Assessment of serum YKL-40 as a diagnostic biomarker and its discriminating power between different liver diseases

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The aim of this study was to assess the performance of serum YKL-40 (Chitinase 3-like 1) as a diagnostic biomarker and its discriminating power between different liver diseases. And assessment of the diagnostic value of serum YKL-40 independently and in combination with some of the well-known routine markers by our new equation that we gave it the name (KLT score), and study if this combination leading to diagnostic power improvement and their AUCs elevation or not. Patients were classified into 4 groups: group 1 included 20 with (HCC), group 2 including 28 with (cirrhosis), group 3 included 20 patients with (HBV), group 4 included 20 patients with (HCV), 20 (control group). Our study revealed that to differentiate between cirrhosis and HCC, KLT score is the best for this discrimination, to discriminate between (HCV and HBV) & (HCV and Cirrhosis), YKL-40 had the best AUC followed by KLT score. We gave a power to routine ALT and Albumin to be useful for differentiation between Cirrhosis and HCC by our new equation (KLT score). Also we provided different cut off values for differentiation between HCV & HBV, Cirrhosis & HCC.

Keywords: YKL-40 (Chitinase 3-like 1), HCV, HBV, Cirrhosis & HCC

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm, the major cause of death in patients with cirrhosis, and the third most common cause of cancer-related death in the world (Kunter, I. et al., 2014). Patients with HCC are usually asymptomatic during the early stages of disease. Unfortunately, 80% of patients with HCC will be diagnosed with advanced stage disease (Cahill BA et al., 2004). Therefore, the most patients are presented with incurable disease at the time of detection which makes early diagnosis of HCC critical for a good prognosis. Surgical resection remains the treatment of choice for these tumors, but unfortunately only 10 -20% of primary HCCs are resectable at the time of diagnosis. Continuous researches are ongoing worldwide to find out and evaluate sensitive and specific new markers for HCC diagnosis (Herzer et al., 2014).

The most common cause of HCC is chronic hepatitis or liver cirrhosis caused by HCV and HBV infection. Therefore early detection of patients at risk, such as chronic carriers of HBV and HCV is justified to improve the outcome of
treatment of hepatocellular malignancy (Schutte et al., 2009)

Many surveillance programs that aimed at detecting early stage HCC have been widely recommended. These programs are based on the use of ultrasound and alpha-fetoprotein (AFP) (Wong et al., 2009) the reliability of imaging techniques has greatly improved in the last years but such diagnostic procedures are expensive and subject to misinterpretation. On the other hand, AFP is the only diagnostic serologic test currently used in surveillance programs either in asymptomatic cases or cirrhotic patients (Sterling t al., 2010). But its reliability remains unsatisfactory as its sensitivity (41% - 65 %). Therefore identification of a new biomarkers to establish the risk of cancer and/or detect its appearance at a preclinical stage is urgently needed (ZHU et al., 2013).

YKL-40: The abbreviation “YKL-40” is based on the one letter code for the first three NH2-terminal amino acids tyrosine (Y), lysine (K), and leucine (L). It is a plasma glycoprotein and a highly conserved member of the mammalian chitinase-like proteins and its molecular weight of 40 kDa and it contains a single polypeptide chain of 383 amino acids, also known as chitinase-3-like-1 (CHI3L1), human cartilage glycoprotein-39 (HC gp39), chondrex. Breast regression protein 39 (Brp-39) 38-kDa heparin-binding glycoprotein (Gp38k) and 40-kDa mammary gland protein (MGP-40). (Mygind, N.D. & Kastrup, J., 2015). YKL-40 has a role in cell proliferation and differentiation, inflammation, and protection against apoptosis (Recklies et al., 2002). It considered to be a growth factor for connective tissue cells such as fibroblast, chondrocytes, and synovial cells. Moreover, it initiates a signaling cascade in connective tissue cells that leads to increased cell proliferation. This suggests that ykl-40 plays a very important role in the pathological conditions leading to tissue fibrosis.

Our concern was to study to assess the performance of serum YKL-40 (Chitinase 3-like 1) as a diagnostic Biomarker and its discriminating power between different liver diseases. And assessment of the diagnostic value of serum YKL-40 (direct marker) independently and in combination with some of the well-known (non-invasive) indirect markers by our new combination score equation that we gave it the name (KLT score), and study if this combination leading to diagnostic power improvement and their AUCs elevation or not.

MATERIALS AND METHODS

SUBJECTS
This study was conducted at El Kasr El Ainy Hospital. It included 88 patients (54 males and 34 females) and their ages ranged from (40 to 60 years). These patients were classified in to 4 groups:

Group 1 (patients with HCC; n=20)
This group included 20 patients with hepatocellular carcinoma (HCC), 16 patients were diagnosed as hepatitis c virus (HCV ; n=16) and 4 patients were diagnosed as hepatitis B virus (HBV ; n=4). They were 12 males (60%) and 8 females (40%) .

Group 2 (patients with Liver cirrhosis; n=28)
This group included 28 patients with liver cirrhosis. They were 13 males (46.43%) and 15 females (53.57%). All patients were diagnosed as hepatitis C virus infection and diagnosed by presence of HCV Abs and confirmed by PCR.

Group 3 (patients with HBV; n=20)
This group included 20 patients with HBV. They were 16 males (80%) and 4 females (20%). They were diagnosed by presence of HBs Ag.

Group 4 (patients with HCV; n=20)
This group included 20 patients with HCV. They were 13 males (65%) and 7 females (35%).

+Control group (n=20)
This group included 20 sex and age matched healthy individuals they were 16 males and 4 females, whose ages ranged from 40 to 60 years.

All subjects included in this study were subjected to the following investigations:

Full history taking focusing on previous hepatic disorders or predisposing factors preceding liver disease
Through clinical examination including abdominal examination.
Laboratory investigations including prothrombin time, renal function (urea and creatinine), liver functions (ALT, AST, Albumin and ALP), hepatitis markers (HBs Ag and HCV Abs), AFP

Patients were further subjected to radiological investigations including CT scan and abdominal ultrasound.

SAMPLING
10 ml of venous blood were withdrawn under complete aseptic conditions from all subjects. The obtained sample were taken in plain tube then left for 30 min to clot and centrifuged for 10
min. the serum was then divided into 2 aliquots. The first was stored at -20°C until the assay of YKL-40, the second was used for estimation of renal function, liver function and hepatitis markers.

METHODS:

Quantitative Determination of serum aminotransferases (AST) & (ALT):

The analysis was done on synchron CX9® auto analyzer which measures ALT and AST activity by coupling the transaminase reactions to specific dehydrogenase reaction applying a kinetic rate method. The system monitors the change in absorbance at 340 nm. The change in absorbance is directly proportional to the activity of ALT & AST in the sample (Schumann et al., 2002).

Serum Albumin:

The analysis was done on synchron CX-9 auto analyzer using bromocresol green reaction (BCP) applying a timed endpoint method. The system monitors the change in absorbance of 600 nm. The change in absorbance is directly proportional to the concentration of albumin in the sample (Doumas and Peters, 1997).

Alkaline phosphatase:

The analysis was done on synchron CX-9 auto analyzer applying international federation of clinical chemistry and laboratory medicine (IFCC) modified method. In this method, the action of ALP and magnesium ions, nitrophenyl phosphate is catalyzed to p-nitrophenol, where the increase in absorbance is directly proportional to the activity of ALP (Thomas, 1998).

Urea:

The analysis was done on synchron CX-9 auto-analyzer applying urease-glutamate dehydrogenase, UV method. In this method, urea is hydrolyzed by urease and one of the products, ammonia, helps to turn NADH to NAD⁺ with the catalysis of glutamate dehydrogenase (GLDH). The decrease of absorbance is directly proportional to the concentration of urea (Thomas, 1998).

Creatinine:

The analysis was done on synchron CX-9 auto analyzer applying modified Jaffé method. In this method, creatinine combines with acid at an alkaline solution to form an orange-red colored complex. The absorbance increase is directly proportional to the concentration of creatinine (Thomas, 1998).

Detecting of HCV-Ab and HBsAg:

Hepatitis B virus antigen (HBsAg) was detected by using Murex anti-HBV surface antigen, an enzyme immunoassay for detection of HBs antigen (ELISA). Hepatitis C virus antibodies (HCV-Ab) were detected using Murex anti HCV antibodies, ELISA 4th generation kit. Ortho-HCV 3.0 ELISA system with enhanced sensitivity was a qualitative assay for detection of antibody of hepatitis C virus in human serum or plasma.

Tumor marker or Hepatocellular Carcinoma marker (AFP):

The analysis of serum AFP was done by a kit supplied by Siemens®. It is a solid-phase, two site sequential chemiluminescent immuno-metric assay. The solid phase was a polystyrene bead enclosed within the test unit, coated with monoclonal antibody specific for AFP (Empfehlungen der Deutschen Ärzte, 1972).

YKL-40 (Human Chitinase-3-like 1)

The quantitative sandwich enzyme immunoassay technique (Quantikine colorimetric sandwich ELISA) was used. A monoclonal antibody specific for CHI3L1 has been pre-coated onto a micro plate. Standards and samples were pipetted into the wells and any CHI3L1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for CHI3L1 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of CHI3L1 bound in the initial step. The color development was stopped and the intensity of the color is measured.

Statistical analysis:

Statistical analysis used in this study was non-parametric test (Kruskal-Wallis), ANOVA that was used to test for significance of difference between the studied groups, post hoc test LSD (least significant difference) was applied for the multiple pairwise comparisons, Receiver operating characteristics (ROC) analysis was used to assess the performance of serum (YKL-40) as diagnostic test for different stage of liver diseases. Additionally, it was used to specify the optimal
cutoff values for discrimination between different studied groups and test characteristics at these cutoffs. And Delong method was used for comparing AUCs obtained from ROC analysis of independent serum YKL-40 and also the combined test (KLT score) to evaluate their discriminate ability between different studied groups. Difference between AUCs, SE of difference, test statics (z-value) and associated P-value for each pairwise comparison.

ALT activity in control, HCV, HBV, cirrhosis and HCC groups ranged from 12 to 48, 35 to 196, 54 to 98, 14 to 140 and 20 to 150 respectively, with mean ± SD (30.20 ± 8.79, 81.35 ± 45.87, 83.10 ± 11.76, 52.57 ± 31.24, and 93.10 ± 24.77 respectively) and a median value of 29, 63.5, 87, 41 and 96 respectively.

AST activity in control, HCV, HBV, cirrhosis and HCC groups ranged from 13 to 46, 40 to 222, 40 to 106, 22 to 140 and 50 to 98 respectively, with a mean ± SD (24.85 ± 8.17, 102.15 ± 59.65, 77.30 ± 22.86, 58.46 ± 19.03 and 73.05 ± 15.54 respectively). Albumin concentration in control, HCV, HBV, cirrhosis and HCC groups ranged from 3 to 8.40, 2.20 to 4.20, 2.10 to 4.70, 1.60 to 3.30 and 1.80 to 3.20 respectively, with a mean ± SD (4.03 ± 1.11, 3.19 ± 0.58, 3.06 ± 0.70, 2.49 ± 0.45 and 2.35 ± 0.38 respectively). YKL-40 level in control, HCV, HBV, cirrhosis and HCC groups ranged from 519 to 1050, 601 to 6400, 401 to 1200, 3683 to 6780 and 2837 to 6400 respectively, with a mean ± SD (771.20 ± 156.14, 2742.2 ± 1916.24, 733.35 ± 245.67, 5015.9 ± 866.52 and 5018.8 ± 1048.12 respectively), and median of 726, 2024, 745.50, 4735.50 and 5542.6 respectively (Table 1).

**RESULTS**

<table>
<thead>
<tr>
<th>Group</th>
<th>YKL-40 vs. KLT Score</th>
<th>Difference between AUCs</th>
<th>Z-Static</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs. HCV</td>
<td>0.056</td>
<td>1.05</td>
<td>0.293</td>
<td></td>
</tr>
<tr>
<td>Control vs. HBV</td>
<td>0.395</td>
<td>4.183</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Control vs. Cirrhosis</td>
<td>0.00</td>
<td>-</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Control vs. HCC</td>
<td>0.00</td>
<td>-</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HCV vs. HBV</td>
<td>0.302</td>
<td>3.940</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>HCV vs. Cirrhosis</td>
<td>0.090</td>
<td>0.879</td>
<td>0.379</td>
<td></td>
</tr>
<tr>
<td>HCV vs. HCC</td>
<td>0.084</td>
<td>1.123</td>
<td>0.261</td>
<td></td>
</tr>
<tr>
<td>HBV vs. Cirrhosis</td>
<td>0.179</td>
<td>2.940</td>
<td>0.0033</td>
<td></td>
</tr>
<tr>
<td>HBV vs. HCC</td>
<td>0.015</td>
<td>1.138</td>
<td>0.255</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis vs. HCC</td>
<td>0.301</td>
<td>3.169</td>
<td>0.0015</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1: Biochemical characteristics of study patients and control subjects. (Mean ± SD)**

<table>
<thead>
<tr>
<th>Group</th>
<th>G1 HCC N=20</th>
<th>G2 Cirrhosis N=28</th>
<th>G3 HBV N=20</th>
<th>G4 HCV N=20</th>
<th>G5 Control N=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>93.10 ± 24.77</td>
<td>52.57 ± 31.24</td>
<td>83.10 ± 11.76</td>
<td>81.53 ± 45.87</td>
<td>30.20 ± 8.79</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>73.05 ± 0.28</td>
<td>58.46 ± 19.03</td>
<td>77.30 ± 22.86</td>
<td>102.15 ± 59.65</td>
<td>24.85 ± 8.17</td>
</tr>
<tr>
<td>Albumin (U/L)</td>
<td>2.35 ± 0.38</td>
<td>2.49 ± 0.45</td>
<td>3.06 ± 0.70</td>
<td>3.19 ± 0.58</td>
<td>4.03 ± 1.11</td>
</tr>
<tr>
<td>YKL-40 (U/L)</td>
<td>5018.8 ± 1048.2</td>
<td>5015.9 ± 866.52</td>
<td>733.35 ± 245.67</td>
<td>2742.2 ± 1916.24</td>
<td>771.20 ± 156.14</td>
</tr>
</tbody>
</table>
In control vs. HCV group: KLT score had the best AUC followed by YKL-40 (AUCs= 0.961; 0.905 respectively). The pairwise comparisons of these AUCs unveiled no statistically significant difference in (YKL-40 vs. KLT Score) (P= 0.293), (table 2).
In control vs. HBV group: KLT score had the best AUC followed by YKL-40 (AUCs= 0.967; 0.572 respectively). The pairwise comparisons of these AUCs unveiled statistically significant difference in (YKL-40 vs. KLT Score) (P< 0.0001).
In control vs. Cirrhosis or HCC group: KLT and YKL-40 score had the best AUC (AUCs= 1 separately). The pairwise comparisons of these AUCs unveiled no statistically significant difference in (YKL-40 vs. KLT Score) (P= 1), (table 2).
In HCV vs. HBV group: YKL-40 had the best AUC followed by KLT score (AUCs= 0.915; 0.617 respectively). The pairwise comparisons of these AUCs unveiled statistically significant difference in (YKL-40 vs. KLT Score) (P= 0.0001), (table 2).
In HCV vs. Cirrhosis group: YKL-40 score had the best AUC followed by KLT (AUCs= 0.807; 0.717 respectively). The pairwise comparisons of these AUCs unveiled no statistically significant difference in (YKL-40 vs. KLT Score) (P= 0.379), (table 2).
In HCV vs. HCC group: KLT score had the best AUC followed by YKL-40 (AUCs= 0.899; 0.815 respectively). The pairwise comparisons of these AUCs unveiled no statistically significant difference in (YKL-40 vs. KLT Score) (P= 0.261), (table 2).
In HBV vs. Cirrhosis group: YKL-40 had the best AUC followed by KLT score (AUCs= 1; 0.821 respectively). The pairwise comparisons of these AUCs unveiled statistically significant difference in (YKL-40 vs. KLT Score) (P < 0.0003), (table 2).
In HBV vs. HCC group: YKL-40 had the best AUC followed by KLT score (AUCs= 1; 0.985 respectively). The pairwise comparisons of these AUCs unveiled no statistically significant difference in (YKL-40 vs. KLT Score) (P= 0.255), (table 2).
In Cirrhosis vs. HCC group: KLT score had the best AUC followed by YKL-40 (AUCs= 0.838; 0.537 respectively). The pairwise comparisons of these AUCs unveiled statistically significant difference in (YKL-40 vs. KLT Score) (P< 0.0015), (table 2).

DISCUSSION

In this study we started to evaluate the performance of serum (YKL-40) as a diagnostic test for different liver diseases. Also we provided the optimal cutoff values for discrimination between different studied groups. Then we decided to develop simple score composed of routine biomarkers and YKL-40. In our study A Non-parametric test (Kruskal-Wallis) was used to test for significant differences between the studied groups and it showed statistically significant differences. Furthermore, Mann-Whitney test was used for pairwise comparison. The multiple pairwise comparisons unveiled significantly increased in the median of YKL-40 in HCV, cirrhosis and HCC groups as compared with control group. Also, the median of YKL-40 was elevated significantly in HCV, cirrhosis and HCC group as compared with HBV group, further, the significance increase in the median of YKL-40 in each of cirrhosis and HCC groups as compared with HCV group, while there was no significant differences in the median of YKL-40 between control with HBV and cirrhosis with HCC groups.

In our study, the significant elevation in the median of ykl-40 in (HCV) as compared with control group (P < 0.0001) could be explained as in HCV infection, HCV and its proteins have been shown to activate hepatic macrophages (Kupffer cells) (Ju, C. & Tacke, F., 2016) and it is reported by krussell 2009 that activated macrophages secrete YKL-40, therefore HCV infection associated with significant elevation in ykl-40 level. Macrophages are known to increase in parallel with the progression of liver fibrosis and it considered to be as an activator of hepatic stellate cells (HSCs), it have been reported to play essential roles in liver fibrogenesis (Kumagai, E. et al., 2016). Therefore, there is positive correlation between YKL-40 and liver fibrosis.

In sharp contrast to HCV in our study we found that there is no significant difference in the median of YKL-40 between HBV and control, we can explain that as the following, in the case of HBV infection the viral replication takes place inside the infected hepatocytes within capsids (Boonstra, et al., 2008). Therefore, the viral genome and replication intermediates hidden from pattern recognition receptors (PRRs) that result in prevention of the initial HBV infection from being detected by the innate immune system and that in contrast to HCV as the HCV life cycle is cytoplasmic in replication complexes.
In our study the significant increase in the median of YKL-40 in (HCC) as compared with control group, that could be explained as in case of HCC activation of the PI3K/AKT pathway is found. YKL-40 is considered to be a promoter of angiogenesis, including activating the mitogen activated protein kinase/extracellular signal regulated kinase (MAPK/ERK) pathway in endothelial cells. Activated MAPK and AKT may be negatively regulated by EGFR Both pathways are required for the cells to complete mitosis. As (Chrissouli Set al., 2010) showed that the activation of these pathways stimulates the growth of connective tissue cells. YKL-40 mediates a mitogenic effect through initiation of MAPK and PI3 K signaling pathways by phosphorylation of the ERK1/2 and protein kinase B (AKT) respectively. In addition, we can give an expectation that YKL-40 may play an important role in the initiation of allergic inflammation and it considered to be an acute phase biomarker in fibrosis diseases. Also another expectation is that: as ykl-40 indirectly increase synthesis of hepatic cancer by the activation of the PI3K/AKT, it is considered to be an attractive target in the design of anti-fibrosis therapy.

Also, YKL-40 is elevated in HCC metastasis as (CLU - YKL-40) pathway may play an important role in HCC metastasis, Clusterin (CLU) greatly upregulates expression of YKL-40 gene in primary (HCC H2P) cells. Overexpression of CLU was significantly higher in metastatic HCC than that in primary HCC cases therefore, dramatic overexpression of YKL-40 in metastatic HCCs detected. Also Lau, S.H. et al., 2005 proved the positive correlation between CLU and YKL-40

Our results showed significant increase in the median of YKL-40 in Cirrhosis. In case of cirrhosis there is high risk to develop bacterial infections that significantly increase the mortality rate. YKL-40 is secreted by locally activated macrophages and neutrophils and is released by exocytosis from specific granules at the site of inflammation, when needed for bactericidal activity and due to this local production YKL-40 levels show a more rapid peak and a more rapid decline after initiation of antibiotic treatment, which is opposite to CRP levels that decline slowly (Ostergaard C et al., 2002). Opposite YKL-40, CRP is primarily a systemic inflammation marker secreted by hepatocytes in response to pro inflammatory mediators such as IL-6. Therefore, YKL-40 is specific serologic marker of granulocyte function and macrophage activation at the site of tissue inflammation as a supplement to conventional acute phase proteins.

In our study Receiver operating characteristics (ROC) analysis was used to assess the performance of serum (YKL -40) as diagnostic test for different liver diseases. Additionally, it was used to specify the optimal cutoff values for discrimination between different studied groups and test characteristics at these cutoffs. For discrimination between control and HCV group, ROC analysis estimated a sensitivity, specificity, PPV and NPV (85, 90, 89.5 and 85.7 respectively) at the best cutoff value of > 954 which was determined by calculating Youden Index. The area under the curve (AUC) was 0.905 with significant associated P-value < 0.0001. ROC analysis revealed discrimination between control and HBV group with sensitivity, specificity, PPV and NPV (40, 90, 80 and 60 respectively) at the best cutoff value of ≤ 627. The area under the curve (AUC) was 0.572 with insignificant associated P-value = 0.446

For discrimination between (control and cirrhosis group) or (control and HCC), ROC analysis estimated a sensitivity, specificity, PPV and NPV (100 separately) at the best cutoff value of >1050 which was determined by calculating Youden Index. The area under the curve (AUC) was 1 with significant associated P-value < 0.0001 revealed discrimination between HBV and HCV group with sensitivity, specificity, PPV and NPV (90, 85, 85.7 and 89.5 respectively) at the best cutoff value of > 906. The area under the curve (AUC) was 0.915 with significant associated P-value = 0.0001. ROC analysis revealed discrimination between HCV and cirrhosis group, a sensitivity, specificity, PPV and NPV (100, 70, 82.4 and 100 respectively) at the best cutoff value of > 2837 which was determined by calculating Youden Index. The area under the curve (AUC) was 0.808 with significant associated P-value < 0.0001.

For discrimination between HCV and HCC group with sensitivity, specificity, PPV and NPV (100, 65, 74.1 and 100 respectively) at the best cutoff value of > 2323. The area under the curve (AUC) was 0.815 with significant associated P-value = 446 and For discrimination between (HBV and cirrhosis group) or (HBV and HCC group), ROC analysis estimated a sensitivity, specificity, PPV and NPV (100 separately) at the best cutoff value of >1200 which was determined by calculating Youden Index. The area under the curve (AUC) was 1 with significant associated P-value < 0.0001.

For discrimination between HBV and HCC group, ROC analysis estimated a sensitivity, specificity,
PPV and NPV (100 separately) at the best cutoff value of >1200 which was determined by calculating Youden Index. The area under the curve (AUC) was 1 with significant associated P-value < 0.0001. ROC analysis revealed discrimination between cirrhosis and HCC group with sensitivity, specificity, PPV and NPV (65, 57.14, 52 and 69.6 respectively) at the best cutoff value of > 4763. The area under the curve (AUC) was 0.537 with insignificant associated P-value = 0.678 Since the independent studied biomarkers failed to discriminate between some studied groups, so we decided to develop simple score composed of routine biomarkers and YKL-40.

Regarding the liver biochemical profile In our study we found significantly increased in the mean of AST in HCV, HBV, cirrhosis and HCC groups as compared with control group (P < 0.05). Also, the mean of AST activity in HCV group was significant elevated as compared with HBV, cirrhosis or HCC groups (P < 0.05). There was significant increase in the mean of AST in HBV group as compared with cirrhosis group (P = 0.035), while there were insignificance differences between HBV with HCC, and cirrhosis with HCC groups (P = 0.656 and 0.101 respectively), significant increase in the median of ALT in HCV, HBV, cirrhosis and HCC groups as compared with control group (P < 0.05). Also, the median of ALT was elevated significantly in HCC group as compared with HBV, HCV or cirrhosis groups (P <0.05), while there was significant decreased in the median of ALT in cirrhosis group as compared with HCV or HBV groups (P < 0.05). There was insignificant difference in the median of ALT between HCV and HBV groups (P = 0.214), and we found significant decrease in the mean of albumin in HCV, HBV, cirrhosis and HCC groups as compared with control group (P < 0.0001 separately). The mean level of albumin was significantly decreased in cirrhosis and HCC groups as compared with HCV or HBV group (P < 0.05), while there were no significant difference between HCV with HBV and cirrhosis with HCC groups.

Multivariate regression analysis to identify predictors for different studied groups

In this model, the groups were set as dependent variable while, YKL-40, ALT, AST and albumin as independent variables (predictors). The model explaining 64.8 % of the variability in studied groups, this model shows that YKL-40, ALT and albumin are significant predictors with regression coefficient (β = 0.0001; 0.008; -0.519: P < 0.0001; 0.002; 0.0001 respectively), but AST was excluded from the model p = 0.107.

The combination of YKL-40, ALT and Albumin (KLT) score = 3.123 + 0.0001*YKL-40 + 0.008 * ALT - 0.519 * Albumin

Then we started to study the characteristics of KLT score in the studied groups also we started to compare between independent serum ykl-40 and also the combined test (KLT score) to evaluate their discrimination ability.

Our results revealed that KLT score had the best AUC followed by YKL-40 in (control vs .HBV) and (Cirrhosis vs HCC), with difference between AUCs (0.395 and 0.301) respectively, the pairwise comparisons of these AUCs unveiled statistically significant differences in (YKL-40 vs KLT score (P< 0.0001), (P< 0.0015) respectively.

Our results revealed that YKL-40 had the best AUC followed by KLT score in (HCV vs HBV) and (HBV vs Cirrhosis) with difference between AUCs (0.302 and 0.179) respectively, the pairwise comparisons of these AUCs unveiled statistically significant differences in (YKL-40 vs KLT score (P< 0.0001), (P< 0.0033) respectively.

CONCLUSION

Our study revealed that to differentiate between cirrhosis and HCC, our new established (KLT score) is the best for this discrimination as we found that KLT score had the best AUC in comparison to dependent serum YKL-40. using ykl-40 showed sensitivity , specificity ,PPV and NPV (65 , 57.14 , 52 and 69.6 ) while using KLT score showed improved sensitivity , specificity, PPV and NPV (80.75 , 69.6 and 84). Also, in the detection of HBV, KLT is better for detection as KLT score had the best AUC in comparison to YKL-40 alone. using ykl-40 showed sensitivity , specificity ,PPV and NPV (40 , 90 , 80 and 60 ) while using KLT score showed improved sensitivity , specificity ,PPV and NPV (95, 90 , 90.5 and 94.7).

To discriminate between (HCV and HBV) & (HBV and Cirrhosis), YKL-40 had the best AUC followed by KLT score.

CONFLICT OF INTEREST

No conflict of interest

AUTHOR CONTRIBUTIONS

Prof Abdelgawad. A.F and Dr .Mohamed A.E designed the study and supervised the work. Karima M.N performed the experiment and wrote the manuscript .All authors read and approved the
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Ali Fahmi et al., Assessment of serum YKL-40 as a diagnostic biomarker


