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Research Article

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Diagnostic sensitivity of arginase, alpha-1 antitrypsin and alpha-fetoprotein in hepatitis patients

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Abstract

Chronic hepatitis C virus (HCV) and hepatocellular carcinoma (HCC) are common liver diseases that lead to death in Egypt, especially in men. The current study aimed to evaluate the diagnostic sensitivity of arginase (ARG) activity, alpha-1 antitrypsin (AAT), and alpha-fetoprotein (AFP) in the sera of patients with HCV (with & without viremia) and HCC. A total of 190 men classified as 40 healthy used as control (G1), 100 infected with HCV (subdivided into 50 with viremia (G2) and 50 without viremia (G3), and 50 with HCC (G4). The activity of ARG significantly decreased in HCV and HCC groups along with significant elevation in the level of AAT and AFP as compared with the control. Although a non-significant variation was scored in AST/ALT, significant differences were observed among AST/ARG and ARG/ALT in the pathogenic groups as compared with the healthy group. Moreover, significant variations in ARG, AAT, AFP, AST/ARG, and ARG/ALT were observed between viremia and non-viremia. Although AFP scored significant change among the viremia and HCC, the rest parameters scored non-significant changes between both groups. Furthermore, a receiver operating characteristic curve (ROC) showed the diagnostic ability for the selected parameters with high sensitivity and multiple linear regressions exhibited good associations between those parameters. These findings suggest the using possibility of ARG, AAT, and AFP in the diagnosis and/or follow-up of patients with HCV or HCC.

Keywords: Hepatitis C virus, hepatocellular carcinoma, viremia, arginase, Alpha-1 antitrypsin, alpha-fetoprotein

Introduction

HCV is a health burden that infects nearly 170 million people worldwide [1], accounting for 3% of the world's population with 3-4 million new diseases and 3.5-5 thousand deaths per year. In Egypt, the condition is quite worse as it has the highest prevalence in the world of 22% of HCV [2], and is unique in its mode of treatment history, epidemiology, risk factors and genotype. The key risk factors for this tremendous prevalence of HCV have traditionally been the insufficient sterilization and reuse of needles in the mass campaigns for schistosomiasis care in Egypt between the 1960s and 1980s [3]. HCV viremia subjects constitute the reservoir for ongoing transmission that occurs primarily through the parental path, especially among people who inject drugs the community in many countries is most at risk for HCV infection. HCV causes acute, often subclinical, hepatitis, but the persistence of the infection and the severity of the resulting inflammation can lead to chronic hepatitis that is aggravated by cirrhosis and/or HCCC in about 80% of infected subjects [4]. Many extrahepatic symptoms are often associated with chronic HCV infection, which raises the rate of death in infected patients such as increasing risk of type 2 diabetes mellitus, circulatory disorders of the thyroid, renal disease, esophageal, and prostate cancer [5].

As no drug is available for complete neutralization of HCV, early diagnosis is important for treatment and prevention measures. To date, the main serological tests for HCV diagnosis are indirectly by detecting specific antibodies to HCV and directly by detecting HCV-RNA and core antigen. Molecular virological techniques play a key role in diagnosis and monitoring of treatment for HCV because it is difficult to cultivate the virus in cell culture, molecular techniques were instrumental in first identifying HCV, making it one of the first pathogens to be identified by purely molecular methods [6]. Due to cost concerns, molecular laboratory facilities are either not available or not commonly used. Compared to molecular approaches, we need user-friendly tests, need low scientific knowledge, low cost, facilitate clinical monitoring of a patient's treatment, regardless of the genotype of HCV in addition to high sensitivity as the lower sensitivity of the HCV core Ag assay limits its usefulness as a significant limitation [2].

HCC is a universal health problem with variations in epidemiological data from one location to another. It is the most common cause of death-and morbidity-related cancer in Egypt, particularly in males [7]. Many hospital-based studies have reported changes in the incidence of HCC [8,9]. The cause for increased prevalence may be due to better screening services and testing methods, an improvement in the survival rate of cirrhotic patients who are more likely to develop HCC. Recently, liver transplantation and resection surgery are key surgical care choices for HCC. Although the mortality of HCC has significantly decreased with the development of surgical techniques, about 60%-100% of the patients suffered from HCC recurrence ultimately even after curative resection, and it has become the most important factor that limits the long-term survival of HCC patients [10]. To date, no biomarker mixture has been effective enough to diagnose a lesion as HCC without confirmatory histological or radiological characteristics. Diagnostic accuracy, particularly for early stage HCC, can be enhanced by combining two or three biomarkers to achieve a fair (>80 per cent) sensitivity with a small decrease in specificity. For this cause, the precision of the marker may also be improved by measuring the overtime variability of the marker. Future studies may develop useful biomarkers to monitor treatment activity, diagnose early response to treatment, and classify patients that are more likely to benefit from treatment [11]. This results in the discovery of sensitive markers for early diagnosis and postoperative recurrence of HCC and the availability of effective care for HCC patients.

Arginase (ARG) is a member of the ureohydrolase family of enzymes that catalyze the last stage of the urea cycle contributing to ammonia detoxification in mammals. Two ARG isoenzymes are encoded by separate genes and are called ARG-1 and ARG-2. They have the same biochemical reactions but are different in cellular expression and localisation. ARG-1 accounts for 98% of ARG production is cytoplasmic and is predominantly synthesized in hepatocytes [12]. Therefore, differences in ARG activity may indicate liver disorders, including carcinogenesis [13]. Alpha-1-antitrypsin (AAT) is the most prevalent serum proteinase inhibitor in human plasma. It is primarily synthesized, secreted and cleared by hepatocytes [14]. It is also an acute phase reactant and its plasma concentrations rise in response to tissue damage, including liver disease [15,16]. The literature on AAT deficiency and its relationship with liver and lung disease has been immense, but there is no knowledge of its use as a liver-specific biomarker. Alpha-fetoprotein (AFP) is a glycoprotein synthesized by the fetal liver and a prominent portion of serum proteins in early embryonic existence. The AFP level starts to decline after birth and is low in healthy adults as mature hepatocytes, although it can increase again in patients with liver disease [17], especially HCC [18]. This study aims to determine the level of ARG, AAT, and AFP and their association with other routine biomarkers in HCV-infected patients (with and without viremia) and patients with HCC that may serve in understanding the pathogenesis of this disease and may greatly advise in the diagnosis.

Materials and Methods

Study design, samples collection, and preparation

This study comprised 190 males matched in age (40- 65 years) and under medical follow-up in Maadi Military Hospital, Cairo, Egypt. They were divided into 4 groups; the control group (G1) that has 40 normal healthy subjects. HCV group without viremia (G2): 50 subjects who are HCV-PCR negative or under detectable level and they have HCV-Ab positive. HCV group with viremia (G3): 50 subjects who are HCV-PCR positive. The HCV- patients are not related to any hepatic tumor. HCC group (G4): consisting of 50 patients with malignant hepatic tumor and they are not related with any viral hepatitis. All samples were collected before the beginning of any treatment. Morning blood samples (5 ml) were collected at 8 O'clock in clean dry plastic tubes left to clot for 20 minutes at 37 °and then centrifuged at 3000 xg for 15 minutes. The sera were divided into 4 aliquots and saved at -80 °for further determinations of ARG, AAT, and AFP as well as routine analyses.

Ethical statement

The study approved by the local ethical committee and an informed written consent was taken from all patients.

ARG activity, AAT and AFP concentrations determination

ARG activity was measured using the colorimetric method of [19]. The concentration of AAT was determined using the radial immunodifusion (RID) process, which is typically used to calculate the concentration of different soluble antigens in biological fluids according to [20] as well as [21] procedures. The approach requires an antigen that radially diffuses from a cylindrical well into an agarose gel containing an effective monospecific antibody. Antigen-antibody complexes are formed and, under the right conditions, form a precipitin ring (**Figure 1**). The AAT concentration (mg/L) referring to each ring diameter was

obtained from the RID comparison table included with the kit. AFP level was determined by the ELISA technique according to the method of [21].

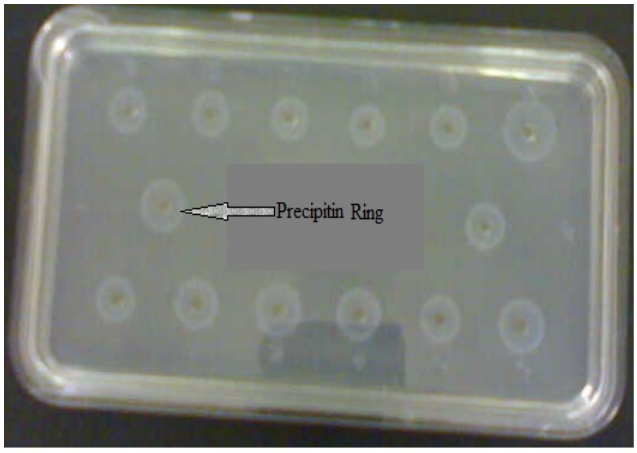

Figure 1. RID plates showed varied precipitin rings.

Determination of AST, ALT, T.BIL, and ALB

Aspartate aminotransferase (AST) activity and Alanine aminotransferase (ALT) were measured using Reitman and Frankel [23] method. Total bilirubin (TBIL) and albumin (ALB) were determined used Jendrassik and Grof [24] and Doumas and Biggs [25] procedures, respectively.

Statistical analysis

In this study, the SPSS program (IBM SPSS-23Inc, USA) was utilized for the data entry and analysis. Clinical data are expressed mean ± SEM in each studied group was made on the obtained values from the numbers of patients in each group. One-way analysis of variance (ANOVA) followed by Turkeyś honestly significant difference (HSD) test was applied to match between the studied groups where $P < 0.05$ value was set as statistically significant. The Receiver operating characteristic (ROC) curves were plotted for each subgroup of patients to assess the diagnostic performance of the biomarkers by determining the area under the curve (AUC) as well as a P-value. The sensitivity and specificity of ARG, AAT, and AFP were calculated using the Youden index method. Multiple linear regressions were used to identify the associated factors for the studied parameters (ARG, AAT, and AFP) with routine analyses (AST, ALT, TBIL, and ALB) as well as enzyme ratios (AST/ALT, AST/ ARG, and ARG/ALT).

Results

Arginase activity, AAT, and AFP concentration

The enzymatic activity of ARG (38.67, 57.96, and 55.65%, respectively) was significantly decreased ($p < 0.05$) in G2, G3, and G4 as matched with control. Although a significant ($p < 0.05$) decrease in ARG activity was observed in G3 and G4 as compared with G2, a non-significant ($p > 0.05$) difference was observed between G3 and G4 (**Figure 2**). Inversely, the serum concentrations of AAT (29.09, 82.89, and 82.45%, respectively) were markedly ($p < 0.05$) increased in the same groups as compared with the control level. While a significant ($p < 0.05$) rise in the level of AAT was observed in G3 and G4 when compared with G2, a non-significant ($p > 0.05$) variation in AAT serum levels was observed when G3 compared with G4 (**Figure 3**). Furthermore, the serum concentration of AFP was significantly increased ($p < 0.05$) in G3 and G4 (2587.53, and 113288.53%, respectively) as compared with G1, while G2 scored a non-significant ($p > 0.05$) increase by 29.57%. A significant ($p < 0.05$) higher level in AFP was observed in G3 and G4 when compared with G2. Also a significant ($p < 0.05$) increased level in AFP serum levels was observed when G4 compared with G3 (**Figure 4**).

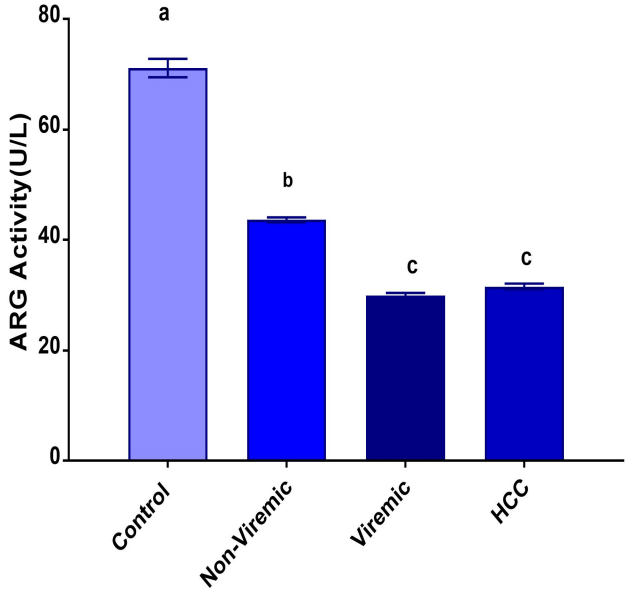

Figure 2. Comparison ARG activities in all studied groups . Each value represents the mean ± SEM.

AST, ALT activities, TBIL and ALB concentrations

A non-significant ($p > 0.05$) increase in enzymatic activity of AST (3.67, 5.33, and 8.64%, respectively) in G2, G3, and G4 as matched with control. Also, a non-significant ($p > 0.05$) variation in AST activity was observed between all pathogenic groups (**Table 1**). G2 and G3 (1.3 and 2.3% respectively) scored a non-significant ($p > 0.05$) increase in ALT activity as compared with G1 while this increase was significant ($p < 0.05$) in G4 (10.73%) as compared with G1. G2 scored a non-significant ($p > 0.05$) decrease in ALT activity as compared with G3 but this decrease was significant ($p < 0.05$) as compared G4 and the non-significant ($p > 0.05$) variation between G3 and G4 continue also in ALT activity (**Table 1**). T.BIL concentrations (79.29, 167.84, and 217.18%, respectively) in G2, G3, G4 were markedly ($p <$

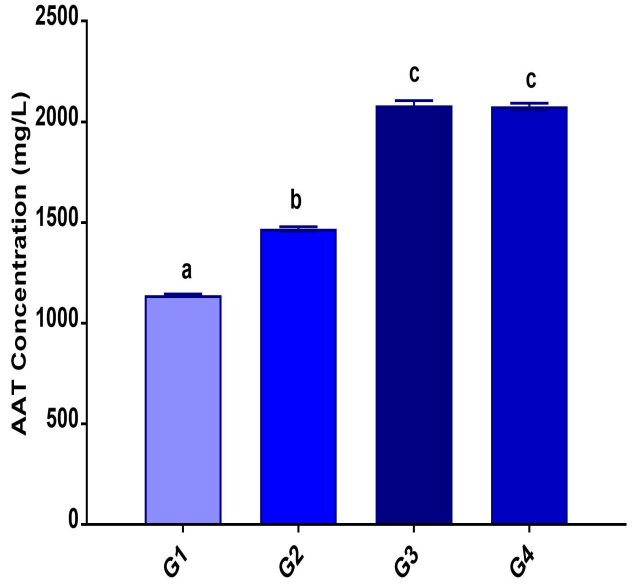

Figure 3. Comparison AAT concentrations in all studied groups. Each value represents the mean ± SEM.

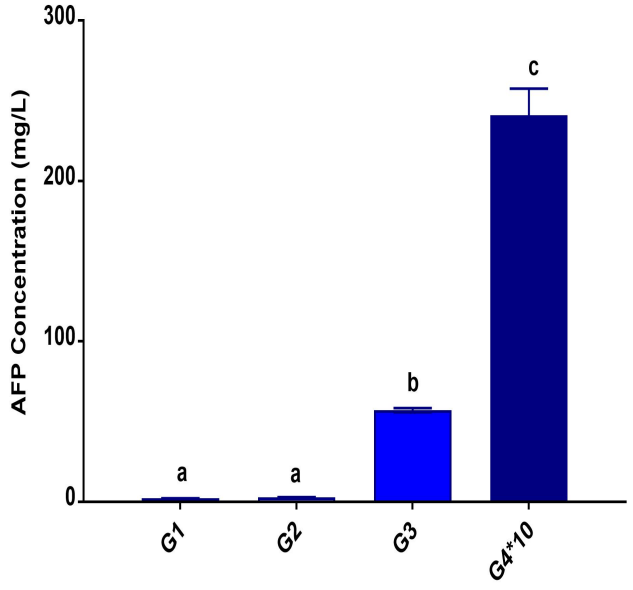

Figure 4. Comparison AFP concentrations in all studied groups. Each value represents the mean ± SEM. Means with different letters indicated the variations between the groups within the same column using Turkeyś honestly significant difference ($p < 0.05$) test. G1: Control group, G2: HCV-PCR negative group, G3:HCV-PCR positive group, G4:hepatocellular carcinoma group.

0.05) increased as compared with the control levels. A significantly higher level of T.BIL was observed in G3 and G4 when compared with G2 but also a non-significant variation in T.BIL serum levels were observed comparing G3 with G4 (**Table 1**). Concerning the serum concentrations of ALB, a significant ($p < 0.05$) decrease levels were observed in G2, G3, and G4 (19.04, 22.73, and 25.31% respectively) as compared with G1. The level of ALB of G2 scored a non-significant ($p > 0.05$) increase as compared with G3 and no statistical variation ($p > 0.05$) was observed between G3 and G4 (**Table 1**).

AST/ALT, AST/ARG, and ARG/ALT

A non-significant ($p > 0.05$) variation in AST/ALT (2.09, 1.24, and 1.69%, respectively) in G2, G3 and G4 as matched with control. Also, a non-significant ($p > 0.05$) variation in AST/ALT was observed between all pathogenic groups (**Table 1**). Inversely a marked ($p < 0.05$) variation in AST/ARG (67.20, 144.55, and 144.26%, respectively) was observed in G2, G3, and G4 as compared with G1. Moreover, a significant variation in AST/ARG was observed between G2 and G3 but a non-significant variation was observed when comparing G3 with G4 (**Table 1**). Interestingly the other ratio of ARG/ALT showed a significant ($p < 0.05$) variation; in all pathogenic groups G2, G3, and G4 (39.41, 58.78, and 59.95% respectively) as compared with G1, in G2 as compared with G3and G4 but no statistical variation ($p > 0.05$) was observed between G3 and G4 (**Table 1**).

ROC curve analysis

ROC curve analysis was used to assess whether the decreased ARG activity or increased levels of AAT, as well as AFP, may serve as a predictor of the severity of HCV and HCC. In comparing ARG activity of G2, G3, and G4 with G1, all AUCs were 1.0 and P values < 0.001 while the associated diagnostic sensitivities were 100 and specificities were 5 at cut-off values of 40.36 U/L, 24.95 U/L, and 26.57 U/L respectively. At the same values of cut-off (24.95 U/L and 26.57 U/L respectively), the ROC curve results differentiate significantly ($P< 0.001$) between G3 as well as G4 when compared with G2 where AUC is also equal unity with associated diagnostic sensitivities were 100 and specificities were 5 for both groups. While comparing ARG activity between G4 with G3, a good association ($P < 0.005$) was observed at AUC 0.756 and the diagnostic sensitivity was 100 but the specificity increased to 45 at ARG activity of 32.04 U/L (**Figure 5** A-F).

Comparing AAT concentrations of G2, G3, and G4 with G1, significant associations ($P< 0.001$) were observed (at 1520 mg/L, 2310 mg/L, and 2175 mg/L respectively), the AUCs were 1, and the sensitivities were 100 while the specificities were 26, 5 and 10 respectively. The AAT concentration significant association ($P < 0.001$) between G3 as well as G4 with G2 took the same trend of G1, the AUC was 1.0 and the associated diagnostic sensitivity was 100 (at 2310 mg/L and 2175 mg/L respectively) and specificity was 5 and 10 respectively. In comparing the AAT level of G4 with G3 a non-significant association ($P = 0.707$) at 2065 mg/L cut off was observed, the AUC was 0.535, the associated sensitivity was 50 and the specificity was 55 (**Figure 5 G-L**).

Table 1. Comparison between ALT, AST, AST/ALT, AST/ARG, ARG/ALT, T.BIL and ALB levels in different studied groups.

	AST (U/L)	ALT (U/L)	AST/ALT	AST/ARG	ARG/ALT	T.BIL (mg/dL)	ALB (g/dL)
G1	27.20 ±0. 972^{a}	30.30 ±0. 864^{a}	0.9102 ±0.042^{a}	0.3872 ±0.01817^{a}	2.3721 ±0.06623^{a}	0.5675 ±0.0298^{a}	4.0700 ±0.0802^{a}
G2	28.20 ±0.851^{a}	30.70 ±0.782^{a,b}	0.9293 ±0.037^{a}	0.6474 ±0.0198^{b}	1.4372 ±0.0364^{b}	1.0175 ±1.137^{b}	3.2950 ±0.0816^{b}
G3	28.650 ±0.762^{a}	31.00 ±0.778^{a,b}	0.9215 ±0.0392^{a}	0.9469 ±0.0308^{c}	0.9778 ±0.0325^{c}	1.5200 ± 0.1448^{c}	3.1450 ±0.0530^{b,c}
G4	29.5500 ±0.908^{a}	33.55 ±0.969^{b}	0.8948 ±0.0389^{a}	0.9458 ±0.0399^{c}	0.9498 ±0.0225^{c}	1.800 ±0.1227^{c}	2.910 ±0.0763^{c}

Each value represents the mean ± SEM. Means with different letters indicated the variations between the groups within the same row using Turkeyś honestly significant difference ($p < 0.05$) test. G1: Control group, G2: HCV-Nonviremic group, G3 HCV-Viremic group, G4 Hepatocellular Carcinoma group.

When analyzing AFP levels in G2, G3 and G4 with G1 a strong associations (P<0 .004, P < 0.001, P <0.001 & AUC = 0.828, 1.0 and 1.0 respectively) were observed (at 3.57 ng/ml, 65.55 ng/ml and 2487 ng/ml respectively) while the diagnostic sensitivities were 100 and the specificities were 5. Moreover significant associations (P < 0.001) also observed when analyzing G3 as well as G4 with G2 (at 65.55 ng/ml & 2487 ng/ml) as well as analyzing G4 with G3 (at also 2487 ng/ml), the AUCs were 1, the associated diagnostic sensitivities were 100 and specificities were 5 (**Figure 5 M-R**).

Multiple regression analysis

The three models of ARG, AAT and AFP are significant (P <0.05) at R square of 0.982, 0.887, and 0.433 respectively. In ARG model, AST, ALT, ALB, AST/ARG & ARG/ALT were significantly ($p < 0.05$) independent variables with regression coefficient (β = 0.098; 0.219; 0.032; - 0.093, and 0.965 respectively), while TBIL and AST/ALT were non-significant predictors (P > 0.05 with β = 0.005; - 0.082 respectively). In AAT model, ALB, AST/ALT, AST/ARG &ARG/ALT were significantly ($p < 0.05$) independent variables with regression coefficient (β = - 0.079; - 0.265; 0.855 and - 0.132 respectively) while AST, ALT and TBIL were non-significant predictors (P > 0.05 with β = - 0.132; - 0.075 and 0.027respectively). While in AFP model, AST, ALT, TBIL, ALB, AST/ARG &ARG/ALT were the significant ($p < 0.05$) independent variables with regression coefficient (β = - 0.499; 0.653; 0.279; - 0.353; 0.682 and 0.633 respectively) while AST/ALT were a non-significant predictor (P > 0.05 with β = 0.349) (**Table 2**).

Table 2. Multiple regression analysis to identify association of serum ARG, AAT and AFP with some routine analyses and enzymes ratios in all studied groups.

Dependent Variable	Independent variable	coefficient	P-value
ARG (R^2=0.982) (P <0.0001) Model (1)	AST	0.098	0.005
	ALT	0.219	0
	TBIL	0.005	0.733
	ALB	0.032	0.034
	AST/ALT	-0.082	0.055
	AST/ARG	-0.093	0.008
	ARG/ALT	0.965	0.0001
AAT (R^2=0.887) (P <0.0001) Model (1)	AST	-0.132	0.132
	ALT	-0.075	0.324
	TBIL	0.027	0.424
	ALB	-0.079	0.039
	AST/ALT	-0.265	0.014
	AST/ARG	0.855	0.0001
	ARG/ALT	-0.132	0.12
AFP (R^2=0.433) (P <0.0001) Model (1)	AST	-0.499	0.011
	ALT	0.653	0.0001
	TBIL	0.279	0.0001
	ALB	-0.353	0.0001
	AST/ALT	0.349	0.147
	AST/ARG	0.682	0.001
	ARG/ALT	0.633	0.001

Discussion

Chronic liver diseases (CLDs) are primarily characterized by the gradual destruction of hepatocytes that can cause alterations in the normal functioning of the liver over time. Worldwide, viral hepatitis is still the leading known etiology of CLDs, especially in Egypt [26] and HCC-related deaths are increasing faster than those for any other cancer [7]. Patients with CLDs express an excessive burden due to their disease which includes substantial healthcare expenses, negative employment impact, and significant impairment in their quality of life [27]. Thus the burden of CLDs should be investigated not only from the epidemiological perspective but also from the economic context.

Circulating diagnostic and prognostic biomarkers could be used in the proper postoperative treatment of patients at the early stages of CLDs development. The most pressing concerns are the detection of sensitive markers for early diagnosis and monitoring of patients with CLDs. ARG is mainly secreted via hepatocytes to catalyze the conversion of L-arginine to L-ornithine and urea which is the final step of urea cycle [28]. The results of this study indicating the lower serum ARG activities in all pathogenic groups as compared with control. This low ARG activity that was observed in the patients' groups may be due to the failure of seriously damaged hepatocytes to synthesize and/or liberate normal enzymes into the bloodstream. Another logic cause for ARG decreased activity particularly in G3 and G4 may be the competition for the substrate between ARG and nitric enzyme synthase (NOS) that catalyze the conversion of L-arginine to nitric oxide (NO) and L-citrulline especially many studies showed a relation between a high level of NO and HCV viral load [29,30] and HCC progression [31,32]. In this study, we observed that a significant decrease in ARG activity in the

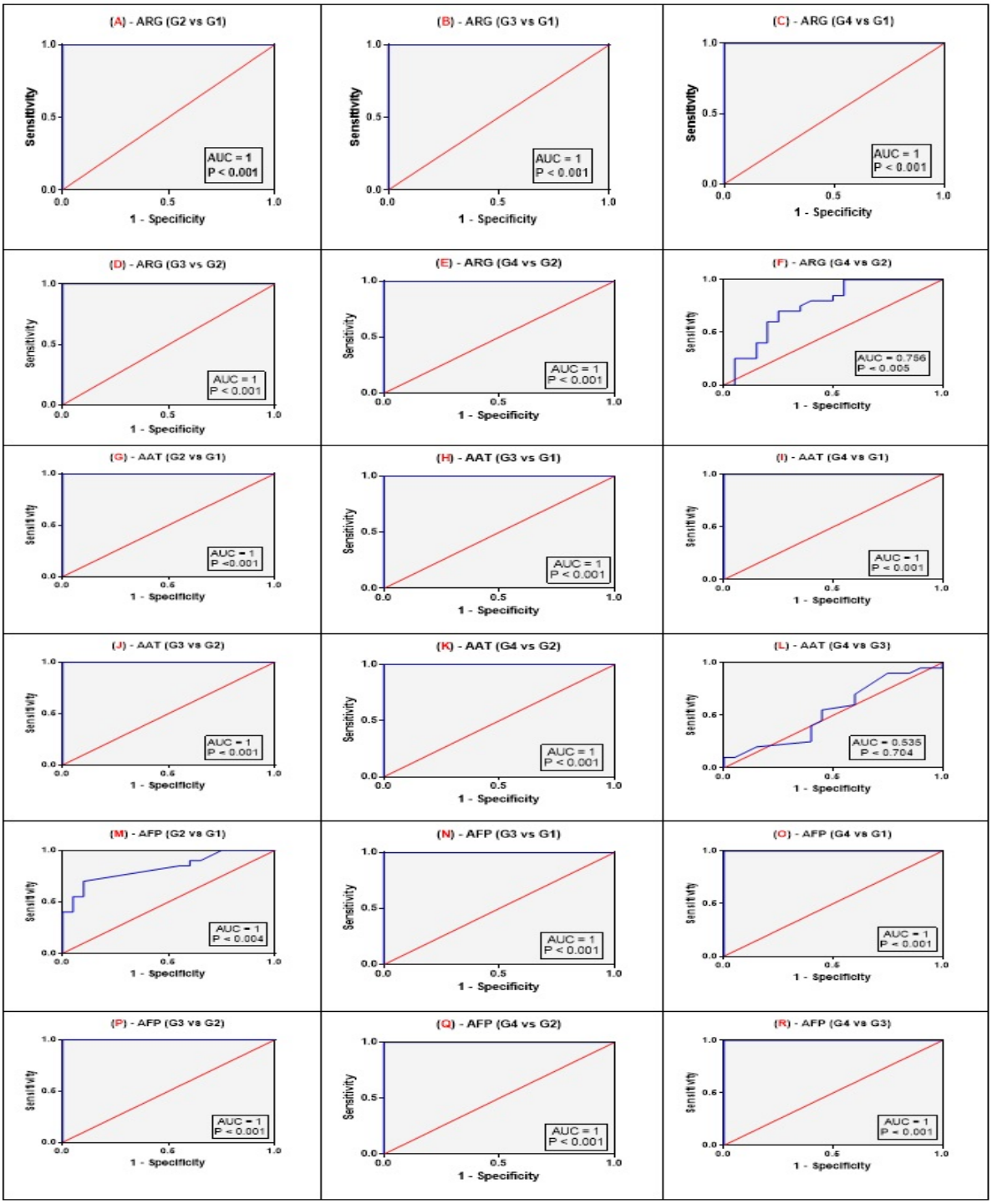

Figure 5. Area under the ROC curve (AUC) and P value for the three studied parameters ((A-F) ARG= Arginase, (G-L) AAT= Alpha-1 antitrypsin and (M-R) AFP= alpha fetoprotein) to discriminated between pathogenic groups (G2, G3 and G4; Nonviremic-HCV, Viremic-HCV and HCC respectively) and control (G1) as well as between pathogenic groups itself.

G3 group as compared with G2. This may be due to the association between viral particles load in serum and increased NO production by NOS that compete with ARG for a substrate.

All pathogenic groups have higher serum AAT concentration as compared with G1. This increase may be due to the massive inflammation of the liver caused by a viral infection and liver cancer. It is probable that during a liver injury in hepatitis there is an increased synthesis along with the increased transport of AAT to the damaged sites, thereby counteracting the release of serine proteases produced by neutrophils to exterminate the microorganisms. Increased neutrophils in the patients might contribute to tissue damage and autoimmune diseases, where the presence of inflammatory mediators is prevalent in both infective and non-infective pathologies [33]. Thus, the high level of AAT in serum may be derived from normal liver cells and represent self-protective responses to the viral particles especially HCV-NS3 which is essential for viral maturation and replication that have serine protease activity when complex with the viral NS4A [34]. Further, Oguz et al., [35] have showed that the release of acute-phase proteins increases with HCV viremia, this may explain the significantly high level of AAT in G3 as compared with G2. Moreover, there has been considerable evidence that the lysosomes of tumor cells produce the proteases [36], which may result in the subsequently enhanced synthesis of AAT in the liver cells to compensate for the activity of proteases. Our results were compatible with the observation of Tan et al., [15] who have showed that AAT is highly expressed in serum samples of patients with HCC and HCV, The authors have suggested the specific secretion of AAT from normal hepatic tissues to serum as they found that the level of AAT gene expression was higher in normal hepatic tissue than HCC and HCV hepatic-diseased tissues.

AFP is widely used as a serum marker for diagnosing HCC although its level was found to be elevated also in benign CLDs such as chronic viral hepatitis without HCC [37]. In this study, the highest level of AFP was observed in the HCC group as compared with control and HCV groups. Cancer cells can modify their surface antigen and change the microenvironment around the tumor lesion to implement their immune escape. Production of AFP by HCC cells can not only promote the angiogenesis and proliferation, but also enhance the anti-apoptosis effect of cancer cells. Moreover, AFP can inhibit the maturation and induce apoptosis of dendritic cells the main antigen-presenting cells that activating T lymphocytes, so that cancer cells could escape from host immune surveillance [38]. We also observed a significant increase in AFP level in G3 as compared with control. Tai et al., [37] showed that the increased AFP in HCV-infected patients positively correlated with the rate of fibrosis in liver cells but not correlated to HCV genotype. Moreover, hepatic stellate cells are the principal hepatic fibrogenic cells generating scar tissue in response to persisting liver injury. These cells are highly expressed in HCV patients where they express high levels of AFP their presence is related to the severity of fibrosis, and expression of these cells has been associated with response to treatment, being higher in non-responders compared with responders [39]. Furthermore, HCV gene products can activate cellular oncogenes [40] such as AFP [41,42]. Additionally, the presence of increased injury and inflammation in case viremic patients may enhance the production of AFP [43] in G3 as compared to G2.

The significant results of ROC curve analysis showed that the decreased ARG activities, as well as the increased levels of AAT and AFP, are good biomarkers with high sensitivity in the diagnosis of HCV (with or without viremia) and HCC patients.

Conclusion

This study demonstrated that decreased serum ARG activity, as well as increased AAT and AFP levels, can significantly differentiate between the viremic and non-viremic status in patients infected with HCV. Therefore, these parameters can be used for follow-up patients infected with HCV and under treatment to evaluate the improvement of treatment with low economical cost. The combination of ARG, AAT alongside AFP may increase the sensitivity of detection and early diagnosis of HCC especially in low-AFP producing HCC. Moreover, ARG ratios with aminotransferases (AST/ARG and ARG/ALT) can be useful in the liver profile of chronic disease than aminotransferase ratio. These findings should be validated in further studies recruiting a larger number of patients.

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References

1. Mehrpour K, Mirzaei SA, Savardashtaki A, Nezafat N, Ghasemi Y. Designing an HCV diagnostic kit for common genotypes of the virus in Iran based on conserved regions of core, NS3-protease, NS4A/B, and NS5A/B antigens: an in silico approach. Biologia. 2021 Jan;76(1):281-96.
2. Gupta E, Bajpai M, Choudhary A. Hepatitis C virus: Screening, diagnosis, and interpretation of laboratory assays. Asian journal of transfusion science. 2014 Jan;8(1):19.
3. Elgharably A, Gomaa AI, Crossey MM, Norsworthy PJ, Waked I, Taylor-Robinson SD. Hepatitis C in Egyptpast, present, and future. International journal of general medicine. 2017;10:1.
4. Kouyoumjian SP, Chemaitelly H, Abu-Raddad LJ. Characterizing hepatitis C virus epidemiology in Egypt: systematic reviews, meta-analyses, and meta-regressions. Scientific reports. 2018 Jan 26;8(1):1-7.
5. Negro F, Forton D, Craxì A, Sulkowski MS, Feld JJ, Manns MP. Reviews in basic and clinical gastroenterology and hepatology. Gastroenterology. 2015;49(6):1345.
6. Kumar A, Rajput MK, Paliwal D, Yadav A, Chhabra R, Singh S. Genotyping diagnostic methods for hepatitis C virus: A need of low-resource countries. The Indian journal of medical research. 2018 May;147(5):445.

7. Rashed WM, Kandeil MA, Mahmoud MO, Ezzat S. Hepatocellular Carcinoma (HCC) in Egypt: A comprehensive overview. Journal of the Egyptian National Cancer Institute. 2020 Dec;32(1): 1-1.
8. Abd-Elsalam S, Elwan N, Soliman H, Ziada D, Elkhalawany W, Salama M, Hawash N, Arafa M, Badawi R, Shehata WM, Khalil HS. Epidemiology of liver cancer in Nile delta over a decade: a single-center study. South Asian journal of cancer. 2018 Jan;7(1):24.
9. Ziada DH, El Sadany S, Soliman H, Abd-Elsalam S, Salama M, Hawash N, Selim A, Hamisa M, Elsabagh HM. Prevalence of hepatocellular carcinoma in chronic hepatitis C patients in Mid Delta, Egypt: A single center study. Journal of the Egyptian National Cancer Institute. 2016 Dec 1;28(4):257-62.
10. Golabi P, Fazel S, Otgonsuren M, Sayiner M, Locklear CT, Younossi ZM. Mortality assessment of patients with hepatocellular carcinoma according to underlying disease and treatment modalities. Medicine. 2017 Mar;96(9).
11. Zacharakis G, Aleid A, Aldossari KK. New and old biomarkers of hepatocellular carcinoma. Hepatoma Research. 2018 Oct 17;4.
12. Munder M. Arginase: an emerging key player in the mammalian immune system. British journal of pharmacology. 2009 Oct;158(3):638-51.
13. Labib OH, Harb OA, Khalil OH, Baiomy TA, Gertallah LM, Ahmed RZ. The diagnostic value of arginase-1, FTCD, and MOC-31 expression in early detection of hepatocellular carcinoma (HCC) and in differentiation between HCC and metastatic adenocarcinoma to the liver. Journal of gastrointestinal cancer. 2020 Mar;51(1):88-101.
14. Kalsheker N, Stockley RA. Alpha-1-antitrypsin Deficiency: Biology, Diagnosis, Clinical Significance, and Emerging Therapies. Academic Press; 2017 Jun 6.
15. Tan XF, Wu SS, Li SP, Chen Z, Chen F. Alpha-1 antitrypsin is a potential biomarker for hepatitis B. Virology journal. 2011 Dec;8(1):1-7.
16. Topic A, Ljujic M, Radojkovic D. Alpha-1-antitrypsin in pathogenesis of hepatocellular carcinoma. Hepatitis monthly. 2012 Oct;12(10 HCC).
17. Manuc D, Preda CM, Sandra I, Baicus C, Cerban R, Constantinescu I, Olteanu AO, Ciora CA, Manuc T, Chiriac DE, Chifulescu AE. Signification of Serum Alpha-Fetoprotein Levels in Cases of Compensated Cirrhosis and Hepatitis C Virus without Hepatocellular Carcinoma. Journal of medicine and life. 2020 Jan;13(1):68.
18. Shabana MA, Abdelkader AH, Afifi SA. Evaluation of HCV-associated Hepatocellular Carcinoma based on Alpha-fetoprotein levels. Afro-Egyptian Journal of Infectious and Endemic Diseases. 2020 Mar 1;10(1):10-5.
19. Marsch W. Determination of arginase activity. Clin. Chem. 1965;11:624.
20. Fahey JL, McKelvey EM. Quantitative determination of serum immunoglobulins in antibody-agar plates. The Journal of Immunology. 1965 Jan 1;94(1):84-90.
21. Mancini GJ, Carbonara AT, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. immunochemistry. 1965 Sep 1;2(3):235-IN6.
22. Hirai H, Chu TM. Biochemical markers for cancer. Marcel Decker Inc., New York. 1982:25-59.
23. Reitman S, Frankel S. Colorimetric methods for aspartate and alanine aminotransferase. Am. J. Clin. Pathol. 1957;28:55-60.
24. Jendrassik L, Grof P. Estimation of total serum bilirubin level by spectrophotometrically in serum and plasma. Biochem Zeitschrift. 1938;297:81-9
25. DOUMAS BT, BIGGS HG, ARENDS RL, PINTO PV. Determination of serum albumin. InStandard methods of clinical chemistry 1972 Jan 1 (Vol. 7, pp. 175-188). Elsevier.
26. Gomaa A, Allam N, Elsharkway A, El Kassas M, Waked I. Hepatitis C infection in Egypt: prevalence, impact and management strategies. Hepat Med. 2017; 9: 1725.
27. Stepanova M, De Avila L, Afendy M, Younossi I, Pham H, Cable R, Younossi ZM. Direct and indirect economic burden of chronic liver disease in the United States. Clinical Gastroenterology and Hepatology. 2017 May 1;15(5):759-66.
28. Hwang HM, Lee JH, Min BS, Jeon BH, Hoe KL, Kim YM, Ryoo S. A novel arginase inhibitor derived from scutellavia indica restored endothelial function in ApoE-null mice fed a high-cholesterol diet. Journal of Pharmacology and Experimental Therapeutics. 2015 Oct 1;355(1):57-65.
29. Machida K, Cheng KT, Sung VM, Lee KJ, Levine AM, Lai MM. Hepatitis C virus infection activates the immunologic (type II) isoform of nitric oxide synthase and thereby enhances DNA damage and mutations of cellular genes. Journal of virology. 2004 Aug 15;78(16):8835-43.
30. Hassan MI, Kassim SK, Ali HS, Sayed ED, Khalifa A. Evaluation of nitric oxide (NO) levels in hepatitis C virus (HCV) infection: relationship to schistosomiasis and liver cirrhosis among Egyptian patients. Disease markers. 2002 Jan 1;18(3):137-42.
31. Eissa LA, Eisa NH, Ebrahim MA, Ragab M, El-Gayar AM. Nitric oxide is a potential diagnostic marker for hepatocellular carcinoma. Scientia pharmaceutica. 2013 Sep;81(3):763-76.
32. Zhang X, Jin L, Tian Z, Wang J, Yang Y, Liu J, Chen Y, Hu C, Chen T, Zhao Y, He Y. Nitric oxide inhibits autophagy and promotes apoptosis in hepatocellular carcinoma. Cancer science. 2019 Mar;110(3):1054-63.
33. Keshari RS, Jyoti A, Dubey M, Kothari N, Kohli M, Bogra J, Barthwal MK, Dikshit M. Cytokines induced neutrophil extracellular traps formation: implication for the inflammatory disease condition. PloS one. 2012 Oct 26;7(10):e48111.
34. Ashfaq UA, Javed T, Rehman S, Nawaz Z, Riazuddin S. An overview of HCV molecular biology, replication and immune responses. Virology journal. 2011 Dec;8(1):1-0.
35. Oguz A, Atay AE, Tas A, Seven G, Koruk M. Predictive role of acute phase reactants in the response to therapy in patients with chronic hepatitis C virus infection. Gut and liver. 2013 Jan;7(1):82.
36. Fennelly C, Amaravadi RK. Lysosomal biology in cancer. Lysosomes. 2017:293-308.

37. Tai WC, Hu TH, Wang JH, Hung CH, Lu SN, Changchien CS, Lee CM. Clinical implications of alpha-fetoprotein in chronic hepatitis C. Journal of the Formosan Medical Association. 2009 Mar 1;108(3):210-8.
38. Wang X, Wang Q. Alpha-fetoprotein and hepatocellular carcinoma immunity. Canadian Journal of Gastroenterology and Hepatology. 2018 Oct;2018.
39. Abdoul H, Mallet V, Pol S, Fontanet A. Serum alpha-fetoprotein predicts treatment outcome in chronic hepatitis C patients regardless of HCV genotype. PLoS One. 2008 Jun 11;3(6):e2391.
40. Emokpae MA, Adejumol BG, Abdu A, Sadiq NM. Serum alpha-fetoprotein level is higher in hepatitis C than hepatitis B infected chronic liver disease patients. Nigerian medical journal: journal of the Nigeria Medical Association. 2013 Nov;54(6):426.
41. Sakata K, Hara M, Terada T, Watanabe N, Takaya D, Yaguchi SI, Matsumoto T, Matsuura T, Shirouzu M, Yokoyama S, Yamaguchi T. HCV NS3 protease enhances liver fibrosis via binding to and activating TGF- type I receptor. Scientific reports. 2013 Nov 22;3(1):1-7.
42. Khatun M, Ray RB. Mechanisms underlying hepatitis C virus-associated hepatic fibrosis. Cells. 2019 Oct;8(10):1249.
43. Yang JD, Dai J, Singal AG, Gopal P, Addissie BD, Nguyen MH, Befeler AS, Reddy KR, Schwartz M, Harnois DM, Yamada H. Improved performance of serum alpha-fetoprotein for hepatocellular carcinoma diagnosis in HCV cirrhosis with normal alanine transaminase. Cancer Epidemiology and Prevention Biomarkers. 2017 Jul 1;26(7):1085-92.