RESEARCH PAPER
INTERNATIONAL JOURNAL OF PURE MEDICAL RESEARCH

Medicine

KEYWORDS: Obesity, Pregnancy, Anesthesia

ROLE OF HIGH MOBILITY GROUP CHROMOSOMAL PROTEIN 1 AND CLUSTERIN AS MARKERS IN PATIENTS WITH HEPATITIS C VIRUS RELATED HEPATOCELLULAR CARCINOMA

Volume-2, Issue-6, June - 2017

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Article History
Received: 12.03.2017
Accepted: 06.05.2017
Published: 10.06.2017

ABSTRACT

CLUSTERIN is A highly conserved heterodimeric disulfide-linked 80K glyco- protein originally named apolipoprotein-J (ApoJ) , which is widely distributed in tissues and body fluids. In 1983 identified a high-molecular weight protein in ram rete testis fluid and named Clusterin CLU because it is a cell-aggregating factor capable of eliciting the "Clustering" of Sertoli cells . In humans, it was firstly purified from serum and the cloned gene was named CLI (complement cytolysis inhibitor) or ApoJ due to similarities with other known. It consists of two polypeptide chains connected by four to five disulfide bonds.

Introduction

Hepatocellular carcinoma (HCC) is the primary liver cancer derived from hepatocytes and accounts for 85–90% of all primary liver cancers. It is the 5th common cancer in men and the 7th common cancer in women overall in the world (Ferlay et al., 2015; Ozakyol., 2017). However, it is the third leading cause of cancer-related death, exceeded only by cancers of the lung and stomach. Even HCC is not in the most frequent cancers in the majority of the world, its high mortality and short survival time causes a serious worldwide health burden. Incidence to mortality rate is 0.95 for HCC and 5 years survival is only 6.9%. Because only a minority of the patients are diagnosed in early stages, over all median survival time is as short as 11 months.Annually, the estimated number of new cases is about 782,000 and causin 600,000 deaths globally per year (Ghouri et al.,2017).

In Egypt the incidence of HCC in the past 10 years has been doubled (Shaker et al.,2013). It is the second most common cancer in and the 6th most common cancers in women. It is estimated that the prevalence of HCC will increase in Egypt, reaching its peak by the year 2018 (Azab et al.,2011). The heavy burden of HCC parallels high rates of HCV and its complications while hepatitis B virus (HBV) rates have declined after the introduction of the vaccine in 1992 .Egypt has a significant prevalence of HBV, HCV, bilharzial infection and smoking, which may allow us to identify a high-risk group of patients with HCC among those with chronic liver disease and cirrhosis (Omran et al.,2015) . HCC prevalence is high in Nile Delta area, more common in males, rural residents and farmers especially in HCV patients (Naglaa et al.,2017).

CLUSTERIN is A highly conserved heterodimeric disulfide-linked 80K glyco- protein originally named apolipoprotein-J (ApoJ) , which is widely distributed in tissues and body fluids. In 1983 identified a high-molecular weight protein in ram rete testis fluid and named Clusterin CLU because it is a cell-aggregating factor capable of eliciting the "Clustering" of Sertoli cells . In humans, it was firstly purified from serum and the cloned gene was named CLI (complement cytolysis inhibitor) or ApoJ due to similarities with other known apolipoproteins (Ishaq et al., 2017). It consists of two polypeptide chains connected by four to five disulfide bonds (Matukumalli et al., 2017).

CLU is thought to play diverse functions both cytoprotective and cytotoxic, thus resulting in conflicting results (Materia et al.,2011).For example, its involvement in numerous physiological processes important for carcinogenesis has been reported, including apoptotic cell death, cell adhesion, tissue remodeling, cell cycle regulation, DNA repair, lipid transportation, membrane recycling and immune system regulation(Andersen et al.,2007).Cytoplasmic CLU immunostaining was noted to correlate with poor prognosis in patients with renal cell carcinoma,11 hepatocellular carcinoma,urothelial bladder carcinoma,and prostate adenocarcinoma. Also increased expression of secreted CLU was associated with radioresistance, chemoresistance, and hormone resistance, making CLU a promising target for antitumor therapeutics (Hassan et al.,2014).

High mobility group box 1 (HMGB1), a member of the high mobility group protein family, was originally character¬ized as a non histone, nuclear DNA binding protein (Cheng et al., 2008). It has been implicated in several disease states, including sepsis, arthritis, ischemia re perfusion injury and cancer. It also functions as an extracellular signaling molecule during inflammation, cell differentiation, cell migration and tumor metastasis (Ito et al., 2007). The signal pathways of HMGB1, its receptors include receptor for advanced glycation end product (RAGE) (Cheng et al.,2014), the toll-

like receptors (TLRs, such as TLR-2, 4 and 9) (Conti et al.,2013), intergrin (Friggeri et al.,2010),α-synuclein filaments(Song et al.,2014), proteoglycans (e.g., heparin sulfate, CD24 (Chen et al.,2009), the T-cell immunoglobulin domain and mucin domain-3 (TIM-3) (Chiba et al.,2012) the member of the G protein-coupled receptors CXCR4(Penzo et al .,2010)N-methyl-D-aspartate receptor (NMDAR)(Pedrazzi et al., 2012) and the triggering receptor expressed on myeloid cells-1(TREM1)

Constant release of HMGB1 as a pro inflammatory cytokine from necrotic tumor cells can create a microenvironment similar to that of chronic inflammation, a condition known to contribute to the development of epithelial malignancies. Epithelial mesen¬chymal transition (EMT) was shown to promote the migratory and invasive capacity of HCC cells (Liu et al.,2015).

The search for a serum biological marker of tumor invasiveness and prognosis in HCC is of clinical importance. In this study, we measured the levels of serum HMGB1 and serum CLU analysed the relationship between the HMGB1, CLU and clinic pathologic parameters in patients with hepatocellular carcinoma.

Patients and Methods

A total of 200 adults at Minia University Hospital, Egypt between November 2014 and December 2017 were candidates for this study, but only 190cases fulfilled our inclusion and exclusion criteria. All subjects (or their legal guardians) gave their informed consent to the study, which was approved by the local ethics committee.

Those subjects were subdivided into four groups based on clinical and laboratory characteristics

-Group 1(G1): Healthy subjects (control group). This group included 30 apparently healthy persons with no history of liver disease and no abnormalities in the laboratory investigation.

Group 2 (G2): 30 patients with chronic hepatitis-C virus infection. The diagnosis is based on the presence of anti-HCV antibodies and detectable HCV-RNA for 6 months or more.

Group 3 (G3): 100 patients with hepatocellular carcinoma on top of chronic HCV infection- related cirrhosis. The diagnosis of HCC based on the criteria of Egyptian Society of Liver Cancer (ESLC) 2011.

Group 4 (G4): 30 patients with chronic hepatitis C virus infection – related cirrhosis are those who had:

(1) Positive serum anti-HCV antibodies
(2) Cirrhosis compatible with HCV origin proved by ultrasound and 3 dimension CT scan
(3) Absence of HCC defined by the absence of a focal liver mass on ultrasonography or CT scan.

Exclusion criteria:

1- Patients with other causes of chronic liver disease are excluded including concurrent hepatitis B virus infection (positive for hepatitis B surface antigen),drug-induced liver disease, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, hemochromatosis, α-1 Antitrypsin deficiency, and Wilson's disease , Bilharziasis (positive anti-Bilharzial anti bodies, history of contact or receiving treatment) .
2- Patient who started to receive antiviral treatment previously.
3- Patient who had a human immunodeficiency virus (HIV), or schistosomal co-infection.
4- History of diabetes or fasting serum glucose P≥ 7.0 mmol/L, or a 2-h postprandial serum glucose ≥ 11.1 mmol/L,
5- History of ischemic heart disease during the previous 6 months uncontrolled hypertension, unstable angina, or severe cardiac arrhythmia
6- Alcohol consumption
7- Inadequate kidney function (Serum creatinine level ≥150 µmol/L)
8- Patient who received concomitant cancer chemotherapy.

All participants were subjected to the following:

(A) Clinical Assessment and sociodemographic data:

1- History:

Clinical and historical details were collected including: age of patient, sex, and residence.

Past history of other chronic medical diseases, disturbed conscious level, previous surgery or long term drug intake.

2-General examination and abdominal examination

(B) Laboratory investigation:

Patient was required to be fast for 8 to 10 hours and venous blood sample was drawn before 9 AM for,

1-Serological analysis:

A-HMGB1 level:

Whole blood was initially collected in non-heparinized tubes and allowed to clot at room temperature for half an hour, then centrifuged for 15 min, and serum collected for storage at –80 C in microfuge tubes until assayed. Serum samples were ultrafiltered with Microcon YM-100 filters (Millipore, Billerica, MA, USA). Filtrate was transferred to a new clean Eppendorf tube, 2× Laemmli sample buffer (Bio-Rad, Laemmli Sample Buffer, Hercules, CA,USA)was made with_-mercaptoethanol (Bio-Rad) according to manufacture's instructions.

B-Serum Clusterin level:

Serum CLU concentration was determined using the ELISA technique according to the manufacturer's instructions (Biovendor-Laboratorni Medicina a.s., Cat. No. RD194034200R).

Hepatitis B surface antigen (HBsAg):

Hepatitis B surface antigen was performed by enzyme-linked immunosorbent assay (Sanofi Diagnostic Pasteur, Marne-la-coquette, France).

Hepatitis C virus antibodies (anti-HCV) in serum:

Hepatitis C virus antibodies were performed with a third-generation ELISA (BIOELISA HCV kit, BIOkit, S.A Barcelona).

Hepatitis C virus RNA (HCV RNA) by quantiative polymerase chain reaction (PCR):

HCV RNA quantitation was carried out by using by two different laboratories, one using Amplicor HCV Monitor version 2 (Amplicor HCV, Roche Diagnostics GmbH, Mannheim, Germany) (lower limit of detection 50 IU/ml), and the other using Abbottm2000 (lower limit of detection 12 IU/ml). Patients were considered as having undetectable serum HCV RNA only if the virus was undetectable by both assays.

HIV virus antibody in serum:

HIV virus antibody in serum was performed with a third-generation ELISA (BIOELISA HCV kit, BIOkit, S.A Barcelona).

2- Liver function tests:

Serum ALT, AST , albumin, and bilirubin. They were done using Integra 400 autoanalyzer. Prothrmbin time,concentration and international normalized ratio (INR) were done using thrombrel-s (human throplastin containing calcium) from Behring diagnostic inc.USA.

3- Complete blood picture:

Measurement was done by sysmex counter k-1000.

5- Serum urea and creatinine level

6 - Serum AFP concentration:

was determined using a two site chemiluminometric immunoassay (ACS–180, Siemens Healthcare Diagnostics, Germany) by the Immulite 1000 Automated Analyzer (Diagnostic Products Corporation).

9- Anti Bilharziale antibodies

(C) ECG (Electrocardiogram)

(E) Abdominal ultrasonography:

(F) Triphasic Computerized Tomography of abdomen:

To detect HFL in liver and tumor description (Number of tumors: 1/2/≥3, Size of main tumor (cm) and Ratio of main tumor of <3/3–5/>5cm). Diagnosis of HCC (early enhancement during arterial phase followed by rapid washout of contrast in delayed phase).

All patients have been assessed using:
The Child–Pugh score
The Model for End-Stage Liver Disease (MELD) Score

Results

The studied subjects comprised 190 individuals (30 apparently healthy volunteers: mean age 41.7 ± 5.96 years; 13 (43.3%) males and 17(56.7%) females, 30 CHC cases: mean age 43.2± 6.3 years; 18 (60%) males and 12 (40%) females, 30 LC cases: mean age 50.8± 4.2 years; 16 (53.3%) males and 14 (46.7%) females and finally 100 HCC cases: mean age 53.1± 4.2 years; 66 (66%) males and 34 (34%) females.

Table 1: Correlation between level of Clusterin and HMGB1 in the four groups

	Group I (CHC) N=30	Group II (LC) N=30	Group III (HCC) N=100	Group IV (Control) N=30	P value					
Clusterin(ng/ml)								*<0.001**		
Range	(64-100)	(54-89)	(120-189)	(71-123)	I vs II	I vs III	I vs IV	II vs III	II vs IV	III vs IV
Mean ± SD	80.8±10.5	72.5±9.7	157.6±20.6	94.8±13.1	*0.234*	*<0.001**	*0.008**	*<0.001**	*<0.001**	*<0.001**
HMGB1(ng/ml)								*<0.001**		
Range	(28-46)	(27-52)	(41-89)	(2-11)	I vs II	I vs III	I vs IV	II vs III	II vs IV	III vs IV
Mean ± SD	38.2±5.1	40.4±8	65.4±13.7	6.3±2.6	*0.856*	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**

Serum CLU (ng/ml) level showed a significant increase in the HCC group compared to the control group (157.6± 20.6 vs.94 ±13.1) (p< 0.001) ,to the CHC group (157.6± 20.6 vs. 80.8 ±10.5) and LC group (157.6± 20.6 vs. 72.5±9.7) (p< 0.001), while a significant decrease in serum CLU level was found in the LC group compared to the control group (72.5±9.7 vs. 94 ±13.1) (p< 0.001).There were no significant differences between CLU levels in patients with CHC (80.8±10.5 ng/ml) and LC patients (72.5±9.7 ng/ml; p =0.23).

Table 9: Simple Discriminant functional analysis for prediction of patient with HCC (LC is the reference group)

	Wilk's lambda	P value	Constant	Coefficient	Sectioning point	Accuracy (%)
Clusterin	0.212	**<0.001***	-7.363	0.053	-1.222	100
AFP	0.943	**0.006***	-0.515	0.002	-0.156	76.9

Table 2: Simple Discriminant functional analysis for prediction of patient with HCC (LC is the reference group)

	Wilk's lambda	P value	Constant	Coefficient	Sectioning point	Accuracy (%)
Clusterin	0.212	**<0.001***	-7.363	0.053	-1.222	100
HMGB1	0.586	**<0.001***	-4.721	0.079	-0.533	88.5
AFP	0.943	**0.006***	-0.515	0.002	-0.156	76.9

Table 3: Multiple Stepwise Discriminant functional analysis for prediction of patient with HCC (LC is the reference group)

	Wilk's lambda	P value	Constant	Coefficient	Sectioning point	Accuracy (%)
Clusterin(ng/ml)				0.052		
HMGB1(ng/ml)	0.188	**<0.001***	-8.093	0.027	-1.317	100
D. bilirubin				-0.53		

Table 4: ROC curve analysis for prediction of patient with HCC (LC is the reference group)

	Clusterin	HMGB1	AFP
Optimal cutoff point	>89	>44	>68
AUC	1	0.939	0.881
95% CI	0.972-1	0.882-0.973	0.812-0.931
P value	***<0.001****	***<0.001****	***<0.001****
Sensitivity	100	99	90
Specificity	100	68.97	72.41
PPV	100	91.7	91.8
NPV	100	95.2	67.7
Accuracy	100	92.25	86

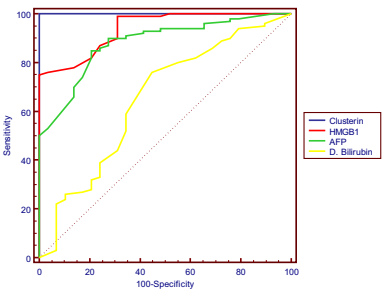

Fig 1 : ROC curve for sensitivity and specificity of AFP and serum clusterin and HMGB1 for diagnosis of HCC

Table 5: Correlation between serum AFP and serum Clusterin and HMGB1 levels of the studied groups.

	AFP			
	Clusterin		HMGB1	
Group	r	P value	r	P value
HCV	-0.141	*0.456*	0.128	*0.500*
LC	0.111	*0.559*	-0.178	*0.346*
HCC	-0.011	*0.913*	**0.411**	***<0.001****
Control	0.067	*0.725*	-0.116	*0.543*

There was a significant positive correlation shown between the levels of serum HMGB1 and AFP among HCC group only (r =0.411, p < 0.001). But there was no significant statistical difference in serum CLU and AFP among all groups (Table 5).

Table 6: Comparison between serum CLU and HMGB1 in child class A&B and class C HCC patients

	Child class		
	A & B (n=49)	C (n=51)	P value
Clusterin(ng/ml)			
Range	(120-187)	(122-189)	***<0.001****
Mean ± SD	147.4±17.9	167.4±18.3	
HMGB1(ng/ml)			
Range	(41-73)	(46-89)	***<0.001****
Mean ± SD	56±8.1	74.4±11.8	

There was a significant statistical difference in serum CLU among the different Child-Pugh classes in HCC patients as its mean level in child class A&B 147.4±17.9 and 167.4±18.3 in class C with p<0.001.Also there was a significant increase in the level of HMGB1 between child A&B HCC patients (56±8.1) and child C HCC patients (74.4±11.8) with p<0.001 (Table18)

Table 6: Correlation between serum CLU and HMGB1 and extrahepatic spread

	Extrahepatic focal spread		
	No (n=69)	Yes (n=31)	P value
Clusterin (ng/ml)			
Range	(120-189)	(122-185)	*0.624*
Mean ± SD	156.9±21.2	159.1±19.7	
HMGB1(ng/ml)			
Range	(41-89)	(49-88)	***<0.001****
Mean ± SD	60.7±12.5	75.8±10.1	

Moreover, when sorting HCC patients into those with extrahepatic spread of HCC included 31 cases (31%) and 69 cases (69%) without extrahepatic spread , there was significant positive correlation between serum HMGB1 and extrahepatic spread (P<0.001). And no significant increase in the level of serum CLU (p=0.624) (Table 6).

Table 7: ROC curve analysis for prediction of patient with extrahepatic focal spread

	HMGB1
Optimal cutoff point	>67
AUC	0.808
95% CI	0.717-0.880
P value	***<0.001****
Sensitivity	83.87
Specificity	76.81
PPV	61.9
NPV	91.4
Accuracy	79

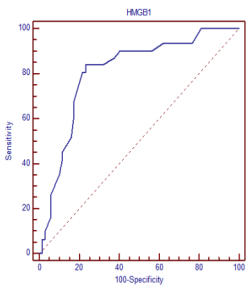

Fig2: ROC curve for sensitivity and specificity of serum HMGB1 in prediction of extrahepatic spread

ROC curve showing increase in level of HMGB1 with extrahepatic spread at the cutoff value >67ng/ml serum HMGB1had 83.87 sensitivity, 76.81specificity, 61.9 PPV% and 91.4% NPV for HCC prognosis (Table 7)

Table 8: Correlation between focal lesions characters and serum Clusterin and HMGB1 levels

	Clusterin		HMGB1	
	r	P value	r	P value
Tumor size	**0.212**	***0.035****	**0.523**	***<0.001****
Tumor number	0.108	*0.285*	**0.266**	***0.007****

We divided the tumors into three groups by their maximum size: <3 cm; 3–5 cm and >5 cm to compare the serum HMGB1 and CLU levels in patients with different size of HCC. There was a significant positive correlation between the levels of serum HMGB1 and the size of tumor ($r = 0.523$, $p < 0.001$). Also significant correlation was found between serum CLU and the size of tumor ($r = 0.212$, $p=0.035$).

We divided the tumors into three groups by their number of focal lesions: 1; 2 and > 2 to compare the serum HMGB1 and CLU levels in patients with different number of focal lesions. There was a significant positive correlation between the levels of serum HMGB1 and the number of tumors ($r = 0.266$, $p = 0.007$). But no significant correlation was found between serum CLU and the number of tumors ($r = 0.108$, $p=0.285$) (Table 8).

Discussion

Worldwide, HCC is the third-leading cause of cancer deaths with more than 500,000 people affected (Luca et al.,2015). It is considered a health burden in Egypt due to its rising incidence Treatment options for HCC are very limited, as it is often diagnosed at a late stage (De Giorgi et al., 2013) leaving this disease with unfavorable prognosis with five-year survival rate of <5%, if diagnosed late with unresectable disease (Debruyne et al.,2010). Therefore, it is highly recommended to find new markers for diagnosing HCC at an early stage and to predict the prognosis of HCC.

In the present study, serum CLU and serum HMGB1 were measured by the ELISA technique in a cohort of Egyptian patients (healthy controls, liver cirrhosis patients, chronic hepatitis patients and HCC cases on top of HCV related liver cirrhosis). We found a significant decrease in serum CLU level in the HCV related cirrhosis patients when compared to the control group,and higher in HCC group than the control group ,LC group and CHCgroup .with highly significant difference between them ($p<0.001$). This was in agreement with Nafee et al., 2012 and Ramadan et al., 2014 ,who reported significant lower level of serum CLU in cirrhotic patients which may be due to reduced liver cell mass or regenerating nodule incapable of expressing CLU similar to normal and malignant cells. Another study done by Wang et al., 2012, reported that serum CLU levels in patients with HCC post HBV infection were significantly lower than in those with chronic hepatitis and healthy subjects. In the present study, serum clusterin was significantly increased with the progression of child pugh staging systems of HCC with $p<0.001$. A handful of studies tackled the issue of the diagnostic performance of biomarkers in HCC. In the present study, serum AFP with a cutoff level of >68 ng/ml ,had 90% sensitivity, 72.41% specificity, 91.8% PPV and 97.7% NPV outperformed serum CLU with a cutoff level of >89ng/ml in diagnostic sensitivity100%, specificity 100% , and positive and negative predictive values 100% .

Its significant increase with appearance of HCC can be explained by its anti-apoptotic function (Koltai., 2014)which suggests that malignant cells when develop can over express CLU to survive and grow. This is reinforced by our findings in analysis of HCC subgroups which showed significant and progressive increase in serum CLU with advance in tumor stage. The relation of CLU to HCC progression was also variable in different reports. In vitro study using HCC cell lines by Lau et al. (Lau et al., 2009) demonstrated that overexpression of CLU increased cell migration and formation of metastatic tumor nodules, Also significant correlation was found between serum CLU and the size of tumor ($r = 0.212$, $p=0.035$). But no significant correlation was found between serum CLU and the number of tumors ($r = 0.108$, $p=0.285$).Wang et al. and Nafaa et al. found no significant difference of CLU serum levels between different tumor sizes.

In our study we did not find a significant increase in serum CLU in HCCs with extrahepatic spread when compared to HCC without extrahepatic spread.

We have also demonstrated that serum HMGB1 levels were significantly increased in patients with HCC than those in patients with chronic hepatitis and cirrhosis. These results suggest that HMGB1 may play a pivotal role in the development of HCC. Several factors may contribute to elevation in serum HMGB1 levels in the patients with HCC. One is that HCC cells produce and secrete HMGB1. Stimulation of neoplastic cell lines with promoters such as 12-O-tetradecanoyl phorbol acetate (TPA) and the binding of c-Myc to the HMGB1 promoter induces the transcription of HMGB1 (Cheng et al.,2008) .

HMGB1 secretion is dependent on active translocation of HMGB1 from the nucleus to the cytoplasm before being released into the extra cellular milieu (Rendon-Mitchell et al.,2003 ;Liu et al.,2015)

On the other hand, it was well known that HMGB1 can be passively released by necrotic and ischeamic cells (Rovere-Querini et al.,2004) . Our results also showed that serum HMGB1 levels were strongly correlated with tumor stage , tumor size, number of focal lesions, extrahepatic spread, known HCC marker (AFP) and child pugh score .

These data suggest that HMGB1 may play an important role not only in development of HCC but in metastasis and poor outcome. In addition, overexpression of HMGB1 has been shown to cause modulation of the transcriptional expressing of many groups of genes reported to play key roles in different biological processes of neoplasm progression and metastasis. Enhanced expression of

HMGB1 and RAGE has been strongly associated with atypical and increased size of colorectal adenomas, colorectal metastases to lymph nodes and distal organs, and poor prognosis at any colorectal cancer stage (Jiao et al.,2011),correlating with invasiveness and poor outcome (Lin et al.,2012).

Thus, in our study, elevation of HMGB1 in patients with HCC can contribute to HCC genesis and metastasis by altering gene expression with cells.

In conclusion, HMGB1 could be a useful and specific marker for evaluating the tumour differentiation grade, tumour stage and predicting prognosis in patients with HCC. This relatively new concept helps us to understand the pathogenesis of HCC and might provide insights that targeting HMGB1 production or release might have substantial potential applications for HCC treatment. And we concluded that serum clusterin was more sensitive than AFP for HCC diagnosis so it might be used as a useful biomarker in screening of high risk populations and for diagnosis of HCC which is one of the most serious malignancies all over the world and specially in Egypt.

REFERANCES

1. Luca Cicales, John Geibel, Francisco TA-Lavera and Burcagir: Hepatocellular Carcinoma treatment and management-http://emedicine.medscape.com/article/197319-overview, 2015.
2. De Giorgi, V., Buonaguro, L., Worschech, A., Tornesello, M. L., Izzo, F., Marincola, F. M., ... & Buonaguro, F. M. (2013). Molecular signatures associated with HCV-induced hepatocellular carcinoma and liver metastasis. PloS one, 8(2), e56153.
3. Debruyne, E. N., Vanderschaeghe, D., Van Vlierberghe, H., Vanhecke, A., Callewaert, N., & Delanghe, J. R. (2010). Diagnostic value of the hemopexin N-glycan profile in hepatocellular carcinoma patients. Clinical chemistry, 56(5), 823-831.
4. Ramadan, R. A., Madkour, M. A., El-Nagarr, M. M., & Abourawash, S. N. (2014). Serum clusterin as a marker for diagnosing hepatocellular carcinoma. Alexandria Journal of Medicine, 50(3), 227-234.
5. Nafee, A. M., Pasha, H. F., Abd El Aal, S. M., & Mostafa, N. A. (2012). Corroboration of Serum Apolipoprotein J (Clusterin) as a Biomarker for Evaluating Hepatocellular Carcinoma. Afro-Egypt J Infect Endem Dis, 2(1), 16-24.
6. Wang, Y., Wang, X., Zhao, H., Liang, B., & Du, Q. (2012). Clusterin confers resistance to TNF-alpha-induced apoptosis in breast cancer cells through NF-kappaB activation and Bcl-2 overexpression. Journal of chemotherapy, 24(6), 348-357.
7. Lau, S. H., Sham, J. S. T., Xie, D., Tzang, C. H., Tang, D., Ma, N., ... & Zhang, W. M. (2006). Clusterin plays an important role in hepatocellular carcinoma metastasis. Oncogene, 25(8), 1242.
8. Koltai, T. (2014). Clusterin: a key player in cancer chemoresistance and its inhibition. OncoTargets and therapy, 7, 447.
9. Cheng, B. Q., Jia, C. Q., Liu, C. T., Lu, X. F., Zhong, N., Zhang, Z. L., ... & Li, Y. Q. (2008). Serum high mobility group box chromosomal protein 1 is associated with clinicopathologic features in patients with hepatocellular carcinoma. Digestive and Liver Disease, 40(6), 446-452.
10. Rendon-Mitchell, B., Ochani, M., Li, J., Han, J., Wang, H., Yang, H., ... & Sama, A. E. (2003). IFN-γ induces high mobility group box 1 protein release partly through a TNF-dependent mechanism. The Journal of Immunology, 170(7), 3890-3897.
11. Rovere-Querini, P., Capobianco, A., Scaffidi, P., Valentinis, B., Catalanotti, F., Giazzon, M., ... & Bianchi, M. E. (2004). HMGB1 is an endogenous immune adjuvant released by necrotic cells. EMBO reports, 5(8), 825-830.
12. Liu, Z., Dou, C., Wang, Y., Jia, Y., Li, Q., Zheng, X., ... & Song, T. (2015). High-mobility group box 1 has a prognostic role and contributes to epithelial mesenchymal transition in human hepatocellular carcinoma. Molecular medicine reports, 12(4), 5997-6004.
13. Jiao, L., Taylor, P. R., Weinstein, S. J., Graubard, B. I., Virtamo, J., Albanes, D., & Stolzenberg-Solomon, R. Z. (2011). Advanced glycation end products, soluble receptor for advanced glycation end products, and risk of colorectal cancer. Cancer Epidemiology and Prevention Biomarkers, 20(7), 1430-1438.
14. Lin, L., Zhong, K., Sun, Z., Wu, G., & Ding, G. (2012). Receptor for advanced glycation end products (RAGE) partially mediates HMGB1-ERKs activation in clear cell renal cell carcinoma. Journal of cancer research and clinical oncology, 138(1), 11-22.
15. Materia, S., Cater, M. A., Klomp, L. W., Mercer, J. F., & La Fontaine, S. (2011). Clusterin (apolipoprotein J), a molecular chaperone that facilitates degradation of the copper-ATPases ATP7A and ATP7B. Journal of Biological Chemistry, 286(12), 10073-10083.
16. Andersen, C. L., Schepeler, T., Thorsen, K., Birkenkamp-Demtröder, K., Mansilla, F., Aaltonen, L. A., ... & Ørntoft, T. F. (2007). Clusterin expression in normal mucosa and colorectal cancer. Molecular & Cellular Proteomics, 6(6), 1039-1048.
17. Hassan, M., Watari, H., AbuAlmaaty, A., Ohba, Y., & Sakuragi, N. (2014). Apoptosis and molecular targeting therapy in cancer. BioMed research international, 2014.
18. Ito, N., DeMarco, R. A., Mailliard, R. B., Han, J., Rabinowich, H., Kalinski, P., ... & Lotze, M. T. (2007). Cytolytic cells induce HMGB1 release from melanoma cell lines. Journal of leukocyte biology, 81(1), 75-83.
19. Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., ... & Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer, 136(5).
20. Ozakyol, A. (2017). Global epidemiology of hepatocellular carcinoma (HCC epidemiology). Journal of gastrointestinal cancer, 48(3), 238-240.
21. Ghouri, Y. A., Mian, I., & Rowe, J. H. (2017). Review of hepatocellular carcinoma: Epidemiology, etiology, and carcinogenesis. Journal of carcinogenesis, 16.
22. Shaker MK, Abdella HM, Khalifa MO, El Dorry AK. Epidemiological characteristics of hepatocellular carcinoma in Egypt: a retrospective analysis of 1313 cases. Liver Int. 2013 Nov;33(10):1601-6.
23. Azab, M., Zaki, S., El-Shetey, A. G., Abdel-Moty, M. F., Alnoomani, N. M., Gomaa, A. A., ... & Atia, F. (2011). Radiofrequency ablation combined with percutaneous ethanol injection in patients with hepatocellular carcinoma. Arab Journal of Gastroenterology, 12(3), 113-118.
24. Naglaa A. El Sherbinya , Samy Zakyb, Ahmed A. Gommac, Essam A. Hassand, Nancy A. Attae. Epidemiology of Hepatocellular Carcinoma in Fayoum Governorate-Egypt .International Journal of Sciences: Basic and Applied Research (IJSBAR) (2017) Volume 33, No 1, pp 21-32 .
25. Omran, D. A. E. H., Awad, A. H., El, M. A., Mabrouk, R., Soliman, A. F., & Aziz, A. O. A. (2015). Application of Data Mining Techniques to Explore Predictors of HCC in Egyptian Patients with HCV-related Chronic Liver. Asian Pacific Journal of Cancer Prevention, 16(1), 381-385.
26. Ishaq, S., Kaur, H., & Bhatia, S. (2017). Clusterin: It's Implication in Health and Diseases. Annals of Applied Bio-Sciences, 4(1), R30-34.
27. Matukumalli SR, Tangirala R, Rao CM. Clusterin: full-length protein and one of its chains show opposing effects on cellular lipid accumulation. Sci Rep. 2017 Jan 25;7:41235.
28. Pedrazzi, M., Averna, M., Sparatore, B., Patrone, M., Salamino, F., Marcoli, M., ... & Melloni, E. Potentiation of nmda receptor-dependent cell responses by extracellular high mobility group box 1 protein. PLoS ONE 2012, 7, 31.
29. Penzo, M., Molteni, R., Suda, T., Samaniego, S., Raucci, A., Habiel, D. M., ... & Palumbo, R Inhibitor of nf-kappa b kinases alpha and beta are both essential for high mobility group box 1-mediated chemotaxis. J. Immunol. 2010, 184, 4497–4509.
30. Chen, G.Y.; Tang, J.; Zheng, P.; Liu, Y. Cd24 and siglec-10 selectively repress tissue damage-induced immune responses. Science 2009, 323, 1722–1725.
31. Chiba, S., Baghdadi, M., Akiba, H., Yoshiyama, H., Kinoshita, I., Dosaka-Akita, H., ... & Hirashima, M.. Tumor-infiltrating dcs suppress nucleic acid-mediated innate immune responses through interactions between the receptor tim-3 and the alarmin hmgb1.Nat. Immunol. 2012, 13, 832–842.
32. Friggeri, A.; Yang, Y.; Banerjee, S.; Park, Y.J.; Liu, G.; Abraham, E. Hmgb1 inhibits macrophage activity in efferocytosis through binding to the alphavbeta3-integrin. Am. J. Physiol. Cell Physiol. 2010, 299, 8.
33. Song, J.X.; Lu, J.H.; Liu, L.F.; Chen, L.L.; Durairajan, S.S.; Yue, Z.; Zhang, H.Q.; Li, M. Hmgb1is involved in autophagy inhibition caused by snca/alpha-synuclein overexpression: A process modulated by the natural autophagy inducer corynoxine b. Autophagy 2014, 10, 144–154.
34. Conti, L.; Lanzardo, S.; Arigoni, M.; Antonazzo, R.; Radaelli, E.; Cantarella, D.; Calogero, R.A.; Cavallo, F. The noninflammatory role of high mobility group box 1/toll-like receptor 2 axis in the self-renewal of mammary cancer stem cells. FASEB J. 2013, 27, 4731–4744.
35. Chen RC, Yi PP, Zhou RR, Xiao MF, Huang ZB, Tang DL, Huang Y, Fan XG. The role of HMGB1-RAGE axis in migration and invasion of hepatocellular carcinoma cell lines. Mol Cell Biochem 2014;390:271-٢80.