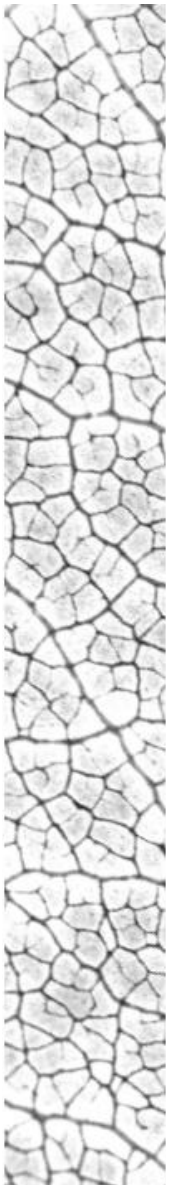

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Study the relationship between Interleukin-35 and clusterin with Mda, Gsh, Cat and Sod among male Iraqi chronic Hepatitis C patients

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Abstract

Background: Chronic Hepatitis C (CHC) disease is known as a main public health problem through the world. CHC disease might lead to fibrosis of hepatocytes. In present paper, we attempted to measure whether there is any association among IL-35 and CLU with MAD, GSH, CAT and SOD, and several biochemical variables such as AST, ALT, GGT, ALP, T. BIL, and albumin. **Methods:** This study has included 44 patients from the Al-Fallujah Teaching Hospital, who was having CHC disease, and 44 healthy controls (HCs) were registered. Serum AST, ALT, GGT, ALP, T. BIL, albumin concentrations were estimated by colorimetric methods, IL-35 and CLU, MAD, GSH, CAT and SOD levels were estimated by ELISA technique. **Results:** Compared with healthy controls, the serum IL-35 and CLU, GSH, CAT and SOD concentrations were lower of patients with CHC and the serum MDA values were higher ($P<0.0001$, all), the serum IL-35 and CLU concentrations related positively with serum GSH, CAT, SOD and albumin, and negatively correlated with MDA, AST, ALT, GGT, ALP and T. BIL concentrations (all $P<0.01$). The area under the receiver operating characteristic (AUROC) curve of serum for MDA, CLU, SOD, CAT, IL-35 and GSH levels were 0.9904, 0.9403, 0.9274, 0.9246, 0.8515 and 0.82, respectively. **Conclusion:** Correlation among IL-35 and CLU with MAD, GSH, CAT and SOD may be attractive markers to predict disease development in CHC patients**.**

Keywords: Interleukin-35, clusterin, oxidative stress, chronic Hepatitis C

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INTRODUCTION

Hepatitis C virus (HCV) which detected in 1989, it has been a worldwide concern of public health.(Houghton, 2019; Mokhtari, et al. 2016). About 3% of the world populace is now HCV diseased. HCV contagion often over time progresses into chronic hepatitis C, annually claiming about 500 000 person (Moosavy, et al. 2017). Hepatocellular carcinoma and cirrhosis, two of the mutual threatening of life problems of CHC are assessed to have affected 8% and up to 30% of the CHC diseased persons, respectively (Baumert, et al. 2017). Interleukin (IL)-35 is recently recognized IL-12 family members, which consist of binary subunits of heterodimeric, IL27β chain and IL12α chain p35 (Collison, et al. 2007). It is chiefly excreted by $CD8^+$ and $CD4^+$ regulatory T cells (Tregs), stimulated regulatory B cells, in addition to, dendritic cells and exhibits functions of immunosuppressive in several contagious diseases, autoimmune disorders and many types of cancers (Zhang, et al. 2019). The activity of immunosuppressive of IL-35 was chiefly regulated via Tregs proliferation function and stimulation, inhibition of, $CD8^+$ T cell cytotoxicity and differentiation Th17 in chronic and acute viral hepatitis (Teng, et al. 2019). Clusterin (CLU) is a chaperone molecular liable for sustaining folding of protein of excreted proteins and it has three isoforms which differentially involved in processes of anti - or proapoptotic, CLU is participate in various diseases linked to oxidative stress (OS), such as inflammatory diseases, aging, neurodegenerative diseases and many types of cancers (Sansanwal, et al. 2015). CLU action is similarly participating in contagious diseases, involving CHC. It is encouraged via the stress of CHC contagion, which disorders regulation of glucose. Immunostaining cytoplasmic CLU was distinguished to relate with poor diagnosis in hepatocellular carcinoma patients. Contagion encouraged OS consequences in the producing of reactive oxygen species (ROS) that stimulate reactions cascade of, lipids, nucleic acids or proteins, producing poisonous reactive types (Kang, et

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al. 2004). Chains of polyunsaturated fatty acid (PUFA) in membranes may be turn out via ROS into peroxides of lipid that change the permeability and fluidity of membrane, or whose smashing products effectiveness many types of proteins and change their biological roles. Antioxidants, for example E vitamin remove free radicals and inhibit their harmful properties. Many previous papers indicated the encouragement of replication of HCV via many lipophilic antioxidants and E vitamin, and its suppression via PUFAs, together in primary liver cultures and in hepatoma cells (Yamane, et al. 2014)., (Huang, et al. 2007)..Peroxidation of lipids disturbs the morphogenesis of membranous web (Huang, et al. 2007). Moreover, OS is related with the growth of carcinoma of hepatocellular (Fujinaga, et al. 2011). which occurrence is similarly augmented in CHC disease (Fattovich, et al. 2004). The main organelles in the manufacture of OS are the mitochondria, through dehydrogenases enzymes of mitochondria and their electron transport chain (ETC) centers.(Rigoulet, et al. 2011). These sites are the best targets of the HCV which leads to CHC disease.(Barbaro, et al. 1999). Chronic inflammation of liver leads to OS and peroxidation of lipid, producing additional ROS, and many types of aldehydes which may interact with nitrogen bases of DNA to produce DNA promutagenic adducts by either exogenous or endogenous abuses (Bartsch, & Nair, 2006). Thus, OS biomarkers may guess CHC danger and moreover the CHC reappearance. Methods for the quantitative estimation of OS may be placed in three groups: (1) compounds changed via OS evaluation (2) the action of antioxidant enzymes evaluation (3) OS signs having factors of transcription evaluation. Quantification of serum reactive oxygen metabolites derivatives concentration, a simple process for determining ROS, is establish to guess the risk of reappearance of CHC after ablation of radiofrequency or surgical resection.(Suzuki, et al. 2013). For that reasons, our study aims to estimate serum IL-35, CLU, MAD, GSH, CAT and SOD concentrations and calculation of the existence of any relationship among CHC patients with these biomarkers, namely relating among IL-35 and CLU with liver enzymes, catalase (CAT) and super oxide dismutase (SOD), also glutathione (GSH) and malondialdehyde (MDA) in CHC patients.

MATERIALS AND METHODS

Present study involved 44 male Iraqi patients with CHC contagion who joined the outpatient clinic of the Al-Fallujah Teaching Hospital (Fallujah / Anbar / Iraq) from July 2018 to February 2019. Criteria of exclusion were hepatitis B surface antigen positivity, human immunodeficiency virus (HIV) coinfection, primary kidney diseases, leukocytosis (total leukocyte count>12000 cells/μL), and addicts of drug, smokers or have taken vaccine of HBV, or previous history of CHC therapy, patient with carcinoma of hepatocellular or any additional cancers were excepted from the data. Forty four seemingly healthy employees in line in age, sex and ethnic background were used as HC group. All subjects provided informed printed agreement before registration, and the search was permitted by the University of Anbar ethics board and was completed with detail to the Helsinki Statement. Tests of routine laboratory such as serum concentrations of AST, ALT, ALP, GGT, total bilirubin (T.BIL) and albumin were done by a commercial kits equipped from Spanish company Linear and using color measurement methods; furthermore serum levels of IL-35, CLU, MAD, GSH, CAT and SOD were measured by ELISA technique by using kits manufactured by U.S.A company MyBioSource.

Statistical Analysis

All biomarkers were stated as mean, standard deviation (S.D) and mean of standard error (MSE). All statistical tests were 2-tailed, and data were using Paired t test for compared and to calculate correlations between all parameters, Pearson's correlation coefficient (r) was performed, we applied Receiver operating characteristic (ROC) curve study to evaluate the general investigative performance of each assessment, and evaluated of method was done by the exploration of area under the receiver operating characteristic curve (AUROC). P-values less than or equal 0.05 were showed as a statistical significant differences. Data statistical investigation was done by the statistical software of Graphpad Prism version 7.04 and Statistical Package for Social Science (SPSS version 25.0).

RESULTS

This study involved 88 males (44 patients and same number of healthy persons as control group). The mean age (years) of patients and HCs was 48.33 and 46.98 (p=0.34) respectively as shown in **Table 1**, the mean value of AST, ALT, GGT and ALP (U/L) of patients was 86.93, 81.27, 97.34 and118.3 which decreased in the healthy group of values 26.34, 21.52, 50.95 and 86.64 (<0.0001) respectively, as shown in **Table 1** and **Figs. 1**, **2**, **3**, **4**, respectively, while the mean level of T.BIL (mg/dL) of patients was1.384 which decreased in the healthy group to value 0.8491 (<0.0001) as shown in **Table 1** and **Fig. 5**, but the mean level in patients with CHC of albumin (g/dL) was higher as compared to HCs 3.473 *vs* 4.352 (<0.0001) as shown in **Table 1** and **Fig. 6**.

Table 1. Distribution of Studied variables in CHC patients and healthy control group

	Healthy controls			Patients (CHC)			
Parameter	**Mean**	**SD**	**SEM**	**Mean**	**SD**	**SEM**	**p-value**
Age years	48.33	11.673	1.343	46.98	10.425	1.192	0.1434
AST U/L	26.34	6.397	0.9645	86.93	18.06	2.722	<0.0001
ALT U/L	21.52	7.911	1.193	81.27	20.53	3.094	<0.0001
GGT U/L	50.95	18.59	2.803	97.34	34.35	5.178	<0.0001
ALP U/L	86.64	19.52	2.943	118.3	23.01	3.468	<0.0001
T.BIL mg/dL	0.8491	0.2156	0.03251	1.384	0.351	0.05353	<0.0001
Albumin g/dL	4.352	0.727	0.1096	3.473	0.669	0.1009	<0.0001
IL-35 pg/ml	28.39	13.28	2.002	10.17	5.264	0.7937	<0.0001
CLU ng/ml	6.436	0.8885	0.1339	4.6	0.8195	0.1235	<0.0001
MAD ng/ml	7.095	1.651	0.2489	18.93	6.331	0.9545	<0.0001
GSH µg/ml	6.132	2.013	0.3035	3.625	1.396	0.2104	<0.0001
CAT µg/ml	39.47	11.62	1.752	20.49	5.918	0.8921	<0.0001
SOD µg/ml	53.09	14.61	2.203	30.77	5.757	0.868	<0.0001

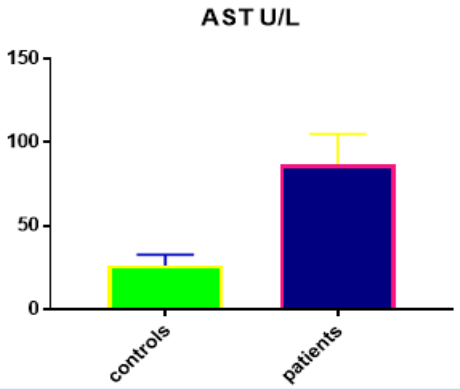

Fig. 1. mean+ S.D for AST

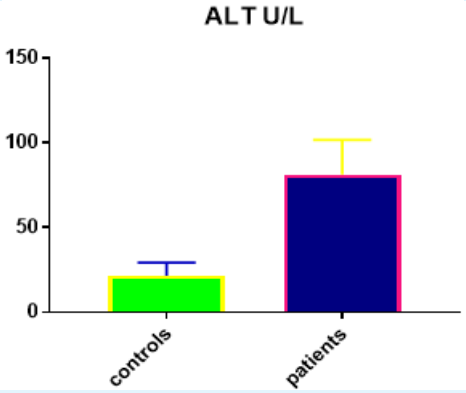

Fig. 2. mean+ S.D for ALT

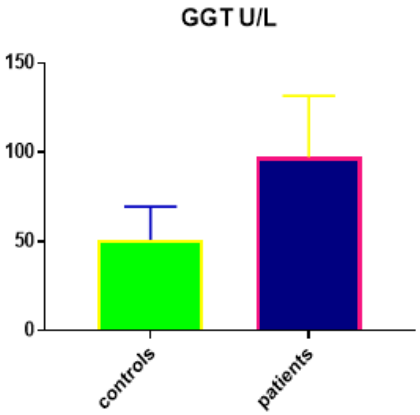

Fig. 3. mean+ S.D for GGT

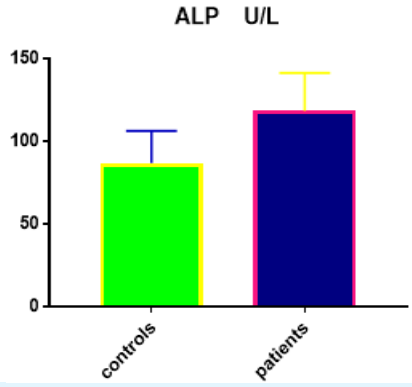

Fig. 4. mean+ S.D for ALT

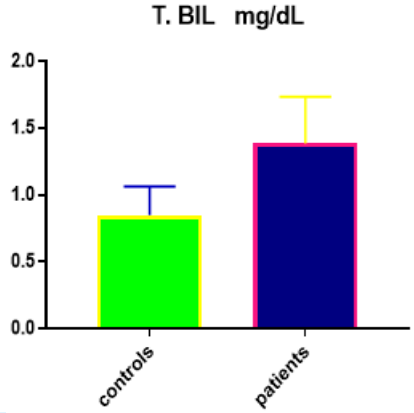

Fig. 5. mean+ S.D for T. BIL

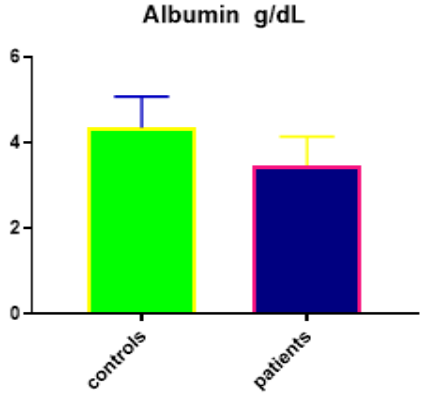

Fig. 6. mean+ S.D for Albumin

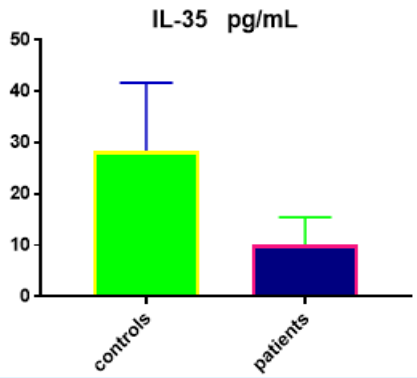

Fig. 7. mean+ S.D for IL-35

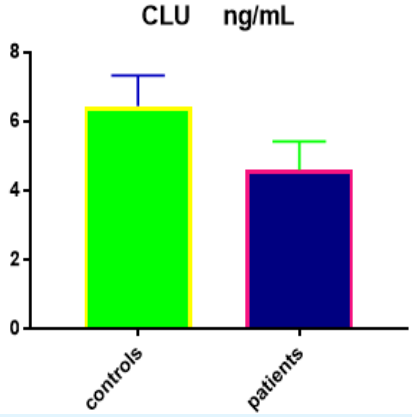

Fig. 8. mean+ S.D for CLU

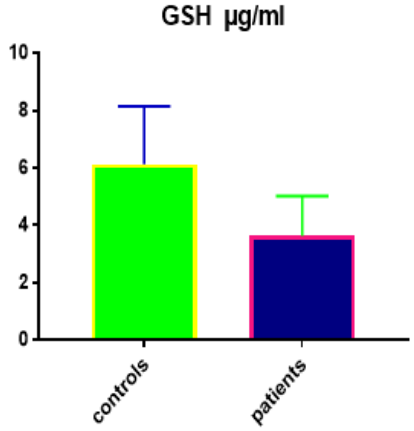

Fig. 9. mean+ S.D for GSH

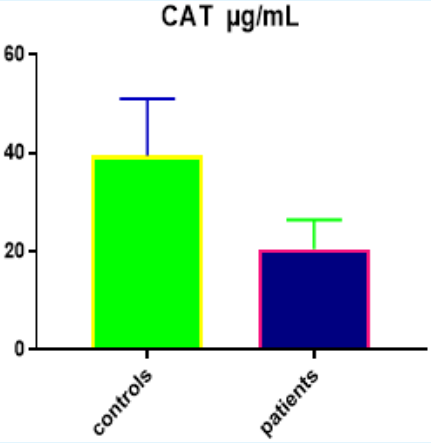

Fig. 10. mean+ S.D for CAT

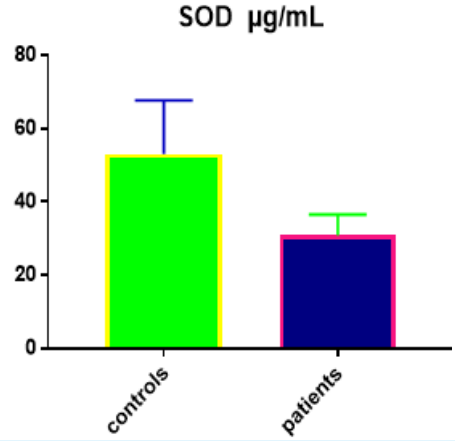

Fig. 11. mean+ S.D for SOD

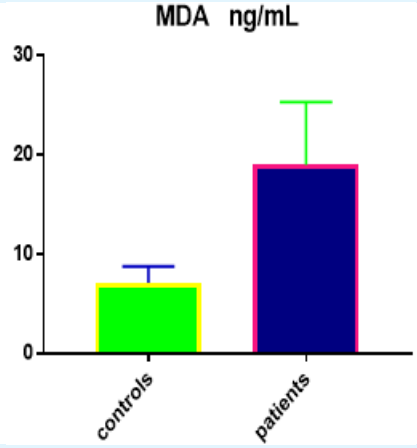

Fig. 12. mean+ S.D for MDA

Table 2. Correlation of IL-35 and CLU with all studied parameters

parameter	IL-35	CLU
IL-35	1	0.461*
CLU	0.461*	1
GSH	0.567*	0.401*
CAT	0.433*	0.465*
SOD	0.542*	0.528*
MAD	-0.520*	-0.614*
AST	-0.636*	-0.643*
ALT	-0.572*	-0.718*
GGT	-0.367*	-0.591*
ALP	-0.432*	-0.386*
T. BIL	-0.465*	-0.485*
Albumin	0.419*	0.466*

*P is significant; it was less than 0.01 for all variables

It is clear from the present study that levels of serum IL-35 (pg/mL), CLU (pg/mL), GSH (µg/mL), CAT (pg/mL) and SOD (pg/mL) were significantly higher in HCs group compared with CHC patients (28.39, 6.436, 6.132, 39.47 and 53.09 *vs* 10.17, 4.6, 3.625, 20.49 and 30.77) (<0.0001) as shown in **Table 1** and **Figs. 7**, **8**, **9**, **10**, **11**, respectively, whereas, MDA (µg/mL) was significantly decreased in HCs group compared with CHC patients (7.095 *vs* 18.93) (<0.0001) as shown in **Table 1** and **Fig. 12**.

Table 2 shows the relationship among serum IL-35 and CLU with all studied parameters in CHC patients. A

Table 3. Diagnostic Criteria of the ROC Curves for Tested Variables in CHC patients

Parameter	AUC	Std. Error	95% confidence interval (CI)	P-value
AST IU/mL	1	0	1 to 1	<0.0001
ALT IU/mL	1	0	1 to 1	<0.0001
GGT IU/mL	0.8856	0.03405	0.8188 to 0.9523	<0.0001
ALP IU/mL	0.8468	0.03941	0.7696 to 0.9241	<0.0001
T. BIL mg/dL	0.9043	0.03004	0.8455 to 0.9632	<0.0001
Albumin g/dL	0.8035	0.04542	0.7144 to 0.8925	<0.0001
IL-35 pg/mL	0.8515	0.04301	0.7672 to 0.9358	<0.0001
CLU ng/mL	0.9403	0.022	0.8972 to 0.9835	<0.0001
MAD ng/mL	0.9904	0.006443	0.9778 to 1.003	<0.0001
GSH µg/mL	0.829	0.0419	0.7469 to 0.9112	<0.0001
CAT IU/mL	0.9246	0.02655	0.8726 to 0.9766	<0.0001
SOD IU/mL	0.9274	0.02745	0.8736 to 0.9812	<0.0001

moderate positive association was noticed between CLU, GSH, CAT and SOD (0.461, 0.567, 0.433 and 0.542; p<0.01 for all variables) respectively, with IL-35, but MDA displayed a moderate negative association (-0.520; p<0.01) with IL-35, also CLU was presented a moderate positive association with IL-35, GSH, CAT and SOD (0.461, 0.401, 0.465 and 0.528; p<0.01 for all variables) respectively, and showed a moderate negative association with MAD (-0.614; p<0.01), while moderate negative association was identified between AST, ALT, GGT, ALP and T.BIL (-0.636, -0.572, -0.367, -0.432 and -0.465; p<0.01 for all variables) respectively with IL-35, but it was exhibited a moderate positive association with serum levels of albumin (0.419; p<0.01), also CLU was presented a moderate negative association with AST, ALT, GGT, ALP and T.BIL (--0.643, -0.718, -0.591, -0. 386 and -0.485; p<0.01 for all variables) respectively, but it was showed a moderate positive correlation with serum levels of albumin (0.466; p<0.01).

Between AST, ALT, GGT, ALP and T.BIL (-0.636, -0.572, -0.367, -0.432 and -0.465; p<0.01 for all variables) respectively with IL-35, but it was exhibited a moderate positive association with serum levels of albumin (0.419; p<0.01), also CLU was presented a moderate negative association with AST, ALT, GGT, ALP and T.BIL (--0.643, -0.718, -0.591, -0. 386 and -0.485; p<0.01 for all variables) respectively, but it was showed a moderate positive correlation with serum levels of albumin (0.466; p<0.01).

Receiver operating characteristic curves were done to evaluate the diagnostic abilities for all studied biomarkers as shown in **Table 3**.

The AUC values of AST and ALT were 1 (95% CI 1-1), **Figs. 13** and **14**, respectively and this was expected because these two variables are very specialized for liver function, while GGT, ALP, T.BIL and albumin showed the following AUC values 0.8856 (95% CI 0.8188 to 0.9523), 0.8468 (95% CI 0. 0.7696 to 0.9241), 0.9043 (95% CI 0.8455 to 0.9632) and 0.8035 (95% CI 0.7144 to 0.8925), (**Figs. 15**, **16**, **17** and **18**, respectively). The AUC values of IL-35 and CLU were 0.8515 (95% CI 0.7672 to 0.9358) and 0.9403 (95% CI 0. 0.8972 to 0.9835), (**Figs. 19** and **20**, respectively), but MDA, GSH, CAT and SOD showed the following AUC values 0.9904 (95% CI 0.9778 to 1.003), 0.829 (95% CI 0. 0.7469 to 0.9112), 0.9246 (95% CI 0.8726 to 0.9766) and 0.9274 (95% CI 0.8736 to 0.9812), (**Figs. 21**, **22**, **23** and **24**, respectively).

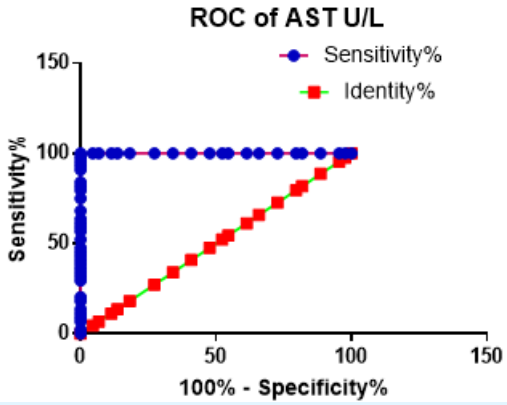

Fig. 13. ROC curve displaying AUC of AST in CHC patients

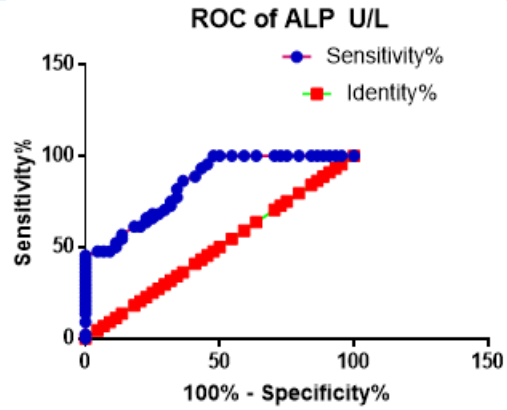

Fig. 14. ROC curve displaying AUC of ALT in CHC patients

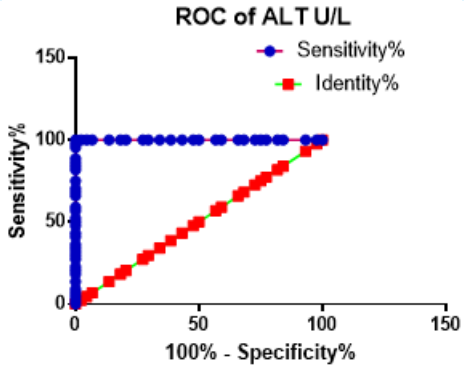

Fig. 15. ROC curve displaying AUC of GGT in CHC patients

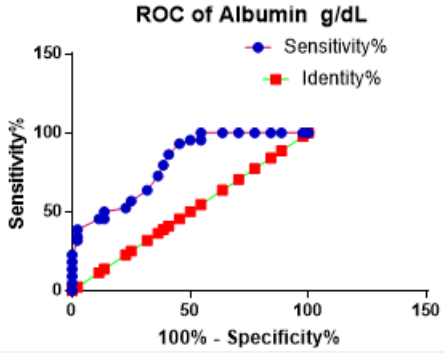

Fig. 16. ROC curve displaying AUC of ALP in CHC patients

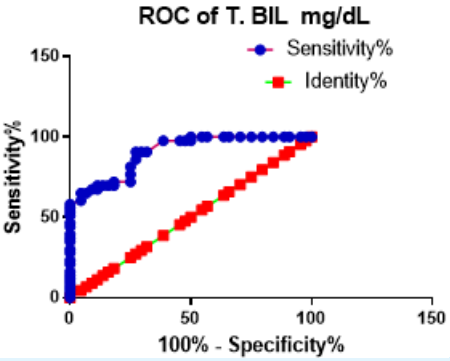

Fig. 17. ROC curve displaying AUC of T.BIL in CHC patients

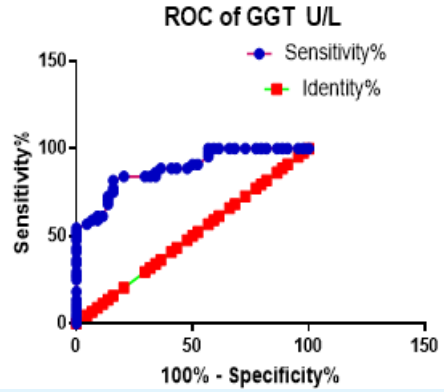

Fig. 18. ROC curve displaying AUC of albumin in CHC patients

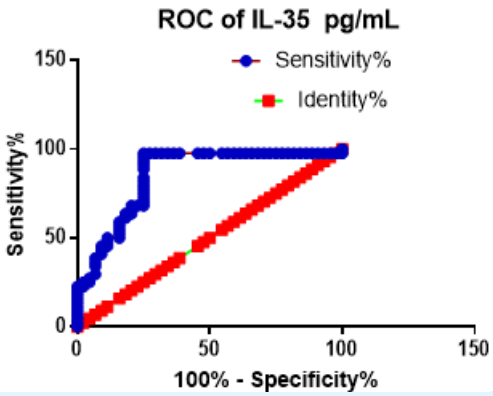

Fig. 19. ROC curve displaying AUC of IL-35 in CHC patients

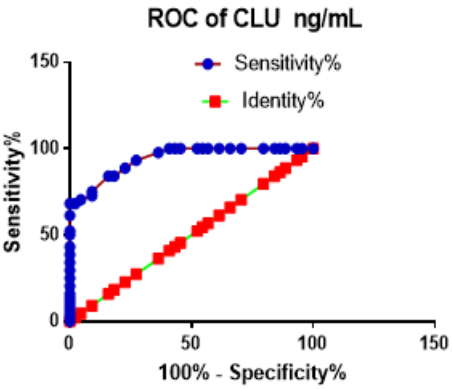

Fig. 20. ROC curve displaying AUC of CLU in CHC patients

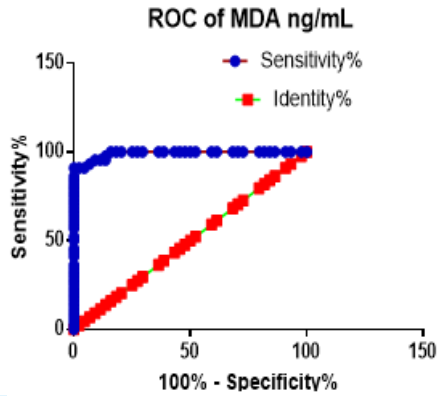

Fig. 21. ROC curve displaying AUC of MDA in CHC patients

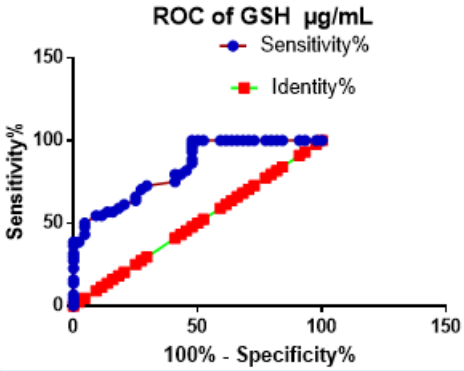

Fig. 22. ROC curve displaying AUC of GSH in CHC patients

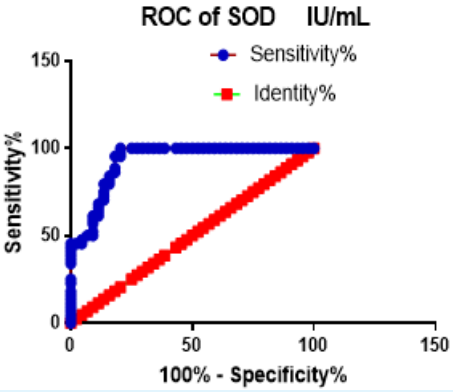

Fig. 23. ROC curve displaying AUC of CAT in CHC patients

DISCUSSION

In the present study, raised serum IL-35 was detected in patients with in as compared to HCs. Furthermore, IL-35 concentration was also positively related with CLU, GSH, CAT, SOD and shows negative relationship with MDA, telling a potential correlation among IL-35 and CHC disease. Previous data showed that IL-35 overexpression prevented growth of cell and augmented sensitivity of apoptosis of different cancer cell lines in human such as cancer of liver, which is the main reason of CHC of liver cells (Long, et al. 2013). More recent article similarly extended the controlling role of IL-35 in $CD25^{+}$, CD127 $CD4^{+}$dim/− Tregs in CHC disease (Liu, et al. 2017). Interleukin35 is noticeable in peripheral CD4T cells and is assumed to exert vital roles in prevention of the immune reply through CHC disease (Li, et al. 2015) proposing it can play possible therapeutic goal to regulator CHC disease (Xiang, & Xie, 2015). Therefore, we examined the biological roles of IL-35 in CHC disease, we demonstrate that high levels of IL-35 may inhibit creation and viral replication in patients with CHC. Serum CLU exhibited an important decline in the CHC group compared to HCs. Also, CLU level was positively associated with IL-35, GSH, CAT, SOD and shows negative association with MDA, telling a possible correlation among CLU and patients with CHC. This may explain a promising defensive function of CLU in CHC which eventually leads to cirrhosis. Previous study reported a reduction in serum CLU in hepatitis B viral liver cirrhosis and alcoholic liver cirrhosis (Wang, et al. 2010). It is a chaperone of Golgi molecular contribute stimulation of the protein kinase B / phosphatidylinositol 3-kinase pathway, extra-cellular signal regulated kinase (ERK) signaling variation, angiogenesis activation, the nuclear factor kappa B (NF-κB) pathway facilitation and processes of antiapoptotic, another biological roles, such as adhesion cell and membrane recycling, apoptosis (activation of p53 by down regulation which leads to apoptosis), transport of lipid, (Sansanwal, & Sarwal, 2015). a very recent data reported an important elevation in the serum CLU concentration in primary period in patients with CHC as regard to last period of CHC (Abd El-Latef, et al. 2019). In CHC infection, the making of ROS is a multifactorial process. Necroinflammatory of longstanding environments may lead to ROS creation, regardless of the steatosis existence. For example, in CHC and irritation and continuing liver cells necrosis are related with an augmented manufacture of ROS (Farinati, et al. 1999). This OS promote liver cells destruction and peroxidation of lipid membrane, leading to reduction of reduced glutathione (GSH) and release of malondialdehyde (MDA) (De Maria, N., Colantonl, A., Fagiuoli, et al. 1996). In our study, CHC disease was related primary with a decline of GSH, and secondary with MDA growth. Our data was established that the serum MDA concentration of CHC patients is greater than that of the HCs. This data was in agreement with the previous article that the patients with CHC have greater level of MDA (Trevisani, et al. 2002), and proposed that further OS and fragile protection by antioxidants system occur in the patients with CHC than the HCs. Telling that level of MDA can rise regularly in CHC physiological process. OS in liver cells are main sign of hepatic stellate cells (HSCs) stimulation and increase of CHC disease. Additionally, this paper indicated that MDA concentration in CHC patients have inverse relationship with IL-35 and CLU. This data established that the difference in concentration of MDA correlate to CHC severity. This data was confirmed that GSH, catalase (CAT), superoxide dismutase (SOD) concentration was declined in serum of patients with CHC. It perhaps reduced the efficiency of antioxidant barrier in studied CHC patients. At what time the action of the antioxidants stated above are inadequate, an animal can not to suppress the excess of ROS which are formed. Therefore, this lead to damage of liver cells.(Chrobot, et al. 2000). In agreement with our results this study demonstrated that CAT and SOD concentrations declined CHC patients (Chrobot, et al. 2000). Another study showed that CHC patients had importantly greater OS, which may lead to CHC disease.(El-Kannishy. et al. 2012)

The current paper presented an important decline in the CAT and SOD activities in CHC patients as regard to HCs. Similarly, an important positive relationship was established among IL-35 and CLU concentrations with CAT and SOD enzymes. It has been recommended that improved OS in patients with CHC might be attributed to the augmented manufacture of many types of cytokines stated to happen in this situation. Stimulated macrophages, Kupffer cells and neutrophils have been stated to be main causes of ROS in the development the process of inflammatory in CHC patients.(Wisniewska-Ligier, et al. 2004)

Catalase is the main antioxidant enzyme has been studied in many diseases related with OS (e.g., neurodegenerative diseases atherosclerosis, hyperlipidaemia, hypertension and diabetes mellitus). OS is associated in these diseases pathogenesis. CAT decays hydrogen peroxide produced via superoxide dismutation which catalysed via SOD. Even if it seems that augmented activities of CAT which could be useful in the metabolic diseases and atherosclerosis pathogenesis, CAT function in these diseases pathogenesis beside with many other enzymes degrading hydrogen peroxide (such as peroxiredoxins and glutathione peroxidase) and SOD should be measured and calculated accurately (Kodydková, et al. 2014). SOD is the first line of protection in the system of cellular antioxidant against oxidative destruction facilitated via superoxide radicals and it is may be the best effective antioxidant (Shaban, et al. 2003).

Decreased activity of SOD in CHC patients in the current study shows the augmented creation of $O_2^{-\cdot}$ radicals in hepatocytes, this in agreement with our results (Ramakrishnan, et al. 2006). Antioxidant biomarker decline, (GSH, CAT and SOD) in CHC patients in this study agree with the previous study which stated that these antioxidants are reduced in CHC patients (Osman, et al. 2007). Conversely, the rise in peroxidation of lipid, in the current study led to membrane permeability increase and inflammation of liver cells which led to the rupture liver cells and contents leakage of liver cells, as a result T.BIL level and AST and ALT activities were raised in patients with CHC as regard to HCs. Conversely the concentrations of albumin were declined as a result of biosynthesis decrease. The discriminatory ability of the biomarkers was evaluated in the study through drawing ROC curves and matching AUC; in the foreground was MDA with the best diagnostic value in patients with CHC through using AUROC examination. Furthermore, OS detected in patients with CHC. OS is formed by the virus itself, by changes in the homeostasis of trace metal and its influence on many types of cytokines, for instance TNF-α.(Sevastianos, et al. 2020). CLU was ranked second in terms of diagnostic ability of the CHC disease, as has been previously mentioned; HCV leads to high levels of oxLDL (Nakhjavani, et al. 2011). Other variables showed the following descending order according to the diagnostic ability of a CHC disease SOD, CAT, IL-35 and GSH respectively. This study has several limitations: firstly, size of sample and some data cannot reach statistical differences, we think that our results may encourage future studies on a greater number of subjects, secondly single center only was used for this study, thirdly the lack fibrosis and histological assessment of liver cells which is a public problem in many studies that are speaking about this infection, fourthly other liver dysfunction biomarkers, such as hepatic stellate cells or alpha fetoprotein was not assessed in patients with CHC, present outcomes must established in a multicenter study with big sample size. Our data only show, but not yet prove, the fundamental relations among serum IL-35 and CLU markers with MAD, GSH, CAT and SOD in patients with CHC. The biological activities of IL-35 and CLU in the CHC pathogenesis and their predictive values in CHC result similarly need many other studies. In conclusion, this research established that serum concentrations of IL-35, CLU, MDA, GSH, CAT and SOD are beneficial indicators for CHC diagnosis. In CHC patients, MDA was a superior biomarker to predicator of disease, serum MDA, GSH, enzymes of antioxidant and enzymes of liver function investigations are beneficial in monitoring damage of liver cells in CHC patients. The current paper was showed the antioxidant barrier insufficiency can lead to OS in CHC patients and, so antioxidant cure may be suitable for patients with CHC, moreover we detected great concentrations of AST, ALT, GGT, ALP, T.BIL and low albumin. Generally, the current data detected important positive relationship of IL-35 and CUL with GSH, CAT, SOD, whereas important negative relationship was conformed to MDA. The above outcomes detect that IL-35 and CLU can control immunity in CHC patients, also we conformed lower serum of antioxidant enzyme levels in CHC patients than in HCs, IL-35, GSH, CAT, SOD and CLU level was a importantly decreased in CHC patients, proposing that serum level of these variables can be using in the diagnosis of CHC disease. MDA is a stable parameter of OS. Oxidative alterations in LDL lipids and proteins produced by ROS. Our study detect greatly important rise in serum MDA concentrations in patients with CHC than in HCs.

Future studies of the IL-35, CLU, GSH, CAT and SOD may lead to novel healing methods of CHC infection, which can exert a vital function in both the detoxification of CHC drug and ROS scavenging. Present study confirmed the role of the OS encouraged through the host cell and the virus; so that we may be probable to found novel and further active healing goals for cure of CHC disease. OS is an important face of CHC disease, the extra noticeable of OS variables, which means the higher viral load. Serum MDA concentrations can reflect a possible predictive variable for the progress of CHC disease and should be confirmed in prospective studies in the future. We can conclude that serum CLU did superior than serum IL-35 in all features of predictive performance of CHC disease. However, additional studies of bigger sample populaces and with different functions status of liver will be necessary to test whether serum CLU is related with etiologies of specific liver disease**.** We also confirmed augmented MDA and declined GSH concentrations, antioxidant enzymes activity may lead to CHC disease, in consent the view that damage caused by OS exerts a main function in CHC disease. Still, the correlation among MDA and GSH concentrations, the activity of antioxidant enzymes, and variations of liver function are fairly humble.

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