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ORIGINAL ARTICLE

Correlation between Serum Levels of HCV Core Antigen and Liver Enzymes for Assessment of Disease Activity in Chronic Hepatitis C Patients

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ABSTRACT

Background: *Chronic HCV infection and the associated complications represent a significant threat to the medical community particularly in highly endemic areas. HCV core antigen (HCVcAg) in serum or plasma is a marker of HCV replication and viral kinetics. HCVcAg becomes detectable few days after HCV RNA and can be used as a substitute for HCV RNA for the diagnosis of acute or chronic HCV infection.* ***Objective:*** *The objective of this study was to correlate between serum levels of HCVcAg and liver enzymes (surrogate markers of liver damage) for assessment of disease activity in chronic HCV-infected patients among Egyptians.* ***Methodology****: This study involved 28 patients (10 females & 18 males) from those attending the Outpatient Clinic of Shebin El-Kom Fever Hospital with chronic HCV infection. They were classified according to Child Pugh classification into Child-Pugh stage A (71.4%) and stage B (28.6%). All patients were subjected to full history taking, clinical examination, abdominal ultrasound, liver function tests, renal function tests, CBC, fasting blood sugar and measurement of serum HCVcAg level by enzyme linked immunosorbent assay (ELISA).* ***Results:*** *Serum levels of ALT as well as AST were significantly elevated in liver cirrhotic patients. There was a highly significant positive correlation between HCVcAg and ALT, AST, GGT and ALP (P-value <0.001) while there was no statistically significant correlation between HCVcAg and total bilirubin, albumin, AFP levels, PT time, PT concentration and INR (P-value > 0.05).****Conclusion:*** *HCVcAg concentrations had an excellent correlation with liver enzymes levels. HCVcAg could be believed as prognostic marker for disease severity in chronic HCV infection.*

INTRODUCTION

Hepatitis C virus (HCV) infection is wide spread in Egypt and threatens the lives of many Egyptians. Egypt has the highest prevalence of HCV infection in the world among adults (14.7%) [1].

The disease causes chronic hepatic inflammation leading to liver fibrosis, cirrhosis and hepatocellular carcinoma[2].HCV infection is often further complicated by underlying hepatitis B and Shistosoma mansoni co-infection that ultimately precipitates to higher morbidity and chronicity[3].

The HCV core antigen (HCVcAg) is a marker of HCV infection, which is recently developed. It exists in both RNA-free core protein structures and complete virions and has been detected in infected patients' serum. Hence, these assays were used as an alternative to HCV RNA for the diagnosis of active HCV infection as well as for the evaluation of disease activity[4].

Liver is one of organs with the most abundant aminotransferase content. Serum aminotransferase is a sensitive marker to detect liver damage. Alanine aminotransferase (ALT) exists in large quantities in liver cells and is expelled to the circulation during the process of damage. Inflammation reaction caused by infectious or non-infectious agents have great impact on its level[5].

ALT is found in the cytosol, as a liver enzyme it is widely used as an indicator of hepatocellular damage in acute and chronic hepatitis[5, 6].The purpose of the current study is to recognize the correlation between HCVcAg and aminotransferase levels in patients with chronic HCV infection.

METHODOLOGY

Study population:

This study was approved by the Ethical Committee of Faculty of Medicine, Menoufia University in collaboration with Shebin-Elkom Fever Hospital during the period from June 2017 to May 2019 and all patients gave an informed consent. It involved 28 patients from

those attending the Outpatient Clinic of Shebin El-kom Fever Hospital. All patients suffered from compensated liver disease and documented chronic HCV infection by serum hepatitis C antibody testing. According to HCV viral load, the studied individuals were arranged into 3 groups including:

- *Group 1*: Comprised 14 patients (10 males and 4 females) with mild viremia.
- *Group 2*: Comprised 8 patients (5 males and 3 females) with moderate viremia.
- *Group 3*: Comprised 6 patients (3 males and 3 females) with severe viremia.

The patients were subjected to the following investigations:

- Clinical examination and history: Including, jaundice, ascites, hepatic encephalopathy, variceal hemorrhage, bilharziasis, fever, abdominal pain and associated co-morbidities (diabetes & hypertension). Data about exposure to previous operations, dental manipulations, drug abuse, shared razors for males and needle pricks were also collected.
- Assessment of severity of liver affection: According to Child–Pugh score[7].
- Routine laboratory investigations including: Complete blood count (CBC), kidney function tests, serum urea &creatinine.
- Special laboratory investigations including: Liver function tests including serum total bilirubin (TBIL), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-transferase (GGT), alkaline phosphatase (ALP), alpha fetoprotein (AFP), International Normalized Ratio (INR) and prothrombin time (PT) & concentration (PTC). All previously mentioned tests results and HCV viral loads were collected from patient's files.
- **Measurement of serum HCVcAg level by Enzyme linked immunosorbent assay (ELISA):** Venous blood samples (5ml) were aseptically withdrawn in plain vacutainer tubes from all studied groups. The samples were left to clot for 4 hours at room temperature and centrifuged at approximately 1000 x g for 15 min. The clear sera kept frozen at -80°C. Repeated freeze-thaw cycles were avoided.
- ***Principle of the assay:*** Human HCVcAg *Sinogeneclon Co., Ltd* ELISA kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. Purified human HCVcAg antibody has been precoated onto 96-wells of the microtitre plates, made solid-phase antibody, then HCVcAg was added to wells. Combined HCVcAg which with enzyme labeled, become enzyme-antibody antibody-antigen complex, after washing completely, substrate was added, substrate was converted to blue color at HRP enzyme-catalyzed, reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of HCVcAg in the samples was then determined by comparing the O.D. of the samples to the standard curve.

Statistical analysis:

Data collected were tabulated and analyzed by Statistical Package of Social Science (SPSS, version 20; SPSS Inc., Chicago, Illinois, USA) on IBM personal computer. The following statistics were applied: descriptive statistics: e.g. percentage (%), mean ($\overline{X}$) and standard deviation (SD), range and analytic statistics: e.g. Chi-square test, Mann-Whitney test, ANOVA (f) test, Kruskal-Wallis test and The ROC (receiver operating characteristic) curves. P value <0.05 considered to be significant. Spearman correlation coefficient (r) was used to measure the relation between two quantitative variables.

RESULTS

The mean age was 39.1±11.8, 40.4±15.6 and 40.0±15.2 years old for group 1, group 2 and group 3 respectively. No statistically significant difference was found among the three studied groups regarding age, gender distribution, residence, smoking and socioeconomic status (P > 0.05) (table-1).

Table 1: Socio-demographic characteristics of the studied groups

Socio-demographic characteristics	Total cases (No.=28)			Test of significance	P value
	Group 1 (Mild viremia) (No.=14)	Group 2 (Moderate viremia) (No.=8)	Group 3 (Severe viremia) (No.=6)		
Age (year) Mean±SD	39.1±11.8	40.4±15.6	40.0±15.2	**Kruskal Wallis test**=0.01	0.99
Gender					
Male	10 71.4	5 62.5	3 50.0	χ^2=0.86	0.65
Female	4 28.6	3 37.5	3 50.0		
Residence					
Urban	4 28.6	3 37.5	3 50.0	χ^2=0.85	0.65
Rural	10 71.4	5 62.5	3 50.0		
Smoking					
Yes	7 50.0	2 25.0	0 0.0	χ^2=5.08	0.08
No	7 50.0	6 75.0	6 100		
Socioeconomic level					
Low	9 64.3	4 50.0	4 66.7	χ^2=0.55	0.76
Moderate	5 35.7	4 50.0	2 33.3		

χ2: Chi square test

A highly significant statistical difference was observed between the three studied groups regarding ALT, AST, ALP and GGT levels (P<0.001). On the other hand, no statistically significant difference was detected among the three studied groups regarding total bilirubin, albumin, AFP levels, PT time, PT concentration and INR (P> 0.05) (table-2).

Table 2: Specific laboratory investigations of the studied groups

Specific investigations' findings	Total cases (No.=28)			Test of significance	P value
	Group 1 (Mild viremia) (No.=14)	Group 2 (Moderate viremia) (No.=8)	Group 3 Severe viremia (No.=6)		
	Mean±SD	Mean±SD	Mean±SD		
ALT (IU/ml)	59.6±7.8	85.0±20.6	192.0±66.4	**Kruskal Wallis test**=17.6	<0.001**
AST (IU/ml)	46.1±13.3	65.0±23.3	174.2±67.6	**Kruskal Wallis test**=17.14	<0.001**
ALP (IU/ml)	25.8±14.8	75.1±24.4	195.8±68.8	**Kruskal Wallis test**=21.24	<0.001**
GGT (IU/ml)	12.7±7.5	32.1±10.8	63.3±12.1	**Kruskal Wallis test**=20.14	<0.001**
Total bilirubin (mg/dl)	1.1±0.27	1.2±0.84	1.8±1.2	**Kruskal Wallis test**=0.73	0.69
Albumin (g/dl)	3.6±0.54	3.7±0.39	3.4±0.37	**ANOVA test**=0.85	0.44
PT (Seconds)	14.7±2.1	14.5±1.6	14.9±2.2	**ANOVA test**=0.08	0.92
PT Concentration (%)	86.3±11.5	86.8±9.4	84.9±13.1	**ANOVA test**=0.05	0.95
INR	1.2±0.19	1.2±0.14	1.2±0.18	**ANOVA test**=0.09	0.91
AFP (ng/ml)	5.9±2.5	5.1±3.5	5.3±2.9	**Kruskal Wallis test** =0.59	0.74

**highly significant difference

About 78.6% (11/14),75% (6/8) and 50% (3/6) of group 1, group 2 and group 3 respectively were class A as per Child-Pugh score, while 21.4% (3/14), 25% (2/8) and 50% (3/6) of group 1, 2 and 3 respectively were class B. Collectively 71.4 % (20/28) and 28.6% (8/28) of the studied patients belonged respectively to class A and B of Child-Pugh classification but with no significant statistical difference (P> 0.05) (figure-1).

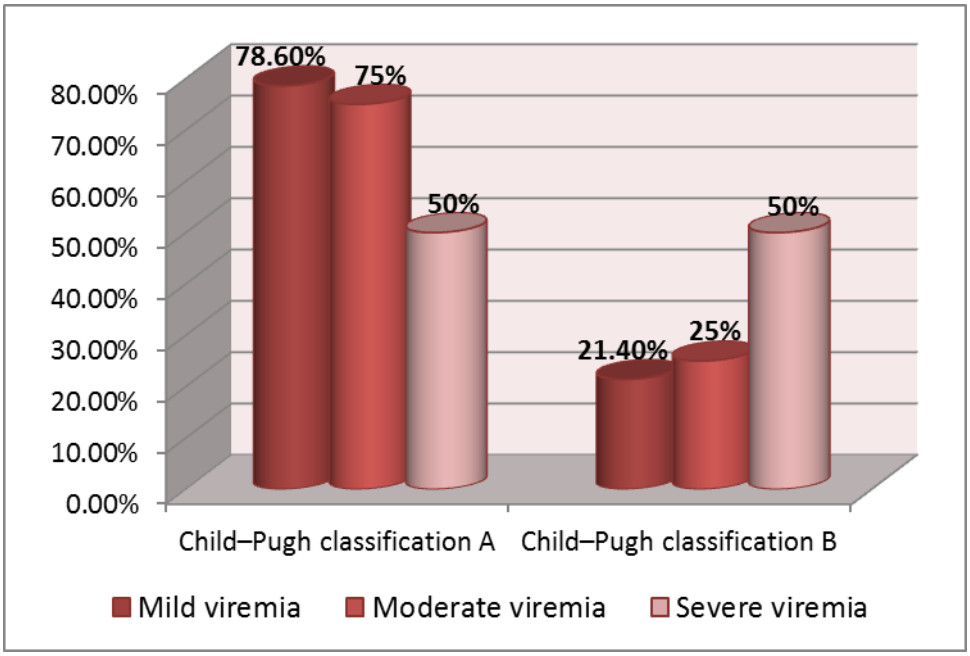

Fig. 1: Comparison between the three studied groups regarding Child-Pugh classification

A highly significant positive correlation was noted between HCVcAg and specific laboratory investigations regarding ALT, AST, ALP and GGT levels (P< 0.001). However, no significant statistical correlation was found between HCVcAg and total bilirubin, albumin, ALP levels, PT time, PT concentration and INR (P> 0.05) (table-3) and (figure-2).

Table 3: Correlation between of HCVcAg level and specific laboratory investigations of the studied groups

Specific laboratory investigations	Baseline HCV core antigen (Peiu/ml)	
	r	P value
ALT (IU/ml)	0.885	<0.001**
AST (IU/ml)	0.874	<0.001**
ALP (IU/ml)	0.990	<0.001**
GGT (IU/ml)	0.954	<0.001**
Total bilirubin (mg/dl)	0.024	0.91
Albumin (g/dl)	-0.316	0.10
PT (Seconds)	0.204	0.29
PT Concentration (%)	-0.227	0.24
INR	0.227	0.25
AFP (ng/ml)	0.022	0.91

**highly significant difference

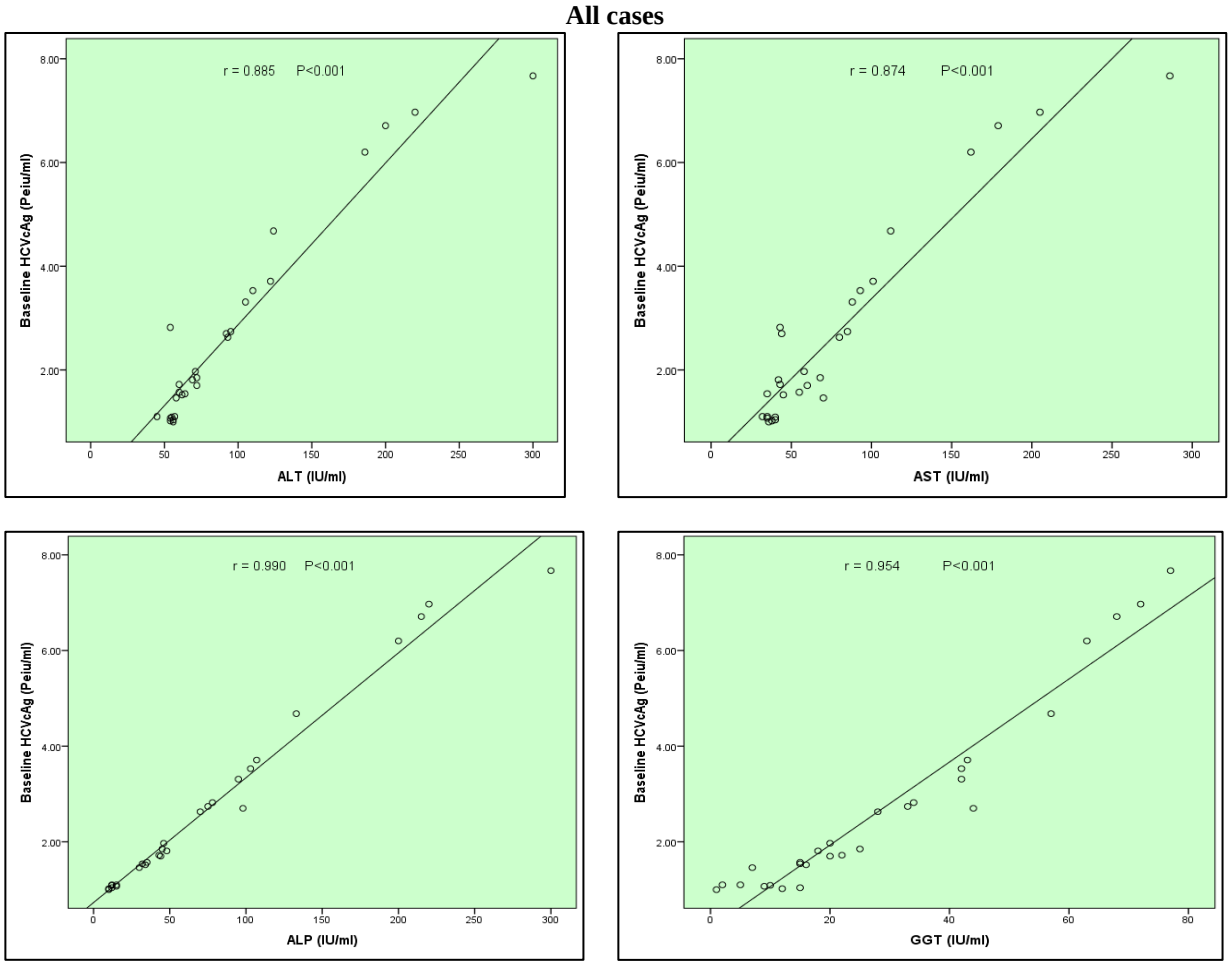

Figure 2: Correlation between of HCVcAg level and specific laboratory investigations of the studied groups

The mean values of HCVcAg in Child's class A and Child's class B patients were 2.5±1.9 and 3.4±2.1 Peiu/ml respectively but with no significant statistical difference between the two classes(P> 0.05) (table -4).

Table 4: Comparison between Child Pugh A and B classifications regarding HCVcAg baseline

Parameter	Total cases (No.=28)		Mann Whitney test	P value
	Child class A (No.=20) (71.4%)	Child class B (No.=8) (28.6%)		
	Mean±SD	Mean±SD		
Baseline HCVcAg	2.5±1.9	3.4±2.1	1.37	0.17

DISCUSSION

Assays for the detection of the HCVcAg, a viral protein released into the plasma during viral assembly, have been developed as a more stable, affordable, alternative to HCV nucleic acid tests. Several commercial HCVcAg assays are now available and have demonstrated highly sensitive and specific diagnoses of active HCV infection in a range of populations[8].

A quantitative HCVcAg test has been developed for the confirmation of viremia in patients with hepatitis C. This test can detect total nucleocapsid core antigen whose sequence is highly conserved across HCV genotypes[9].

The liver is an organ that contains many aminotransferase enzymes, this enzyme will be released in large quantities to the circulation during liver damage due to inflammation. High level of ALT serum in blood indicates the occurrence of necro- inflammation. Liver damage appears in the process of immune clearance, long response of immune processes will increase the risk of liver cirrhosis. The examination of ALT enzymes gives important information about liver function state.

According to *Fabris et al*[10] the levels of ALT enzyme increase after 10-14 weeks of initial infection and the elevation reaches up to 20 times of the normal limit level but a slight increase in ALT levels is often found mainly in chronic infections, as found in this study.

In the present study, the percentages of male CHC patients were higher than females representing 71.4% (10/14) for group 1, 62.5% for group 2 (5/8) and 50% (3/6) for group 3 while female patients comprised 28.6% (4/14) for group 1, 37.5% (3/8) for group 2, 50% for group 3.This result was confirmed by Rong et al[11]who documented that the percentage of male donors who were HCV viremic was about 3.8 times as many as that of the female donors (79.2% versus 20.8%). It has been reported that women are more likely to clear the virus spontaneously after acute infection. However, men are more likely to develop chronic hepatitis and continue to be HCV viremic[12].

It is firmly believed that the outcomes of HCV infection among women are much better than among men. In support of this belief, there exist additional lines of evidence: 1) HCV is more likely to infect men. In the USA, the prevalence of anti-HCV among men was twice as that among women [13]. 2) The male gender has been considered to be one of the key factors in promoting the hepatic fibrosis progression as a consequence of chronic HCV infection[14].3) Female hormones have been documented to act as inhibitors against HCV[15, 16].

According to the current results, there was no statistically significant difference between the three viremic groups (mild, moderate and severe) regarding the age and gender. This comes in agreement with the results documented by other Egyptian studies[17, 18]. In another study by *Rong et al*[11], statistical analyses on 299 voluntary blood donors revealed that the viral loads were significantly different between males and females. The mean viral load among male donors was 6.06 $\log^{10}$ IU/ml comparing to 5.69 $\log^{10}$ IU/ml among female donors (t=2.785, P=0.006). The author documented that, the viral loads were independently correlated with the detected gender, which disagreed with our results. This could be explained by larger study population for *Rong et al*[11]who also reported no statistically significant difference between viral load and age (P=0.177) which agreed with our results (P>0.005).

According to current results, serum levels of ALT as well as AST (surrogate markers for liver damage) were significantly elevated in liver cirrhotic patients where, the mean serum level of ALT was 59.6±7.8, 85.0±20.6 and 192.0±66.4 IU/L and AST was 46.1±13.3, 65.0±23.3 and 174.2 ±67.6 IU/L respectively for group 1, 2 and 3with a statistically significant difference among the three groups (<0.001). This comes in agreement with other published data that showed a highly significant increase of ALT and AST levels in patients with chronic HCV infection[19].

Schuppan and Afdhal[19]explained this observation by leakage of these enzymes from damaged hepatocytes. Liver function tests (ALT and AST) were done for the cases and the results ranged from normal values to four times the normal value.

The average levels of ALT was higher compared with AST levels in the present study, it is caused by the fact that ALT as a cytosolic enzyme that is found with high concentrations and more specific for the liver. AST enzymes are found in the liver, also in heart muscle, brain, kidney, skeletal muscle, lung and pancreas. This enzyme is less specific for the liver. Such observation came in parallel with Yerizel et al[20] who also reported that the average levels of ALT was higher compared with AST levels in chronic HCV patients.

Regarding other liver enzymes, there was a highly significant statistical difference between the three studied groups regarding ALP and GGT levels (P<0.001). Sayed et al[17]also documented a highly significant statistical difference between the three studied viremia groups regarding ALP and GGT levels (P<0.001). Yerizel et al[20]and Maheshwari et al[21] postulated that, there was no significant correlation between viral load and aminotransferase levels (both ALT and AST) which disagreed with our study.

In the existing data, serum albumin, a surrogate marker of liver synthetic capacity, was within normal values. Its mean value was 3.6±0.54 g/dl for group 1, 3.7±0.39 g/dl for group 2 and 3.4± 0.37 g/dl for group 3. This coincided with the results of *Alboraie et al*[22]who documented that fibrosis stage was negatively correlated with albumin (albumin, g/l, Mean ± SD 45.91 ± 3.11 for fibrosis stage 0 (F0), 45.83 ± 3.32 for F1, 44.63 ± 1.99 for F2, 44.62 ± 3.08 for F3 and 40.35 ±5.95 for F4). *Schuppan* and *Afdhal*[19] explained this observation by decreased hepatic production of albumin.

Also, in our study, INR was mildly elevated in the studied groups of patients with a mean level of 1.2±0.19 for group 1, 1.2±0.14 for group 2 and 1.2±0.18 for group 3.This came in agreement with Wiegand and Berg[23], who explained this finding by impaired hepatic biosynthesis of factor V/VII (while thrombin production is maintained).

In the present study, total bilirubin, albumin, AFP levels, PT time, PT concentration and INR, surrogate markers of liver synthetic capacity has no statistically significant difference between the three studied groups (P> 0.05). These results were in consistence with Sayed et al[17] and Baraka et al[18]who reported also no statistically significant difference between HCV viremia and all previous parameters (P> 0.05).

In our study the liver disease severity was estimated according to Child Pugh classification.71.4 % of the studied patients have displayed a Child-Pugh stage A and 28.6% belonged to Child-Pugh stage B, but there were no Child-Pugh stage C patients. This is attributed to exclusion criteria according to Supreme Council and

NCCVH Hepatitis C updated Treatment Protocol December, 2019 which excluded Child-Pugh score stage C. This came in contrast with an Egyptian study conducted by Attia et al[24], in which about 60% of patients have displayed a Child-Pugh score C that amounts to severe liver disease. This could be owing to that infection with hepatitis B virus was the cause of cirrhosis in 70% of the cases and the fact that most of their patients were admitted at a late stage of the disease for different reasons; lack of insurance coverage, delay in hospital visit because of financial problems or cultural considerations.

In the present study, the mean values of HCVcAg baseline levels were 2.5±1.9 and 3.4±2.1 Peiu/ml respectively for Child's class A and Child's class B patients but with no significant statistical difference between the two classes (P> 0.05).This could be attributed to the fact that most of our patients were admitted at an early stage of the disease so they almost had mild liver disease.

The current study proved a highly significant positive correlation between HCVcAg and ALT, AST, GGT and ALP with a correlation coefficient of r=0.885 (P<0.001) for ALT, r=0.874(P<0.001) for AST, r=0.990, (P<0.001) for ALP and r=0.954(P<0.001) for GGT. These results were in consistence with Sayed et al[17]who reported also a highly significant positive correlation with ALT (r=0.788, P=0.000) AST (r=0.797, P=0.000), GGT(r=0.657, P=0.000) and ALP (r=0.564, P=0.000).

Baraka et al[18]documented a significant correlation between HCVcAg and AST serum level in chronic HCV patients. Fan et al[25] also found that the baseline HCVcAg levels were significantly correlated with levels of ALT (r=0.416, P=0.001), AST (r=0.453, P=0.001) and AST/ALT ratio (r=0.201, P=0.001).On the contrary, Duy Thong et al[26] revealed that HCVcAg levels were not correlated with serum ALT levels (r=–0.045, p=0.542) which disagreed with our results. The author explained this observation by the fact that, in some chronic hepatitis patients, serum ALT levels are intermittently elevated**,** but the height of elevations correlates poorly with disease activity and at least one third of infected persons have persistently normal ALT levels despite the viral load [27].

CONCLUSION

Based on the outcome of this study, it can be concluded that a highly significant positive correlation between HCVcAg levels with liver enzymes levels indicates that HCVcAg estimation could have an important role in predicting severity of liver disease in CHC patients. However, further research on a wider population scale is to be recommended.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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