Cytokeratin-18 Fragment Levels as a Biomarker of Non-Alcoholic Fatty Liver Disease in Egyptian Patients with Type 2 Diabetes Mellitus

Prof. Salem Soliman Ahmed Salama, Prof. Abd El-Monaem Mohamed Barrak, Prof. Hassan Abdul-Aziz Hassan Gabber, Prof. Rabie Fathy Abbas, Mohamed Fathy Al-Araby

Internal Medicine Department, Faculty of Medicine, Al Azhar University, Egypt
doctormedo2030@gmail.com

Abstract: Background: Non-alcoholic fatty liver disease **(NAFLD)** is the hepatic pandemic of the **21th** century**,** being the number one cause of chronic hepatic disease in the occidental world ***(Bellentani et al., 2010).*** Although usually benign**,** fatty liver may associate with serious injury**,** with inflammation and hepatocyte necroapoptosis**,** non-alcoholic steatohepatitis **(NASH),** in **20 - 30%** of subjects**.** Those patients are at risk of developing fibrosis**, 1/5** progressing to liver cirrhosis**.** It is apparently more slowly progressive than other chronic liver diseases**,** such as alcohol or viral-induced disease**.** However, because **NAFLD** is so common**,** occurring in **1** out of **3** persons in the developed world**,** it is the **3rd** cause of liver transplantation in **USA** ***(Charlton et al 2011).*** Moreover**,** the problem of hepatocytes being fatty**,** overcomes the liver itself**,** as it increases the risk for cardiovascular disease and death and duplicates the risk for **T2DM,** independently of the severity of liver injury**. Aim of the Work:** It is planned to evaluate the concentration of serum **CK-18** as a non-invasive bio-maker of **NAFLD** in patients with **T2DM. Subjects and Methods:** All subjects were selected from the outpatient clinic of internal medicine and inpatient internal Medicine departments of Sayed-Galal Hospital Al-Azhar University and Al-Matariah Educational Hospital**.** They were Egyptians**;** their age ranged from **19 - 55** years old**.** The study was performed in the period from May 2016 to June 2017. **Results:** Eighty subjects were enrolled in this study, they were classified into 4 groups: Group 1: Includes 20 patients with T2DM and having NAFLD, Group 2: Includes 20 patients with T2DM and *not* having NAFLD, Group 3: Includes 20 patients *without* T2DM and having NAFLD, Group 4: Includes 20 age-matched healthy controls (i.e., neither having T2DM nor having NAFLD). **Conclusion: CK-18** is increased in patient with **NAFLD** either with or without **DM.** Increase **CK-18** level is might be a possible diagnostic biomarker for **NAFLD** among **T2DM** patients**.**

[Salem Soliman Ahmed Salama, Abd El-Monaem Mohamed Barrak, Hassan Abdul-Aziz Hassan Gabber, Rabie Fathy Abbas, Mohamed Fathy Al-Araby. **Cytokeratin-18 Fragment Levels as a Biomarker of Non-Alcoholic Fatty Liver Disease in Egyptian Patients with Type 2 Diabetes Mellitus.** *Nat Sci* 2018;16(4):47-54]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 9. doi:10.7537/marsnsj160418.09.

Keywords: Cytokeratin-18, Non-Alcoholic Fatty Liver Disease, Type 2 Diabetes Mellitus

1. Introduction

Non-alcoholic fatty liver disease **(NAFLD)** is the hepatic pandemic of the **21th**century**,** being the number one cause of chronic hepatic disease in the occidental world ***(Bellentani et al., 2010).*** Although usually benign**,** fatty liver may associate with serious injury**,** with inflammation and hepatocyte necroapoptosis**,** non-alcoholic steatohepatitis **(NASH),** in **20 - 30%** of subjects**.** Those patients are at risk of developing fibrosis**, 1/5** progressing to liver cirrhosis ***(Angulo et al., 1999).*** It is apparently more slowly progressive than other chronic liver diseases**,** such as alcohol or viral-induced disease ***(Fassio et al., 2004).*** However, because **NAFLD** is so common**,** occurring in **1** out of **3** persons in the developed world ***(Bellentani et al., 2010),*** it is the **3rd** cause of liver transplantation in **USA** ***(Charlton et al 2011).*** Moreover**,** the problem of hepatocytes being fatty**,** overcomes the liver itself**,** as it increases the risk for cardiovascular disease and death and duplicates the risk for **T2DM,** independently of the severity of liver injury ***(Musso et al., 2011).***

Patients with **T2DM** have a higher risk of development of **NAFLD** ***(Leite et al., 2009)*** than those without **T2DM** ***(Hossain et al., 2009).*** The prevalence of **NAFLD** is increasing mostly likely due to the rise in obesity and diabetes ***(Kojima et al., 2003).*** It is reported that **13.3%** of deaths among diabetic patients are attributable to liver diseases**,** representing the increasing prevalence of **NAFLD** in patients with **T2DM** ***(Okanoue et al., 2011).*** Patients with **T2DM** can develop **HCC** from **NAFLD** without exhibiting significant liver fibrosis or cirrhosis ***(Paradis et al., 2009). In addition,*** the coexistence of **T2DM** and **NAFLD** is associated with increased cardiovascular mortality and morbidity ***(Targher and Arcaro, 2007). Therefore,*** providing early diagnosis and follow up of **NAFLD** inpatients with **T2DM** is important**.**

NAFLD encompasses a wide spectrum of conditions associated with over accumulation of fat in

the liver ranging from **NAFD** or simple steatosis to non-alcoholic steatohepatitis **(NASH)** and cirrhosis ***(Bacon et al., 1994)*.**

Although **NAFLD** typically follows a benign non-progressive clinical course**, NASH** is a potentially serious condition**;** as many as **25% of** patients may progress to hepatic cirrhosis and its sequelae ***(Farrell and Larter, 2006)*.** It was suggested that progression from **NAFL** to **NASH** andto advanced fibrosis results from **2** distinct events**; *first*,** insulin resistance **(IR)** leading to the accumulation of fat within hepatocytes and ***second*,** mitochondrial reactive oxygen species causes lipid peroxidation and cytokine induction ***(Marchesini, 1999)*.**

With the increasing prevalence of obesity and metabolic syndrome **(MS)** an increase of **NAFLD** is obvious**;** a trend reflected in the increasing number of scientific publications on **NAFLD** and **NASH** ***(Haima, 2014)*.** Patients with **IR** and other symptoms of **MS** should ***therefore*** be screened for **NAFLD** and its progressive and chronic form **NASH (non-alcoholic steatohepatitis)** ***(Bernsmeier et al., 2011)*.** The diagnostic challenge is to predict **NAFLD** patients that are likely to progress into severe liver disease**,** initiate therapy and life style changes and to monitor the efficacy of the measures**.**

The classical gold standard for diagnosing and staging **NAFLD** and assessing fibrosis is liver biopsy **(LB).** However, it has important sample error issues and subjectivity in the interpretation**,** apart from a small but real risk of complications**.** The decision to perform an **LB** is even harder in a condition so prevalent such as **NAFLD,** in which the probability of finding severe liver injury is low**.** In an attempt to overcome **LB** and to subcategorize patients with **NAFLD** in different prognoses allowing better management decisions**,** several non-invasive methods have been studied in the last decade ***(Machado and Cortez-Pinto, 2013)*.**

Cytokeratins are proteins of keratin-containing intermediate filaments found in the intra-cytoplasmic cytoskeleton of epithelial tissue**.** In 2006; a new systematic nomenclature for keratins was created and now proteins previously called **'*cytokeratins*'** are simply called ***keratins (Schweizer et al., 2006)*.**

When hepatocytes are chronically exposed to oxidative stress and toxic substances**,** they become ballooned**;** accumulate fat**,** show a disruption in keratin intermediate filament network and form Mallory bodies ***(Pei et al., 2004)*.**

A Mallory body is composed of abnormally phosphorylated and cross-linked keratins**,** such as **CK-18** and stress-induced proteins ***(Matteoni et al., 1999)*.**

Since hepatocytes containing Mallory bodies are susceptible to apoptosis**,** so those levels of Mallory body-associated proteins released from hepatocytes into peripheral blood may be increased in **NASH** patients and change in accordance with disease activity ***(Calvert et al., 2007)*.** During apoptosis**,** following the production of epithelial effector caspases **3, 6** and **7, CK-18** is cleaved into proteolytic fragments that are released into the blood ***(Ueno et al., 2005)*.**

Recently*,** a monoclonal antibody that selectively recognizes a neoepitopes of ***cytokeratin-18 after caspase-induced cleavage during apoptosis has become available ***(Miyasato et al., 2014)*.** ***Wieckowska et al., (2006)*** found a correlation between the concentration of caspase-cleaved ***CK-18*** and the histological stage in **NAFLD** patients and concluded that the ***CK-18*** concentration can be used to differentiate **NASH** from simple steatosis**.** ***Moreover*,** it was found that determination of **CK-18** fragments in blood**,** predicts and correlates with histological **NASH** in which there is development of lobular inflammation**,** cell ballooning and fibrosis**,** supporting its usefulness in clinical practice ***(Maher et al., 2015)*.** ***In addition*,** elevated concentration of circulating **CK-18** have been reported among **NAFLD** patients compared with those observed in controls**,** mostly non-diabetic subjects ***(Tsutsui et al., 2010); however*,** the level of **CK-18** have not been explored in patients with **T2DM.**

Aim of the Work

It is planned to evaluate the concentration of serum **CK-18** as a non-invasive bio-maker of **NAFLD** in patients with **T2DM.**

Subjects and Methods

Subjects:

Eighty subjects were enrolled in this study, they were classified into 4 groups:

Group 1: Includes **20** patients with **T2DM** and having **NAFLD.**

Group 2: Includes **20** patients with **T2DM** and ***not*** having **NAFLD.**

Group 3: Includes **20** patients ***without*** **T2DM** and having **NAFLD**

Group 4: Includes **20** age-matched healthy controls **(i.e., neither having T2DM nor having NAFLD)**

All subjects were selected from the outpatient clinic of internal medicine and inpatient internal Medicine departments of Sayed-Galal Hospital Al-Azhar University and Al-Matariah Educational Hospital**.** They were Egyptians**;** their age ranged from **19 - 55** years old**.** The study was performed in the period from **May 2016** to **June 2017.**

Exclusion criteria:

Patients with the following will be excluded:

- Any liver disease other than fatty liver

- Heart failure
- Hyper- or hypothyroidism
- Any autoimmune disease
- Malignancy
- Infection
- Pregnancy
- Use any type of medications other than used of **T2DM** treatment.

(All of which have been suggested to affect the serum CK-18 concentration)

Methods:

All subjects enrolled in the study were subjected to:

(I) Clinical assessment:

- **Full history taking including:** Age, sex.
- **Thorough clinical examination including:**
- **Body mass index (BMI)** was calculated on the basis of body weight **(in kilograms)** divided on height **(in meters square).**

Body weight classification according to BMI (kg/m^2) *(Eknoyan, 2007)*:

- **< 18.5:** Underweight
- **18.5 - 24.9:** Healthy normal weight range
- **25.0 - 29.9:** Overweight
- **30.0- 34.9:** Obesity class I
- **35.0 - 39.9:** Obesity class II
- **≥ 40.0:** Obesity class III

- **Waist circumference (WC)** was measured at the mid-point between the lower border of the rib cage and the iliac crest. **WC** should measure no more than **94 cm** for **men, 80 cm** for **women *(Grundy, 2005)*.**

(II) Laboratory investigations:

Laboratory analysis including:

Serum lipid profile evaluation:

TC (enzymatic method) and TG (enzymatic method without glycerol blocking) and HDL (dextran sulfate-$Mgcl_2$ precipitation) were measured on a Hitachi-911automated analyzer using reagent kits supplied by manufacturer of the analyzer ***(Stien and Myers, 1995)*. LDL-c** concentration will be calculated according to ***Friedewald equation*** **(LDL-C = [TC - HDL-C - TG/5)** ***(Maggio and Pi-Sunyer, 1997)*.**

ALT, AST, serum bilirubin, total protein, albumin **and Serum creatinine** was assessed by **Using Hitachi, 911**automatic analyzer.

PT, PT concentration **& INR** will be measured **by Advia 212-01.**

CBC (Hb, WBC and platelet count) using system XT-1500.

FPG, 2h-PPG (hexokinase method) and HA1c

Viral markers **(HBsAg, HBcAb, HCV-Ab), and** autoimmune marker **ANA, ASMA, anti-LKM-Ab** will be measured by **Cobas-E-411**.

(III) Quantification of serum CK-18 fragments (CK-18 M30 neoepitopes):

Methodology of CK-18 assessment: Serum CK-18 was measured using

(a) Sample collection:

5 ml of venous blood were taken from each subject. The serum was separated by centrifugation. Samples were stored at **-20°C.** Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze-thaw cycles, which may cause erroneous results. Hemolysed or lipemic samples were avoided.

Serum Cytokeratin-18 assay:

The assay was done using commercially available enzyme-linked immunosorbent assay **(ELISA)** kit by **Flow cytometry.**

(1) Test principle:

The assay is based on a sandwich **ELISA** technique for the quantitative level of **CK-18** in the sample. The purified anti-**CK-18** antibody was pre-coated onto **96**-well plates and the **HRP**conjugated anti-**CK-18** antibody was used as detection antibodies. The standards, test samples and HRP conjugated detection antibody were added to the wells subsequently, mixed and incubated, then, unbound conjugates were washed away with wash buffer. **TMB** substrates **(A & B)** were used to visualize **HRP** enzymatic reaction. **TMB** was catalyzed by **HRP** to produce a blue colour product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the **CK-18** amount of sample captured in plate. The concentration of **CK-18** can be calculated after reading the **O.D** absorbance at **450 nm** in a microplate reader.

Statistical Methods

Data were collected, revised, coded and entered to the Statistical Package for Social Science **(IBM SPSS)** version **20.** The qualitative data were presented as number and percentages while quantitative data were presented as mean, standard deviations and ranges when parametric distribution, while non-parametric distribution were presented as median with interquartile range **(IQR).**

3. Results

See **Figure (1)-(4)**.

4. Discussion

Non-alcoholic fatty liver disease **(NAFLD)** is defined by the presence of liver fat accumulation exceeding **5%** of hepatocytes, in the absence of significant alcohol intake, viral infection or any other specific etiology of liver disease ***(Tetri and Caldwell, 2003)*.** It is characterized by excessive fat in the form of **TG (steatosis)** in the liver **(> 5% of hepatocytes histologically).** A subgroup of **NAFLD** patients has liver cell injury and inflammation in addition to excessive fat **(steatohepatitis).** The latter condition,

designated **NASH,** is virtually indistinguishable histologically from alcoholic steatohepatitis**.** While the simple steatosis seen in **NAFLD** does not correlate with increased short-term morbidity or mortality**,** progression of this condition to that of **NASH** dramatically increases the risks of cirrhosis**,** liver failure and **HCC** ***(Chalasani et al., 2012 and Angulo, 2010).*** It is estimated that **NAFLD/NASH** will increase the **5-year** direct and indirect medical costs by **26%** ***(Vernon et al., 2011 and Sanyal et al., 2011).***

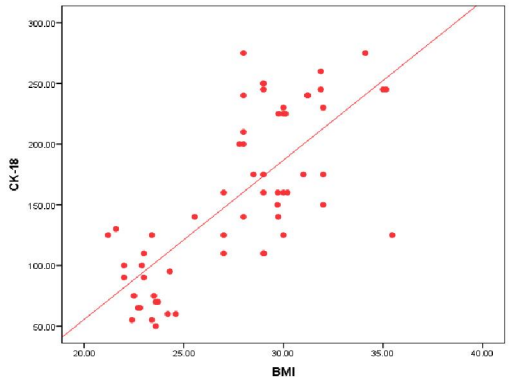

Figure (1): +ve correlation between *BMI* and *CK-18*, i.e., the greater the degree of BMI, the greater the degree of CK-18.

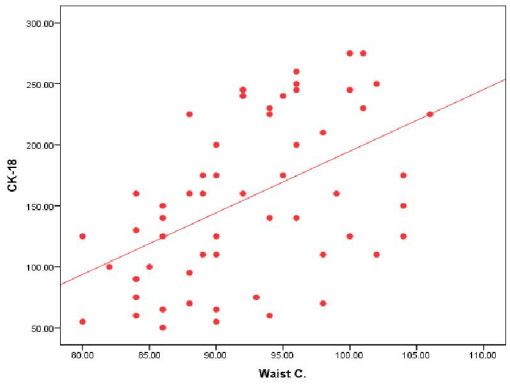

Figure (2): +ve correlation between *WC* and *CK-18*, i.e., the greater the degree of WC, the greater the degree of CK-18.

NAFLD affects **1** in every **3** subjects in the occidental world**.** The vast majority will not progress**,** but a relevant minority will develop liver cirrhosis and its complications ***(Machado and Cortez, 2013).*** The major risk factors for **NAFLD,** central obesity**,** **T2DM,** dyslipidemia and **MS,** are common in Western societies**.** **NAFLD** is the most common liver disorder in Western industrialized countries**,** affecting **20 - 40 %** of the general population ***(Chitturi et al., 2007).***

The clinical consequence of **NAFLD** is not limited to liver related morbidity and mortality**,** but is also associated with **CVD, T2DM** and **MS** ***Adams et al. (2009) and Lonardo and Loria (2009).*** The incidence of **NAFLD** is rapidly increasing**,** with huge clinical and economic burdens ***(Younossi et al., 2016).*** Identifying risk factors with potential therapeutic implications is important in managing **NAFLD** and decreasing these burdens**.** Development of **NAFLD** is a complex process that includes genetic susceptibility and environmental exposures ***(Sookoian and Pirola, 2016).***

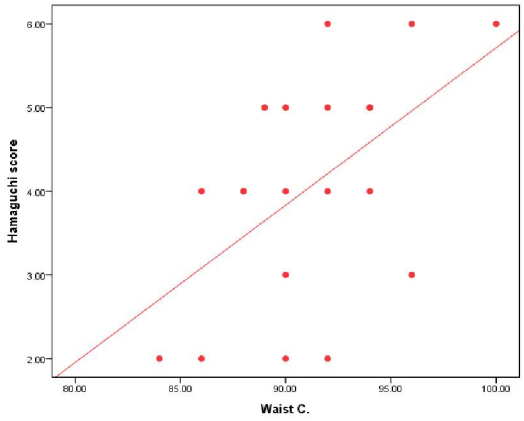

Figure (3): +ve correlation between WC and Hamaguchi score, i.e., the greater the degree of WC, the greater the degree of Hamaguchi score.

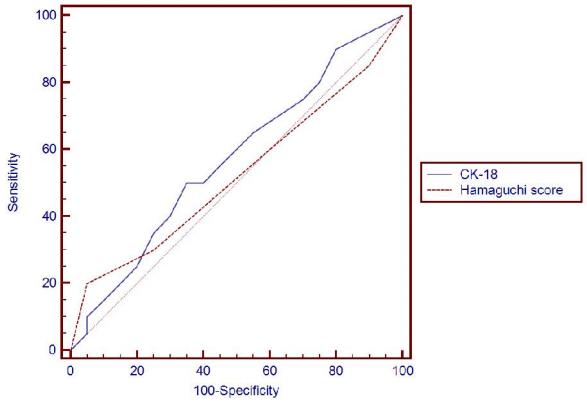

Figure (4): ROC curve: Sensitivity and specificity of CK-18 and Hamaguchi scores.

NAFLD is the most common cause of elevated **LFTs** results**,** after the commonly investigated causes have been excluded**,** and frequently coexists with **T2DM** because the conditions have common risk factors**.**

As both **T2DM** and **NAFLD** are related to adverse outcomes of the other**,** diagnosis and valuation of fatty liver is an important part of the

management of **DM.** Although noninvasive methods**,** such as biomarkers**,** panel markers and imaging**,** may support a diagnostic evaluation of **NAFLD** patients**,** accurate histopathological findings cannot be achieved without a liver biopsy**.** As it is important to know whether steatohepatitis and liver fibrosis are present for the management of **NAFLD,** liver biopsy remains the gold standard for **NAFLD** diagnosis and evaluation**.** ***Therefore*****,** new investigations of the pathogenesis of **NAFLD** are necessary to develop useful biomarkers that could provide a reliable noninvasive alternative to liver biopsy ***(Obika and Noguchi, 2012).***

Cytokeratins **(CKs)** are proteins of keratin containing intermediate filaments found in the intra-cytoplasmic cytoskeleton of epithelial tissue**.** Evidence has now occurred that hepatocellular apoptosis plays a central role in chronic liver disease apoptotic cell death ***(Najimi et al., 2009)*****.** **CK-18** is the major intermediate filament protein in the liver and one of the most prominent substrates of caspases during hepatocyte apoptosis ***(Linder et al., 2004)*****.**

It is planned to evaluate serum **CK-18** level as a non-invasive bio-maker of **NAFLD** in patients with **T2DM.** For these purposes **80** subjects were selected**,** ***they were classified into 4 groups*****: *group 1* (includes 20 diabetics having NAFLD),** ***group 2*** **(includes 20 diabetics and not having NAFLD),** ***group 3*** **(includes 20 non diabetics and having NAFLD)** and ***group 4*** **(includes 20 age-matched healthy controls).**

All subjects enrolled in this study were subjected to history taking**,** clinical examination**, BMI** and **WC** assessment**, CBC, FPG, 2-hr-PPPG, TC, TG, HDL, LDL, AST, ALT, GGT,** serum bilirubin**,** albumin**, PT, INR,** serum creatinine**,** serum **CK-18** measurement and abdominal ultrasonography with ***Hamaguchi***scoring system**.**

- **The obtained results in the current study showed that:**

The mean serum **CK-18** levels in group **1 (diabetics NAFLD)** is **198.50 ± 50.76 U/L,** in group **2 (diabetics non-NAFLD)** is **83. 25 ± 25. 25 U/L,** in group **3 (non-diabetics NAFLD)** is **185. 50 ± 51. 71 U/L** and group **4 (healthy controls)** is **112.50 ± 17.66 U/L. *From these results*****,** it is noted that**, CK-18** levels is significantly increased in **NAFLD** patients either diabetic or non-diabetic compared to control group**. *Also*****,** there is significant difference between groups regarding serum **CK-18** levels with being highest in **NAFLD** groups with mean **198.5 ± 50.76 U/L** and lowest in control group with mean **83.25 ± 25.25 U/L (Table 15; P < 0.001).**

This result is in agreement with ***Zwolak et al., (2016)*** who reported that **CK-18** levels were highest in **NAFLD** group compared with control group as a marker of apoptosis of hepatocyte**.**

Similar results were found in a study done by ***Liang et al., (2015)*** who found that**,** the mean serum **CK-18** levels in **NAFLD** groups was **614.48 ± 471.43 U/L,** which was significantly higher **(P < 0.01)** compared with that of non-**NAFLD** groups **374.5 ± 231.4 U/L.** This indicates that apoptosis and inflammation in **NAFLD** were more evident than those without **NAFLD** and **CK-18** levels have high capability of detecting it**.**

Also ***Yang et al. (2015)*** showed that levels of **CK-18** were significantly higher in **NAFLD** group **(with median 180.3 U/L)** compared with controls **(with median 140 U/L). *Moreover*****;** this is positively correlated with pathologic characteristic of **NAFLD.** These findings showed that **CK-18** levels were correlated to **NAFLD** leading to the preliminary conclusion that **CK-18** could be non-invasive diagnostic marker of **NAFLD.**

In this context**, *Shen et al. (2012)*** reported that**,** the serum levels of **CK-18** were significantly higher **(P < 0.001)** in **NAFLD** group **(with median 354 U/L)** compared with control group **(with median 103 U/L)** with proposing as a good predictor of **NAFLD.**

In addition**, *Miyasato et al. (2014)*** found that**,** the serum concentration of the apoptosis marker **CK-18** were significantly elevated in the subjects with **NAFLD** compared with those observed in subjects without both diabetic and non-diabetic subjects**.**

In the general population**, CK-18** level has been established to be one of the most accurate parameters for diagnosing **NAFLD/NASH *(Shen et al., 2012)*****.** ***Likewise*****,** this study revealed that the **CK-18** concentration is a possible diagnostic biomarker for **NAFLD** among **T2DM** patients**.** The study showed that the diagnostic performance of **CK-18** with sensitivity **90%,** specificity **100%, PPV 100%** and **NPV 99.1%** in diagnosis of **NAFLD** with serum level **> 135 U/L. *However*****,** the range of results are different from other studies**,** this could be attributed to the small sample of the studied groups**.**

In the study done by ***Yang et al. (2015)*** the sensitivity was **80.3%** and specificity was **79.6%** with cut-off value of serum **CK-18** at **170.75 U/L. *In another way*****, *Cusi et al. (2014)*** reported that sensitivity **63%,** specificity was **83%, PPV 95%** and **NPV 31%** with cut-off value at **165 U/L.**

In addition**, *Miyasato et al. (2014)*** found that the best cut-off value of serum **CK-18** predicting **NAFLD** was **180.93 U/L** with sensitivity of **44%** and specificity **97%. *Also*****, *Aida et al. (2014)*** agreed with ***Tsutsui et al. (2010)*** results and proposed cut-off value of **CK-18** in diagnosis of **NAFLD** was **230 U/L,** with sensitivity **89%,** specificity **65 %, PPV 34% and NPV 97%.**

Similar findings obtained by another method done by ***Aida et al. (2014)*** reported that serum **CK-18** levels showed a positive correlation with histologic steatosis **(P = 0.271).** The area under the receiver operating characteristic curve of serum **CK-18** to predict the presence of **NAFLD** was **0.762.** The optimal cut-off point of serum **CK-18** for **NAFLD** was **230.** The sensitivity**,** specificity**, PPV** and **NPV** of serum **CK-18** for **NAFLD** were **0.89, 0.65, 0.34** and **0.97.** Accuracies of diagnosis for both **NAFLD** and definite **NASH** were **0.70.** They concluded that serum **CK-18**could be a clinically useful biomarker to discriminate between **NAFLD** and **NASH.**

In this regards, Miyasato et al. (2014) reported that serum **CK-18** values were significantly higher in **NAFLD** group than in non-**NAFLD** group among both diabetic and non-diabetic subjects**. CK-18**concentration was found to be an independent determinant of **NAFLD** and was positively correlated with ultrasonography score and **AST** and **ALT** concentrations in **T2DM** patients**.** Positive correlations were also identified between **CK-18** and transaminase concentrations in **T2DM** and **NAFLD** cohorts**. CK-18** was found to be significantly associated with **BMI** in **T2DM** patients with **NAFLD.** They concluded that a dose effect between the **CK-18** concentration and severity of **NAFLD** was found in **T2DM** patients**;** ***thus*****, CK-18** concentration is a potentially useful biomarker for assessing efficacy of treatment and improvement in **NAFLD** in patients with **T2DM.**

Finally**,** ***Shen et al. (2012)*** found that the specificity of **CK-18** level in diagnosing **NAFLD** was **90.4%,** sensitivity **84.4%, PPV 94.7%** and **NPV 74.4%** with cut-off value at **180 U/L.** This is significantly and independently associated with increase in **BMI** among **T2DM** patients with **NAFLD.** ***In addition,*** these results in agreement with those of ***Miyasato et al. (2014)*** who found significant association between **CK-18** and **BMI** in **T2DM** patient with **NAFLD.**

In the current study there is significant negative correlation between **CK-18** and **FPG** and **HbA1C,** this means that the greater the degree of **CK-18,** the lesser the degree of **FPG** and **HbA1C.** This finding is in agreement with the result of ***Liang et al. (2015) and Miyasato et al. (2014).***

In this study**,** there is insignificant difference as regards age and sex in the studied groups**.** This is in agreement with the result of ***Zwolac et al. (2016),*** who reported that**,** insignificant difference of age and sex in the studied groups**.** ***Also*****,** ***Liang et al. (2015), Yang et al. (2015), Aida et al. (2014), Cusi et al. (2014) and Shen et al. (2012)*** found the same results**.**

In current study**,** there is highly significant difference **(P < 0.001),** as regards **BMI** in the studied groups**,** being higher in **NAFLD** group **23.44 ± 0.79 Kg/m^2,** also there is significant difference **(P < 0.01),** as regards **WC,** being higher in **NAFLD** group **91.15 ± 3.88 cm** compared with control group **88.6 ± 2.39 cm.**

This means that**,** obesity is an important participant in the pathophysiology of **NAFLD** and progression to **NASH.** ***Moreover*****,** these results also in agreement with those obtained by ***Motamed et al. (2016),*** who found that **BMI** and **WC** were higher in **NAFLD** group compared to non **NAFLD** group**.** ***Also,*** these results are similar to results of ***Yang et al. (2015), Shen et al. (2012) and Joka et al. (2012)*** who reported that **BMI** and **WC** were higher in **NAFLD** group than controls**.**

In the current study**,** there is significant difference **(P < 0.001)** among groups as regards means of **TC, LDL** and **TG** with being higher in **NAFLD** groups **232.70 ± 48.83 mg/dl, 156.04 ± 48.38 mg/dl and 191.9 ± 63.28 mg/dl,** respectively**,** compared with that of controls **127.65 ± 28.45 mg/dl, 63.75 ± 17.92 mg/dl and 104.45 ± 10.72 mg/dl,** respectively **(Table 11).**

These findings are in agreement with those of ***Zwolak et al. (2016), Motamed et al. (2016), Liang et al. (2015) and Akila et al., (2014)*** who reported significant difference between **NAFLD** group and controls as regards **TC, TG** and **LDL.** ***On the other hand*****,** ***Yang et al. (2015)*** showed insignificant difference as regards **TC, LDL** and **TG** between **NAFLD** group and controls**.** ***Also*****,** ***Shen et al. (2012)*** found insignificant difference as regards **TC** between **NAFLD** and controls**,** but there was significant difference as regards **TG.**

In this study**,** there is significant difference **(P < 0.001)** among groups as regards means of **FPG** and **HBA1c** being higher in **NAFLD** groups **140.55 ±40.4 mg/dl and 8.18 ± 1.17%,** respectively**,** compared with those of controls **93.25 ± 5.87 mg/dl and 4.98 ± 0.23%,** respectively **(Table 11).**

These results are similar to the results reported by ***Zwolac et al. (2016), Liang et al. (2015), Akila et al. (2014) and Shen et al. (2012)*** who showed significant difference between **NAFLD** and controls as regards **FPG** and **HBA1c.**

In the current study**,** there is significant difference **(P < 0.001)** among groups as regards mean of **ALT,** being higher in **NAFLD** groups **34.65 ±25.25 IU/L,** compared with that of controls **19.6 ± 3.8 IU/L (Table 11).**

This in agreement with that of ***Zwolac et al. (2016), Yang et al. (2015), Shen et al. (2012) and Joka et al. (2012)*** who reported that there was significant difference between **NAFLD** and controls as regards serum **ALT.**

On the other hand*, *Liang et al. (2015) showed that there was insignificant difference between **NAFLD** and controls as regards **ALT.**

In this study*,** there is ***insignificant difference between the groups as regards **AST (Table 11).** This in agreement with that obtained by ***Liang et al. (2015)*** who reported that there was ***insignificant*** difference as regards **AST** between **NAFLD** and controls**.** ***However, Zwolak et al. (2016) and Yang et al. (2015*)** showed that there was significant difference between **NAFLD** and controls as regards **AST.**

***In the current study*,** there is significant difference **(P < 0.001)** among groups as regards the means of serum **GGT,** being higher in **NAFLD** group **43.75 ± 7.67 IU/L,** than that of controls **24.65 ± 4.55 IU/L (Table 11).**

GGT can be considered an independent predictor for **NAFLD,** since this enzyme increases in **NAFLD** to protect against the adverse effects of insulin resistance **(IR)** due to its antioxidant activity**.**

This finding is in agreement with that obtained by ***Motamed et al. (2016), Zwolak et al. (2016) and Yang et al. (2015)*** who reported that there was significant difference as regards **GGT** between **NAFLD** and controls**.**

In conclusion*, CK-18** has a promising predictive power in the diagnosis of **NAFLD.** It has been also suggested that **CK-18** could be a marker of hepatocyte caspase-directed death ***(Zwolak, 2016). Serum concentrations of **CK-18** fragments correlate with the severity of **NAFLD.**

Conclusion

- **CK-18** is increased in patient with **NAFLD** either with or without **DM.**
- Increase **CK-18** level is might be a possible diagnostic biomarker for **NAFLD** among **T2DM** patients**.**

References

1. Adams L, Waters O, Knuiman M et al., (2009): NAFLD as a risk factor for development of DM and the MS: an 11-year follow-up study. *Am J Gastroenterol; 104: 861 - 867.*
2. Aida Y, Abe H, Tomita Y et al., (2014): Serum CK-18 fragment level as a noninvasive biomarker for NAFLD. *International Journal of Clinical and experimental Medicine; 7(11): 4191 - 4198.*
3. Akila P, Sobba R and Muss T et al., (2014): Study of lipid profile. *International Scientific Research Puplication;22: 50 - 3153/75*
4. Angulo P, Keach J, Batts K and Lindor K et al., (1999): Independent predictors of liver fibrosis in patients with NASH. *Hepatology; 30: 1356 - 1362.*
5. Bacon B, Farahvash M and Janney C et al., (1994): NASH: an expanded clinical entity. *Gastroenterology; 107: 1103 - 1109.*
6. Bellentani S, Scaglioni F and Bedogni G et al., (2010): Epidemiology of NAFLD. *Dig Dis; 28:155 - 161.*
7. Bernsmeier et al., (2011): Nicht-alkoholische Fettleber und Steatohepatitis. Hepatische Manifestation en des metabolischen Syndroms. *Schweiz Med Forum; 11: 43 - 57.*
8. Calvert V, Collantes R and Mendoza M et al., (2007): A systems biology approach to pathogenesis of obesity-related NAFLD using reverse phase protein microarrays for multiplexed cell signaling analysis. *Hepatology; 46: 1315 - 1316.*
9. Chalasani N, Younossi Zand lavine J et al., (2010): The diagnosis and management of NAFLD. *Hepatology; 55: 2002 - 2023.*
10. Charlton M, Burns J and Dierkhising R et al., (2011): Frequency and outcomes of liver transplantation for NASH in the United States. *Gastroenterology; 141: 1249 - 1253.*
11. Chitturi S, Farrell G and Hashimoto E et al., (2007): Asia-Pacific Working Party on NAFLD. *J Gastroenterol Hepatol; 22 (6);778 - 787.*
12. Cusi K, Chang Z and Harrison S et al., (2014): Limited value of plasma CK-18 as a biomarker for NASH and fibrosis in patients with NAFLD. *J Hepatol; 60: 167-74.*
13. Farrell G and Larter C et al., (2006): NAFLD from steatosis to cirrhosis. *Hepatology; 43: S99 - S112.*
14. Fassio E, Alvarez E and Dominguez N et al., (2004): Natural history of NASH: a longitudinal study of repeat liver biopsies. *Hepatology; 40: 820 - 826.*
15. Haima P et al., (2014): Non-invasive detection of Liver Injury and Fatty Liver Disease. *TECO medical Clinical and Technical Review: Group, Switzerland: April: 1 - 16.*
16. Hossain N, Afendy A and Stepanova M et al., (2009): Independent predictors of fibrosis in patients with NAFLD. *Clin Gastroenterol Hepat; 7: 1224 - 1229.*
17. Joka D, Wahl K and Moeller S et al., (2012): Prospective biopsy-controlled evaluation of cell death biomarkers for prediction of liver fibrosis and nonalcoholic steatohepatitis. *Hepatology; 55:455 - 464.*
18. Kojima S, Watanabe N and Numata M et al., (2003): Increase in prevalence of fatty liver in Japan over past 12 years: analysis ofclinical background. *J Gastroenterol; 38: 954 - 961.*
19. Leite N, Salles G and Araujo A et al., (2009): Prevalenceandassociated factors of NALD in patients with T2DM. *Liver Int: 29: 113 - 119.*
20. Liang J, Han T and Gao Y et al., (2015): The expression of serum M30 and M65 in chronic hepatitis B patients with NAFLD. *Eur Rev Med Pharmacol Sci; 19(21):4123-419.*
21. Linder S, Wiesner C and Himmel M et al., (2004): Degrading devices: invadosomes inproteolytic cell

invasion. *Annu Rev Cell Dev Biol.; 2011; 27:185 - 211.*
22. Lonardo A, Bellentani S and Byrne C et al., (2015): Epidemiological modifiers of NAFLD: focus on high-risk groups. *Dig Liver Dis; 47: 997 - 1006.*
23. Machado M and Cortez P et al., (2013): Non-invasive diagnosis of NAFLD: A critical appraisal. *J Hepatology; 58: 1007 - 1019.*
24. Machado M, Ravasco P and Jesus L et al., (2008): Blood oxidative stress markers in NASH and how it correlates with diet. *Scandinavian J of Gastroenterol; 43, 1: 95 - 102.*
25. Maher M, Ibrahim W and Saleh S et al., (2015): CK-18 as a non-invasive marker in diagnosis of NASH and its usefulness in correlation with disease severity in Egyptian patients. *Egyptian J of Medical Human Genetics; 16: 41- 46.*
26. Marchesini G, Brizi M and Bianchi G et al., (2001): NAFLD: A feature of MS. *Diabetes; 50: 1844 - 1850.*
27. Matteoni C, Younossi Z and McCullough A et al., (1999): NAFLD: a spectrum of clinical and pathological severity. *Gastroenterology; 116:1413 - 1419.*
28. Miyasato M, Murase-Mishiba Y and Bessho M et al., (2014): CK-18 fragment level as a biomarker of NAFLD in patients with T2DM. *Clinica Chimica Acta; 433:184 - 189.*
29. Motamed N, Sohrabi M and Ajdarkosh H et al., (2016): Fatty liver index vs WC for predicting NAFLD. *WJG; (22)10: 3023 - 3030.*
30. Musso G, Gambino R and Tabibian J et al., (2014): Association of NAFLD with CKD. *PLoS Med; 11: e1001680.*
31. Obika O and Noguchi H et al., (2012): Diagnosis and Evaluation of NAFLD. *Experimental Diabetes Research; 2012, Article ID 145754, 12 pages doi:10.1155/2012/145754.*
32. Okanoue T, Umemura A and Yasui K et al., (2011): NAFLD and NASH in Japan. *J Gastroenterol Hepatol: 26 (Suppl 1): 153 - 156.*
33. Paradis V, Zalinski S and Chelbi E et al., (2009): HCC in patients with metabolic syndrome often develop without significant liverfibrosis: A pathologic analysis. *Hepatol; 49: 851 - 859.*
34. Pei R, Danbara N and Tsujita-Kyutoku M et al., (2004): Immunohistochemical profiles of Mallory body by a panel of anti-cytokeratinantibodies. *Med Electron Microsc; 37: 114 - 118.*
35. Sanyal A, Chalasani N and Kowdley K et al., (2010): Pioglitazone, vitamin E or placebo for NASH. *N Engl J Med; 362:1675 - 1685.*
36. Schweizer J, Bowden P and Coulombe P et al., (2006): New consensus nomenclature for mammalian keratins. *J Cell Biol; 174 (2): 169 - 174.*
37. Shen J, Ghan H and Wong G et al., (2012): Assessment of NAFLD using serum total cell death and apoptosis markers. *Alimentary pharmacology and therapeutics; (36):1057-1066.*
38. Sookoian S and Pirola C et al., (2016): NAFLD and metabolic syndrome: shared genetic basis of pathogenesis. *Hepatology; 64: 1417 - 1420.*
39. Targher G, Bertolini L and Rodella Set al., (2008): NAFLD increase prevalence of CKD and retinopathy in patient with T2DM. *Diabetologia; 51(3):444 - 450.*
40. Tetri M and Caldwell S et al., (2003): NASH: Summary of an AASLD Single Topic Conference. *Hepatology; 37, 1202 - 1219.*
41. Tsutsui M, Tanaka N and Kawakubo M et al., (2010): Serum CK-18 levels reflect the histological activity score of NAFLD more accurately than ALT levels. *J Clin Gastroenterol; 44: 440 - 447.*
42. Ueno T, Toi M and Linder S et al., (2005): Detection of epithelial cell death in the body by CK-18 measurement. *Biomed Pharmacother; 59 (Suppl.2): S 359 - 362.*
43. Vernon G, Baranova A and Younossi Z et al., (2011): Systematic review: Epidemiology and natural history of NAFLD and NASH in adults. *Aliment Pharmacol Ther; 34:274 - 285.*
44. Wieckowska A, Zein N and Yerian L et al., (2006): In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in NAFLD. *Hepatology; 44: 27 - 33.*
45. Yang M, Xu D and Liu Y et al., (2015): Combined Serum Biomarkers in Non-Invasive Diagnosis of NASH. *PLoS One; 10 (6): e0131664.*
46. Yang S, Lin H and Lane M et al., (1997): Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proceedings of the National Academy of Sciences of the USA; 94 (6); 2557 - 2562.*
47. Younossi Z, Stepanova M and Negro F et al., (2012): NAFLD in lean individuals in USA. *Medicine; 91: 319 - 327.*
48. Zwolak A, Szuster-ciesielska A and Daniluk J et al., (2016): Chemerin, retinol binding protein-4, CK-18 and transgelin-2 presence in sera of patients with NAFLD. *Annuals of hepatology; (15): 6: 862 - 869.*

3/4/2018