Continuous variables were presented as mean values ± SD, whereas categorical variables were presented as percentages.

3. Results:

Table (1) showes that there was statisticaly significant difference between the studied groups regarding their liver function tests except the serum albumin level.

Table (1) Liver function tests among the studied groups:

Items	ACUTE HEPATITIS (no=20)	Fulminant hepatitis (no=20)	Controls (no=25)	P-value
ALT	674.60 ± 565.50 IU a	614.45 ± 516.49 IU a	25.84 ± 7.520 IU c	<0.001*
AST	733.550 ± 232.72 IU a	474.70 ± 346.87 IU a	23.68 ± 7.227 IUc	<0.001*
Bilirubin	8.235 ± 5.773 mg a	4.081 ± 5.815b	0.436 ± 0.250d	0.004*
Serum albumin	3.955 ± 0.3236 mg a	2.845 ± 0.601b	4.088 ± 0.418a	0.115

Table(2) showed that there was statisticaly significant difference between the studied groups regarding their bleeding profile.

Table (2) Bleeding profile among the studied groups:

Items	ACUTE HEPATITIS (no=20)	Fulminant hepatitis (no=20)	Controls (no=25)	P-value
PT	11.52 ± 0.490a	17.55 ± 6.045b	12.136 ± 0.73a	<0.001*
PC	86.75±7.34a	62.25 ± 19.601b	89.44 ± 4.727a	<0.001*
INR	1.055 ± 0.085a	2.277 ± 0.681b	0.896 ± 0.098a	0.004*

Table (3) showed that there was statisticaly significant difference between the studied groups regarding their CK18 level.

Table (3) Comparison between the studied groups regarding the CK18 level :

Items	**(1) ACUTE HEPATITIS (no=20)**	**(2) Fulminant hepatitis (no=20)**	**(3) Controls (no=25)**
CK18	650.585 ± 202.841	998.66 ± 365.042	465.420 ± 79.220
Post hoc (Tukey)	(2)(3)	(1)	(2)(3)
P value	<0.001*		

Table (4) showes that there was insignificant correlation between CK18 and labs in acute hepatitis.

Table (4) Correlation between CK18 and different labs in Acute Hepatitis

Acute Hepatitis (no=20)		C K 18
ALT	R	.229
	P-value	.332
AST	R	.177
	P-value	.455
Bilirubin	R	.200
	P-value	.397
serum albumin	R	-.246
	P-value	.296
PT	R	-.039
	P-value	.869
PC	R	.060
	P-value	.803
INR	R	.115
	P-value	.630

Table (5) illustrated that the CK18 had a significant role in diagnosing of liver disease as Acute Hepatitis and Fulminant Hepatitis with variable sensitivity, specificity, PPV, NPV.

Table (5) Cut off, sensitivity, specificity, PPV, NPV of CK18 in diagnosis of liver disease:

Items	Acute hepatitis	Fulminant hepatitis
P-value	<0.001*	<0.001*
Cut off	502.2	572.9
AUC	0.800	0.986
Sensitivity (95%CI)	75(50.9 - 91.3)	95(75.1 - 99.9)
Specificity (95%CI)	72(50.6 - 87.9)	100(86.3 – 100)
PPV (95%CI)	68.2(52.1 - 80.8)	100.0(86.3 - 100.0)
NPV (95%CI)	78.3(61.9 - 88.9)	96.2(78.7 - 99.4)

Figure (1) Receiver operating characteristic curve for CK18 role in diagnosis of acute hepatitis (vs control)

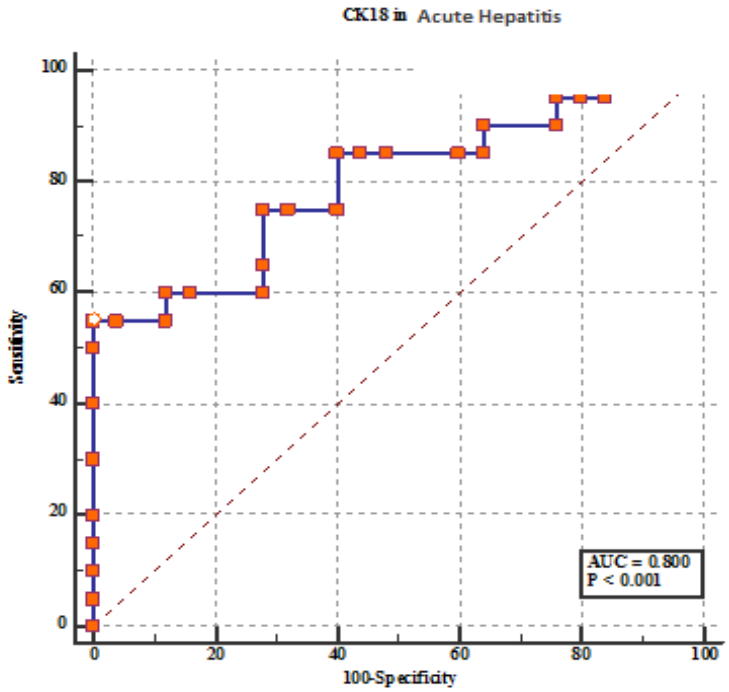
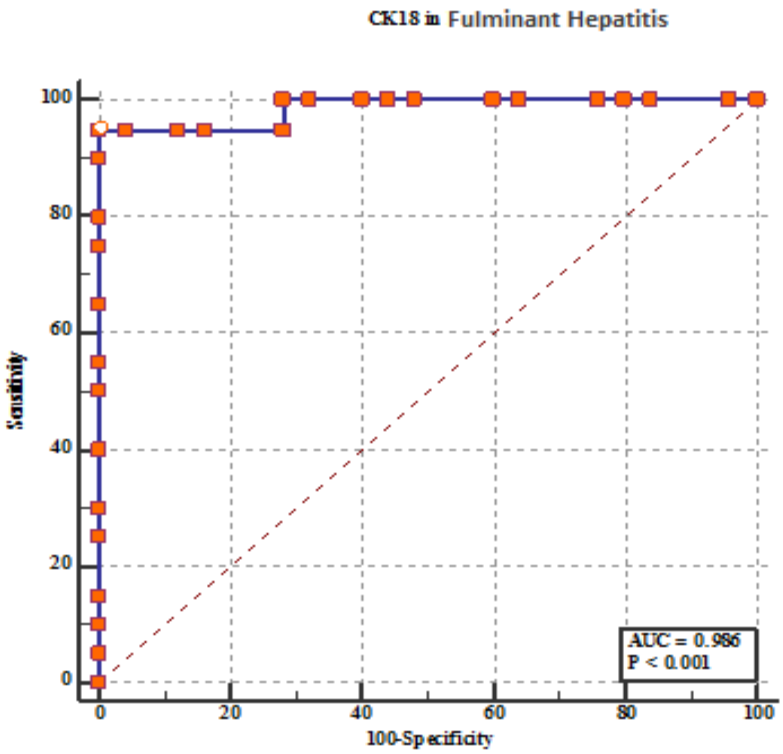


Figure (2) Receiver operating characteristic curve for CK18 role in diagnosis of fulminant hepatitis (vs control)



4. Discussion:

Mandelia et al., (2016)[5] found that Cytokeratin-18 (CK18) is the major intermediate filament protein in the liver.In our study, Dark urine was more prevalent in Acute Hepatitis and fulminant hepatitis.

Ramadan et al., (2023)[6] reported that Jaundice was more prevalent in Acute Hepatitis than the other groups.Jaundice was more common in Acute Hepatitis in comparison with the other cases. Jaundice and dark urine were the essential clinical presentation in acute hepatitis as documented by various studies. **In Elbeltagi, et al. (2023)[7]** study, Jaundice was the commonest clinical manifestations of acute hepatitis

In **Tanwar (2022)[8]** study hepatomegaly is a well-documented sign in children with fulminant hepatitis. **Suttorp & Classen, (2021)[9]** revealed that hepatic cirrhosis is one of the leading causes for elevated portal vein pressure and splenomegaly all over the world. In current study, ALT, AST and bilirubin were more significantly elevated in acute hepatitis, fulminant hepatitis.

Yang et al. (2015)[10], showed that plasma CK18 level in hepatitis cases were elevated, in comparison with healthy control subjects. The results showed the clinical importance of CK18, in addition to other apoptotic markers, to identify the development of hepatitis.**Aida et al. (2014)[11]** findings revealed a significant elevation in plasma CK18 in the cirrhosis group in comparison with control subjects.

ROC curve was plotted to determine the capability of CK 18 for differentiating children with liver diseases. We concluded that the capability of serum CK18 (cut-off 502.2 u/l, sensetivity=75 percent, specificity=72 percent, PPV = 68.2 percent , NPV = 78.3 percent) level to diagnose acute hepatitis was significant (AUC = 0.986, P < 0.001). The ability of serum CK18 (cut-off 572.9 u/l, sensetivity=85 %, specificity=100 %, PPV = 100 %, NPV = 89.3 %) levels to diagnose fulminant hepatitis was significant (AUC = 0.958, P < 0.001).

5. Conclusion:

- CK18 might help in diagnosing acute hepatic diseases of children.
- CK18 levels were significantly elevated in liver disease cases in comparison with controls.
- There was a significant +ve correlation between CK18 and INR in cases of fulminant hepatitis.

Recommendations:

- Further multicenter studies on a greater number of people with serial measurement of CK18 level are recommended to further assess the function of CK18 in diagnosing hepatic diseases of children.
- Further studies are important to compare CK18 in addition to other biomarkers as diagnostic markers in hepatic diseases of children.

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