

Human Aldehyde Dehydrogenase (ALDH) in Cirrhotic and Hepatocellular Carcinoma Patients Related to Hepatitis C Virus

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Aldehyde dehydrogenase (ALDH) is a polymorphic enzyme responsible for the oxidation of aldehydes to carboxylic acids, which leave the liver and are metabolized by the body's muscle and heart. ALDH1 and ALDH2 are the most important enzymes for aldehyde oxidation. These enzymes are found in many tissues of the body but are at the highest concentration in the liver.

AIM: The aim of this study is to evaluate the diagnostic value of serum Aldehyde dehydrogenase level in hepatitis C virus infected Egyptian patients with cirrhosis and hepatocellular carcinoma.

METHODS: This study included 50 patients with cirrhosis, 50 patients with hepatocellular carcinoma and 37 healthy volunteers. For all groups we studied clinical data, liver function tests, viral markers, serum alpha fetoprotein (AFP) and ALDH concentration using enzyme linked immunosorbent assay (ELISA).

RESULTS: Our data showed that ALDH was more sensitive and specific than AFP, ALDH had 74% sensitivity and 82% specificity, P-value (0.000) but AFP had 66% sensitivity and 64% specificity, P-value (0.003).

CONCLUSION: ALDH could be used as useful diagnostic marker for detection of hepatocellular carcinoma and in differentiation between HCC and cirrhosis at a cut off value of 41 U/L.

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Key words: Hepatitis C virus; Hepatocellular carcinoma; Aldehyde dehydrogenase enzyme; Alphafetoprotein

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ABSTRACT

Hepatocellular carcinoma is a primary malignancy of the liver. Hepatocellular carcinoma is now the third leading cause of cancer deaths worldwide, with over 500,000 people affected. In Egypt, hepatocellular carcinoma (HCC) is the second most common cancer in men and the 6th most common cancers in women. This rising incidence and its' poor prognosis make the early detection and surveillance of hepatocellular carcinoma of high importance.

INTRODUCTION

Hepatitis C virus (HCV) infection is a global health problem with an estimated 130-200 million chronic carriers in the world^[1]. It was found that HCV accounts for about 15 % of cases with acute hepatitis^[2], 80 % with chronic hepatitis^[3], 27% with cirrhosis, 25% with hepatocellular carcinoma^[4] and 15-30 % of liver transplantation. Egypt has the highest hepatitis C virus (HCV) prevalence in the world (14.7%). The drivers of the HCV epidemic in Egypt are not well understood, but the mass parenteral antischistosomal therapy (PAT) campaigns in the second half of the 20th century are believed to be the determinant of the high prevalence. Cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver injury, that leads to portal hypertension and end stage liver disease^[5]. The exact prevalence of cirrhosis worldwide is unknown. Cirrhosis prevalence was estimated at 0.15% or 400,000 in the USA^[6], where it accounted for more than 25,000 deaths and 373,000 hospital discharges in 1998^[7]. This may be an underestimation as it is recognized that the high prevalence of undiagnosed cirrhosis in both non alcoholic steatohepatitis (NASH) and hepatitis C. Similar numbers have been reported from Europe, and numbers are even higher in most Asian and African countries where chronic viral hepatitis B or C are frequent. Since compensated cirrhosis often goes undetected for prolonged periods of time, a reasonable estimate is that up to 1% of populations may have histological cirrhosis. Hepatocellular carcinoma (HCC) is a primary malignancy of the liver. Hepatocellular carcinoma is now the third leading cause of cancer deaths worldwide, with over 500,000 people affected. The incidence of hepatocellular carcinoma is highest in Asia and Africa, where the endemic high prevalence of hepatitis B and hepatitis C strongly predisposes to the development of chronic liver disease and subsequent development of hepatocellular carcinoma. liver cancer is the sixth most common neoplasm worldwide, its very poor prognosis makes it the third leading cause of cancer-related mortality, responsible for 600,000 deaths annually^[8]. In the most recently published GLOBOCAN global analysis, it was estimated that, in 2002, 82% of liver cancer cases occurred in developing countries, with 55% in China alone^[8]. In Egypt, hepatocellular carcinoma (HCC) is the second most common cancer in men and the 6th most common cancers in women^[9]. Hospital-based studies from Egypt have reported an overall increase in the relative frequency of all liver-related cancers in Egypt, from approximately 4% in 1993 to 7.3% in 2003^[10]. This rising incidence^[11] may be due to high prevalence of hepatitis C virus (HCV) and its complications^[12] and the fact that people born 20 years ago or earlier in Egypt have not been vaccinated against hepatitis B virus (HBV)^[13]. HCC, as it is a highly malignant tumor with a very poor prognosis so early detection and treatment are required^[14]. Alpha fetoprotein (AFP) is the first serologic assay for detection and clinical follow up of patients with hepatocellular carcinoma which has been the standard tumor biomarker for HCC for many years. The major limitation of AFP testing is that this biomarker is not specific to cancer, AFP levels can also be elevated in people who do not have cancer^[15]. Ultrasonography used in combination with AFP in diagnosis and surveillance of HCC. The problem with ultrasonography is the lack of reproducibility. Ultrasound is operator-dependent, so its reproducibility is poor and has not been studied, which is a major limitation for a surveillance test. In contrast, Aldehyde dehydrogenase is a polymorphic enzyme responsible for the oxidation of aldehydes to carboxylic acids, which leave the liver and are metabolized by the body's muscle and heart. There are three different classes of these enzymes in mammals: class

1 (low Km, cytosolic), class 2 (low Km, mitochondrial), and class 3 (high Km, such as those expressed in tumors, stomach, and cornea). ALDH1 and ALDH2 are the most important enzymes for aldehyde oxidation, and both are tetrameric enzymes composed of 54kDA subunits. These enzymes are found in many tissues of the body but are at the highest concentration in the liver^[16]. The aim of this study is to evaluate the diagnostic and prognostic value of ALDH levels in patients with hepatocellular carcinoma.

MATERIALS AND METHODS

Patients

This study was conducted on 137 cases from National Hepatology and Tropical Medicine Research Institute. Subjects were divided into three groups.

Group 1: 37 healthy volunteers as control. **Group 2:** 50 cirrhotic patients infected with chronic HCV genotype -4 diagnosed on the basis of history, clinical examination, laboratory findings and ultrasonography (US) assessment. **Group 3:** 50 hepatocellular carcinoma patients proven to be infected with chronic HCV genotype -4 by HCV PCR, diagnosed by Ultrasonography (US) assessment, abdominal triphasic Computed Tomography (CT) and serum Alpha-Fetoprotein. This study was approved by the Ethics and Research Committee of Faculty of Pharmacy, Cairo University and National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt.

All Patients were selected to participate in the study gave a written informed consent. All patients included in the study had the procedure thoroughly explained to them. The clinical/pathological data of the patients was recorded, including age, sex, viral infection (Hepatitis C Virus HCV and Hepatitis B Virus HBV), alcohol intakes, biochemical liver function test results (Serum alanine aminotransferase (ALT), Serum aspartate aminotransferase (AST), Total bilirubin, Direct bilirubin, fasting blood glucose, serum creatinine, viral markers including HBs Ag and HCV Ab using ELISA technique, quantitation of HCV-RNA using Real Time PCR for HCV Ab positive cases to detect load of viremia, serum concentration of alpha fetoprotein (AFP) and human aldehyde dehydrogenase total activity using enzyme linked immunosorbent assay (ELISA) technique. The inclusion Criteria of control group were: Adult male or female (30-70 years old), negative serum Hepatitis C virus Antibody by ELISA, negative serum Hepatitis B surface antigen by ELISA and Normal of serum aminotransferases (AST and ALT) levels while the inclusion Criteria of cirrhosis and HCC groups were: Adult male or female (30-70 years old) in or outpatients, positive serum Hepatitis C virus Antibody by ELISA and negative serum Hepatitis B surface antigen by ELISA.

Patients of cirrhosis group approved to have cirrhosis using Ultrasonography, Triphasic computed tomography and Fibrosis score. Patients of HCC group approved to have HCC using Ultrasonography, Triphasic Computed Tomography and AFP while the exclusion criteria of cirrhosis and HCC group were: Positive HBs Ag, Cirrhosis or HCC caused by other than infection of HCV.

Blood sampling and biochemical assays

Fasting venous blood samples (5 ml) were collected by trained laboratory technicians. A portion of blood was allowed to clot and then centrifuged at 3500g for 5 min to separate the serum used for assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, direct bilirubin, liver function tests done using Bechaman CX4. Alpha fetoprotein (AFP) using Biocheck AFP enzyme immunoassay, viral markers using Diasorin kits and finally total aldehyde dehydrogenase (ALDH) concentration using (EIAAB) kits.

Statistical Analysis

Data was coded and entered using the statistical package SPSS (version 15).Data was summarized using number and percent for qualitative variables. Mean and standard deviation for normally distributed quantitative variables. Median and interquartile range (IQR) for quantitative variables which are not normally distributed. Comparisons between groups was done using Chi Square test for qualitative variables. Independent sample *t*-test and Analysis of variance (ANOVA) with Post HOC test for normally distributed quantitative variables while non parametrically Kruskal-Wallis Test and Mann-Whitney Test were used for quantitative variables which are not normally distributed. Correlations were done to test for linear relations between variables. Recessive operating characteristic curve (ROC) were constructed to test the validity of AFP and ADH in discrimination between cirrhosis and HCC groups.

RESULTS

The demographic features and characteristics of the three patients' groups are summarized in table 1. A total of 137 adults, who are comprised of 50 patients with HCC, 50 patients with liver cirrhosis and 37 apparently-normal control subjects were studied. The mean age of control subjects was 42.14±15.93 years with a range between 19 and 77 years. In liver cirrhosis patients the mean age was 62.02±9.69 years with a range between 35 and 82 years. In HCC patients the mean age was 59.28±8.76 with a range between 40 and 76. There was a significant difference in the mean ages of control, HCC patient group and liver cirrhosis group (*p*=0.000). Male predominance among the patients with HCC was 30 men (60.0%) versus 20 women (40.0%) In the liver cirrhosis patients there were 29 (58.0%) males versus 21 (42.0%) females.

Symptoms appearing between groups are summarized in Table 2. There was a statistical significant difference between groups as regards to weight loss (*p*=0.000). In control group, there was no weight loss but in cirrhosis group weight loss was found in 17 patients (34%) and in the HCC group, was found in 25 patients (50%). There was a statistical significant difference between groups as regards degree of encephalopathy (*p*=0.000). In control group there was no encephalopathy but in cirrhosis group 22% moderate and 18% severe encephalopathy were observed. In HCC group 22% moderate and 26% severe encephalopathy were observed. There was a statistical significant difference between groups as regard to bleeding (*p*=0.000). In control group no bleeding cases was found, in cirrhosis group 25 cases (50%) and 26 cases (52%) in HCC group were found. The laboratory findings of the studied groups are summarized in Table 3. There was a statistical significant difference between groups as regards to ALT, AST, T. Bil, D. Bil, glucose, serum creatinine, AFP and ADH values (*p*=0.000). There was no statistical significant difference between cirrhosis and HCC groups as regards to HCV RNA-PCR. As regards to ALDH, the data showed that HCC patients had the highest mean values (50.40±25.33U/L). In cirrhosis group the mean values were (31.63±14.03U/L), in control group the mean values were (17.04±16.14 U/L).

In the receiver operating curve (ROC), the area under curve (AUC) for AFP was 67% when we use 37 ng/mL as a cutoff point which gives the optimum balance between sensitivity, and specificity the sensitivity was 66% and a specificity of 64% (*P*-value= 0.003) were observed.

For ALDH, the area under curve (AUC) was 75.8% when we use 41 U/L as a cutoff point which gives the optimum balance between sensitivity, and specificity the sensitivity was 74% and a specificity of 82% (*P*-value= 0.000) were observed (Table 4, Figure 2).

Table 1 Demographic features of the studied groups.

Characteristics	Control N=37	Cirrhosis N =50	HCC N =50	P -value
Age				0.000
Range	19-77	35-82	40-76	
Mean±SD	42.14±15.93	62.02±9.69	59.28±8.76	
Gender				0.645
Male	25 (67.6%)	29 (58%)	30 (60%)	
Female	12 (32.4%)	21 (42%)	20 (40%)	

P-values < 0.05 were considered significant.

Table 2 Comparison between studied groups regarding clinical symptoms.

Symptoms	Control N=37	Cirrhosis N=50	HCC N=50	P -value
Wt. loss	0 (0%)	17 (34%)	25 (50%)	0.000
Moderate encephalopathy	0 (0%)	11 (22%)	11 (22%)	0.000
Severe encephalopathy	0 (0%)	9 (18%)	13 (26%)	0.000
Bleeding	0 (0%)	25 (50%)	26 (52%)	0.000

P-values < 0.05 were considered significant.

Table 3 Comparison between studied groups regarding laboratory findings.

LAB data	Control N=37	Cirrhosis N =50	HCC N =50	P -value
ALT (IU/L)				0.000
Range	20-70	34-103	34-100	
Mean±SD	31.86±9.35	70.28±17.65	61.28±19.17	
AST (IU/L)				0.000
Range	24-58	40-210	65-310	
Mean±SD	32.81±7.5	134.84±50.7	150.18±71.22	
T. Bil (mg/dL)				0.000
Range	0.4-1.00	0.8-6.00	1.90-5.70	
Mean±SD	0.78±0.19	2.55±1.34	2.82±0.91	
D. Bil (mg/dL)				0.000
Range	0.02-0.3	0.00-1.90	0.30-1.90	
Mean±SD	0.16±0.07	0.65±0.42	0.82±0.38	
Glucose (mg/dL)				0.000
Range	85-142	76-870	76-890	
Mean±SD	101.65±15.42	190.64±133.39	217.94±161.99	
Creat. (mg/dL)				0.000
Range	0.70-1.21	0.60-2.16	0.70-3.10	
Mean±SD	0.94±0.16	1.24± 0.44	1.51±0.61	
AFP (ng/mL)				0.000
Range	2.90-9.00	5.00-651	3.00-1060.00	
Mean±SD	5.72±1.71	129.38±189.91	298.50±275.17	
ALDH (U/L)				0.000
Range	5.60-75.00	8.00-66.00	6.00-99.00	
Mean±SD	17.04±16.14	31.63±14.03	50.40±25.33	
HCVPCR	Not done			0.101
Range		500-2×10 ⁷	20-74×10 ⁹	
Mean±SD		78×10 ⁴ ±30.6×10 ⁵	23×10 ⁶ ±11×10 ⁹	

ALT: Alanine transaminase; AST: Aspartate transaminase; T.Bil: Total Bilirubin; D.Bil: Direct Bilirubin; AFP :alpha-fetoprotein; ALDH: Aldehyde Dehydrogenase *p*-values < 0.05 were considered significant.

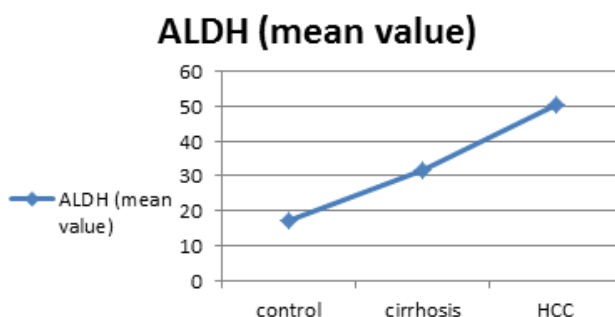
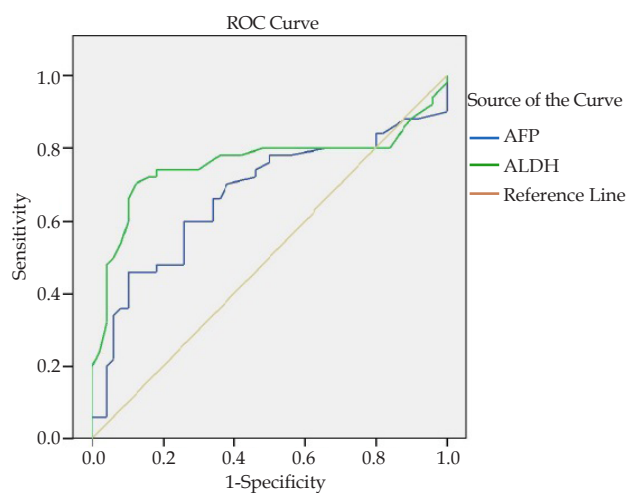


Figure 1 ALDH values among studied groups.

Table 4 ROC curve analysis of ALDH and AFP as markers for HCC.

Test	Cut off	Sensitivity %	Specificity %	AUC %	Asymptotic 95% Confidence interval		P-value
					Lower Bound	Upper Bound	
AFP	37	66	64	67	0.561	0.780	0.003
ALDH	41	74	82	75.8	0.653	0.863	0.000

**Figure 2** ROC curve of AFP and ALDH.

DISCUSSION

Hepatocellular carcinoma is one of the leading causes of cancer-related deaths in the world and in Egypt. The incidence is expected to rise over the next decades owing to the increasing prevalence of chronic liver diseases especially those due to underlying viral hepatitis or non-alcoholic steatohepatitis. Currently, a multitude of markers are available for the diagnosis of hepatocellular carcinoma. However, none of these has adequate sensitivity and specificity. For example, alpha feto protein, the most widely used serum marker for the diagnosis of HCC in clinical practice has a sensitivity and specificity of 41-65% and 80-94% respectively. Furthermore, the sensitivity, specificity and predictive value of AFP is largely dependent upon other variables including the population being studied, the cut-off values used for establishing the diagnosis and the gold standard test used to confirm the diagnosis. In addition, AFP may rise in the background of different clinical situations including viral hepatitis, liver cirrhosis and other malignancies (e.g. cholangiocarcinoma). If a patient has known risk factors for HCC, such as the presence of cirrhosis, increasing levels of AFP have been shown to correlate with the development of HCC^[17]. Unfortunately, AFP serum concentrations do not correlate well with the prognostic values of HCC such as tumor size, stage, or disease progression and ethnic variability may also exist. Furthermore, in some cases of HCC, AFP elevations are not apparent at all^[18]. Hence, was the need to search for a more valid and accurate biological marker for the diagnosis of HCC. The ALDH enzymes are a family of evolutionarily conserved enzymes comprised of 19 isoforms that are localized in the cytoplasm, mitochondria or nucleus. Generally, ALDHs are responsible for oxidizing aldehydes to carboxylic acids^[16,20]. Aldehydes are generated by a wide variety of metabolic processes and most commonly arise from the oxidative degradation of membrane lipids (lipid peroxidation), but can also arise from amino acid, carbohydrate and neurotransmitter catabolism. While many aldehydes play a critical role in physiological processes like vision, neurotransmission and embryonic development, most are cytotoxic

and need to be detoxified, as reviewed by Marchitti *et al.*^[16].

In addition to their important function in aldehyde detoxification, ALDHs perform other functions including ester hydrolysis, serving as binding proteins for various molecules (e.g., androgen and cholesterol) and potentially function as antioxidants by NAD(P)H production, ultra violet light absorption and/or hydroxyl radical scavenging^[16,20,21]. Alcohol dehydrogenase and Aldehyde dehydrogenase which are most abundant in the liver, are the main enzymes involved in ethanol and acetaldehyde metabolism. The activities of total ADH, ALDH, and class I ADH were significantly higher in cancer cells than in healthy tissues^[20].

The aim of this study was to evaluate the diagnostic and prognostic value of ALDH levels in patients with hepatocellular carcinoma. In our study, AFP had a sensitivity of 66% and a specificity of 64% (P -value=0.003) when used at a cut-off value of 37 ng/mL. On the other hand, serum ALDH had a higher sensitivity (74%) and specificity (82%) (P -value=0.000) than AFP when measured at a cut-off value of 41 U/L. To the best of our knowledge, no previous studies have evaluated the diagnostic value of serum ALDH levels in patients with HCC. However, several studies have looked at the different patterns of ALDH isoenzyme activities in patients with HCC. For example, a study by Park *et al.*, revealed increased detection of ALDH-3 but decreased detection of ALDH-2 when measured by 2D electrophoresis and mass spectrometry in a group of patients with HCC. Contrary to our demographic characteristics, none of their patients had hepatitis C. Another study by Jelski *et al.*, demonstrated an increased activity of ALDH-1 in the sera of patients with HCC compared to controls (2.94 mU/L vs 1.43 mU/L) when measured by photometric methods.

We were not able to compare our results with the aforementioned studies for several reasons. First, our study included only patients with hepatitis C-related HCC, while the other studies included subjects with alcohol-related liver disease and viral hepatitis (hepatitis B and C). This might lead to different levels of aldehyde dehydrogenase levels and/or activity. Second, the present study used enzyme linked immunoassay in measuring the ALDH serum levels. On the other hand, more sophisticated technologies (e.g. 2D electrophoresis and mass spectrometry) were used to detect ALDH isoenzyme activities. Third, total ALDH quantitative serum levels were measured rather than specific ALDH isoenzyme activities as demonstrated in the other studies.

Therefore, we believe that our findings need to be confirmed in further studies incorporating a larger cohort of patients with different etiologies of chronic liver disease and HCC.

CONCLUSION

The data showed that ALDH was more sensitive and specific than AFP in detection of HCC, ALDH had 74% sensitivity and 82% specificity, p -value (0.000) but AFP had 66% sensitivity and 64% specificity, P -value (0.003). ALDH could be used as useful diagnostic marker for detection of hepatocellular carcinoma in hepatitis C virus infected patients and in differentiation between HCC and cirrhosis at a cut off value 41 U/L.

IMPLICATION FOR HEALTH POLICY MARKERS/PRACTICE/RESEARCH/MEDICAL EDUCATION

To allow early detection and surveillance of hepatocellular carcinoma which improves disease prognosis and increase survival.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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