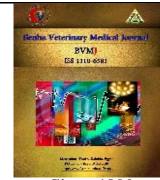
Benha Veterinary Medical Journal 45 (2023) 66-70

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/



Since 1990

Original Paper

Biochemical and Molecular Alterations of Micro RNA 125b and Micro RNA 489-3p as diseases biomarkers in Hepatitis C Virus (HCV)

Amira R. Elweshahi, Mohammed, K. Mahfouz, Samy A. Aziza ,Afaf D. Abdel-Magid

Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Benha University, Egypt

ARTICLE INFO

Keywords

MicroRNA 125b,
MicroRNA 489-3p,
HCV

Received 11/11/2023
Accepted 21/12/2023
Available On-Line 31/12/2023

ABSTRACT

The Hepatitis C virus causes acute and chronic hepatitis. Micro RNA could be used as disease biomarkers. This study looked into Micro RNA125b, Micro RNA 489-3p, and biochemical analysis as a diagnostic for HCV. This study included 20 individuals who had chronic liver disease and were admitted to the Gastroenterology Hospital in Mansoura and the Oncology Institute in Mansoura. All individuals were subjected to full clinical examinations. Following analysis of all patients, polymerase chain reaction (PCR) was confirmed to be positive for chronic HCV in 5 individuals. Five negative control samples were taken from a healthy control group of the same age range and sex. Serum liver function parameters and AFP with gene expression of microRNA125b and microRNA 489-3p were estimated. The HCV group significantly increased AST, ALT, DBIL, TBIL, ALP, GGT, and ALB) while significantly decreasing albumin concentrations. In the HCV group, AFP was substantially higher than in control. The HCV group had a considerable rise in miRNA125b (10.07+ 0.19) but a significant drop in miRNA489-3p (0.36+ 0.02). In addition to biochemical investigation, we found that miRNA125b and miRNA489-3p expression levels were employed as valuable markers for HCV diagnosis.

1. INTRODUCTION

Because the liver is the primary organ for metabolism, detoxification, and secretory functions, it is susceptible to a wide range of disorders (Kalra et al., 2023). Chronic liver diseases are defined as progressive hepatic dysfunction over a six-month (Shah et al., 2020).

Chronic hepatitis C patients are at a significant risk of developing serious consequences, such as cirrhosis, in cirrhotic individuals HCC (WHO, 2020).

HCV is the only species member that causes substantial persistence of hepatic disease, with approximately 110-170 million infected patients worldwide, nearly two-thirds of whom are chronic and at least one-third developing fibrosis and cirrhosis after 20 years of infection; the majority develop various stages of hepatocellular carcinoma (Thrift et al., 2017). The potential treatment for the CHC virus has undergone a significant metamorphosis in recent years; human viral infection discoveries in humans have revealed surprising results which have expanded our knowledge of miRNA role inside the body (Laurence, 2016).

The role of various miRNAs in controlling the viral infection response was investigated, which explains the causes of CHC progression in the majority of infected patients, as well as the consequences of infection in the risk of cirrhosis and HCC development (Cazanave et al., 2011).

Several studies have shown that miRNAs act as tumor suppressors by down-regulating oncogenic targets or tumor promoters by negatively regulating tumor-suppressive target mRNAs (Shenouda and Alahari., 2009). MiRNAs may be employed as biomarkers for the early detection of cancer and other disorders in either case (Wang D et al., 2010). Furthermore, miRNAs and their target genes may be used in anticancer therapy (Hong et al., 2020). Among miRNA families with crucial activities, the miR-125b family has been linked to a range of carcinomas as a tumor suppressor or promoter (Sun et al., 2013).

Hepatitis C virus infection modifies a number of cellular microRNAs, one of which, miR-122, is critical for the HCV replication cycle (Roberts et al., 2011). Other miRNAs prevalent in the liver, such as miR-125b, a highly conserved homolog of lin-4, are being investigated for their involvement in important cellular pathways such as inflammation, fibrogenesis, and hepatocellular oncogenesis (Kim et al., 2013; Shrivastava et al., 2015). Exosomal miR-125b levels have been linked to the prognosis of hepatocellular carcinoma (Liu et al., 2017), and plasma miR-125b levels have been recommended as a non-invasive biomarker in chronic viral hepatitis (Akamatsu et al., 2015).

MiR-489-3p levels were shown to be less in late recurrent HCC patients than in early recurrent instances (You et al., 2018). Low levels of miR-489-3p were linked to malignant clinical characteristics and worse survival rates in HCC patients.

This study objected to exploring MicroRNA 125b and MicroRNA 489-3p potential as precise diagnostic biochemical markers for the identification of chronic HCV infection.

2. MATERIALS AND METHODS

2.1 Materials:

The current investigation follows a cross-sectional design that received approval from the Gastroenterology Hospital in Mansoura and the Oncology Institute in Mansoura. The

Correspondence to: dramiraelweshahi2022@gmail.com

study cohort encompassed 20 individuals diagnosed with chronic liver disease and under treatment at Mansoura Gastroenterology Hospital. Out of these, 5 patients exhibited positive PCR results for chronic HCV infection. The age range of the patients fell between 30 and 50 years, with body weights ranging from 60 to 90 kg. To serve as a comparison, 5 serum samples were obtained from a control group of healthy individuals.

2.2 Methods:

2.2.1 Biochemical Analysis:

Five HCV patients and the control group had serum samples taken for analysis:

A) Liver function variables involved ALT (Murray,1984), AST (Murray,1984), TBil (Pagana et al.,2019), and DBil (Pagana et al., 2019). GGT (Beleta and Gella, 1990) ALP (John 1987) and ALB (Young, 2001). In addition to the analysis of AFP as a tumor marker detector (Johns Hopkins Medicine (2022).

2.2.2 Molecular analysis:

RNA extraction:

The RNeasy Mini Kit Catalogue no.74104

For total RNA extraction and purification derived from initial crude RNA preparations and diverse enzymatic procedures (including proteinase digestion, DNase digestion, labeling reactions, and RNA ligation), 96% ethanol from AppliChem was utilized. Prior to use, this ethanol was diluted to a concentration of 70% by incorporating distilled and deionized water (DDW) (Yuan et al., 2006).

Equipment and apparatuses used for extraction of RNA

1. Epindorff Tubes with a capacity of 1.5 ml
2. Biohit monochannel micropipettes in the range of 20-200 µl and 100-1000 µl.
3. Sterile filter tips with capacities of 200 µl and 1000 µl.
4. A centrifuge (Sigma Sartorius, USA).

The material used for mastermix preparation for SYBR Green real-time PCR

a) Quantitect SYBR green PCR kit Cat. No. 204141

It comprises 1 ml of 2x QuantiTect SYBR Green PCR Master Mix and 2 ml of RNase-Free Water.

b) Revert Aid Reverse Transcriptase (Thermo Fisher) (200 U/µL). Catalog number: EP0441.

The primer and the sequence were illustrated in Table (1).

Table (1): Oligonucleotide primers and probes

Gene	Primer sequence (5'-3')	Reference
U6 (housekeeping)	GCTTCGGCAGCACATATACTAAAAT CGCTTCACGAATTTGCGTGTCAT	Chen et al., 2003
miR-125b	CCCCCGCTAGCTCTTGTTTTGCTTTGCTTTGTC CCCGAATTCACCAAATTTCCAGGATGCAA	Chen et al., 2019
miR-489-3p	CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG AGC TGC CGT	Zheng and Chen., 2020

Source: Biobasic (Canada).

Molecular analysis:

Real time PCR calculation equipment and apparatuses

1. Real time PCR machine (Stratagene MX3005P)

MX3005P QPCR system represents a comprehensive solution for quantitative polymerase chain reaction (QPCR) detection and subsequent data analysis. This system seamlessly integrates an advanced thermal cycler, a cutting-edge optical system featuring a quartz-tungsten halogen lamp along with a singular photomultiplier tube (PMT), and an exceptionally robust analysis software. Inclusive of the package are five carefully chosen filter sets, enhancing the capabilities of the system. The strategic design of the scanning optics ensures unparalleled differentiation between dyes as well as between individual samples, further solidifying its performance.

2. Unichannel micropipettes (100-1000), (2-20), (0.5-10) and (20-200) µl (Biohit)
3. Filter tips of different sizes.
4. Optical tubes (0.2 ml) (Applied biosystem).
5. Optical caps (Applied biosystem).

SYBR green rt-PCR results were evaluated as follows:

To produce amplification curves and determine Ct values, stratagene MX3005P programme was used. The ct value of each sample was compared with the control using the " ΔΔCt " technique published by (Yuan et al., 2006) to examine changes in gene expression across various RNA samples. This entailed using the following ratio: (2-DDct).

Whereas ΔΔCt = ΔCt reference – ΔCt target

ΔCt target = Ct control – Ct treatment and *ΔCt reference = Ct control- Ct treatment.*

3. RESULTS

3.1 Biochemical analysis*:*

The results of the serum biochemical examination are mentioned in Table (2).

Liver function parameters:

The HCV group demonstrated a substantial rise in ALT, AST, GGT, and ALP activity, as well as a significant increase in total bilirubin and direct bilirubin and a noteworthy drop in albumin concentration (Alb) compared to control. However, the HCV group had a substantial elevation (7.76± 0.26) in AFP concentration compared to healthy ones (3.28± 0.21).

Table (2): The biochemical and Molecular changes of HCV and control groups.

Patients' groups	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Albumin (g/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	AFP (ng/ml)	PCR for HCV RNA
Control healthy group	23.79 ± 1.60^{b}	31.12 ± 1.30^{b}	52.20 ± 4.45^{b}	27.59 ± 2.85^{b}	3.91 ± 0.21^{a}	0.90 ± 0.04^{b}	0.23 ± 0.05^{b}	3.28 ± 0.21^{a}	2.65 X 10^{6} ± 0.92^{b}
HCV group	97.83 ± 7.41^{a}	147.82 ± 6.85^{a}	78.25 ± 4.61^{a}	68.48 ± 4.96^{a}	2.55 ± 0.14^{b}	1.96 ± 0.16^{a}	0.62 ± 0.08^{a}	7.76 ± 0.26^{a}	189.71 X 10^{6} ± 3.48^{a}

Data are presented as (Mean ± S.E), Mean values with different superscript letters at the same column are significantly different at (P<0.05).

3.2. Molecular analysis:

Molecular analysis results in (Table 3) The relative expression level of "miRNA-125b" and "miRNA-489-3P" genes, in liver tissue of control and HCV groups.
Concerning miR-125b gene expression of HCV group revealed a substantial elevation (10.07 ± 0.19) ($P < 0.05$) in mRNA125b more than healthy group (1.00 ± 0.05).
However, miR-489-3p Gene Expression: revealed that HCV group had a substantial reduction in mRNA489 average ct (32.46) compared to healthy group, average ct (25.94).

Table (3): Relative expression level of "miRNA-125b" and "miRNA-489-3P" genes, in liver tissue of control and Hepatitis C Virus (HCV) groups of patients.

Patients groups	miRNA-125b Fold change Mean ± SE	miRNA-125b Average Ct	miRNA-489-3P Fold change Mean ± SE	miRNA-489-3P Average Ct
Control healthy group	1.00 ± 0.05[b]	23.04	1.00 ± 0.06[c]	25.94
HCV group	10.07 ± 0.19[a]	19.10	0.36 ± 0.2[b]	32.46

Data were statistically analyzed as (Mean ± S.E), the difference in mean values between superscript letters in the same column is significant at ($P< 0.05$).

4. DISCUSSION

Hepatitis C virus is a risk factor for chronic liver failure and liver cirrhosis progression. Chronic hepatitis C (CHC) is commonly undiagnosed, with normal serologic or biochemical tests diagnosing the majority of cases (Gupta et al., 2014).
In this study, noteworthy high alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-Glutamyltransferase (GGT), and alkaline phosphatase (AlkP) values, significant increase in Total bilirubin (TBIL), Direct bilirubin (DBIL) and alpha-fetoprotein (AFP) concentration with significant decrease in albumin concentration were recorded in HCV group compared to control. These results were supported by Alazzawy (2018). Additionally, Hyder et al. (2013) demonstrated a notable increase in enzymatic functions between viral hepatitis patients compared to the control group. The higher aminotransferase values in the positive HCV marker group were also reported by Mnuti et al. (2011). A wide range of ALT and AST values, from normal to permanently raised, was recorded in chronic HCV, despite studies showing that individuals with normal ALT had a slower development and a reduced occurrence of cirrhosis Hussein. (2016). When hepatocytes are injured in HCV infection, aminotransferases from the liver leak into the bloodstream, leading to high levels of them in the blood. In addition, (Lee et al., 2001), reported that Patients with chronic HCV who have high HCV RNA titers and abnormal ALT levels have active HCV replication with a higher risk of liver damage. ALT levels are used as a marker for monitoring antiviral therapy response in CHC infection (Dufour et al., 2000). ALP and GGT levels rise in chronic liver disorders. As the bile ductules' cell is encouraged to generate ALP, it reaches the bloodstream; this may come from enhanced production and release of the enzyme into serum rather than reduced biliary secretion (Lowe et al., 2017).
Total bilirubin levels were reported to be considerably greater in HCV individuals, revealed to be an indicator of hepatic damage (Min Du et al., 2016). This rise is due to suppression of the conjugation process and the release of unconjugated bilirubin from hepatocytes. A low serum albumin concentration, on the other hand, has been linked to hepatic dysfunction. As albumin concentrations take several weeks to decline after decreased albumin synthesis, albumin reductions begin early in the disease's progression, and the short albumin half-life of about 18-20 days is induced by hepatitis C infection. (Ashraf et al., 2010).
In cirrhosis follow-up, there was a considerable increase in AFP levels, which operate as a marker for HCC development. In HCV patients, there was a link between AFP levels and long-term virological response. This was consistent with the findings of (Masetti et al., 2018). HCV protein expression has been demonstrated to affect multiple potentially carcinogenic pathways through cell signaling transcription modulation, apoptosis, transformation, translational control, and interaction with the translational machinery and post-translational modification system. Aside from the potential effects of the HCV virus on the host genome, HCC may lead to a cycle of inflammation, necrosis, and regeneration in the liver as a result of chronic hepatitis C-induced liver cell injury. In this setting of inflammation, increased cell turnover, as well as oxidative DNA damage, may assist in the accumulation of genetic and epigenetic alterations like activation of cellular oncogenes and telomerase, proliferative signaling pathways, with inactivation of tumor suppressor genes and overexpression of angiogenic and growth factors (You et al., 2018). Elevated AFP levels are also reported in individuals with viral hepatitis without HCC, particularly in those with chronic HCV and liver cirrhosis (Chu et al., 2001).
According to our findings, chronic hepatitis C is an important risk factor for HCC. Given that miRNA dysregulation has been associated with the development and progression of HCC, HCC-associated miRNA signature discovery is of considerable significance for HCC early detection in positive HCV patients prior to disease onset. HCV modifies miRNA expression to promote hepatocyte development toward tumor formation by modulating several signaling pathways (Kanwal et al., 2011).
miRNAs represent a significant focal point in addressing viral hepatitis, presenting a promising avenue for targeting HBV and HCV infections. These infections, in some manner, contribute to the progression of HCC and eventual mortality during persistent infection. By targeting these miRNAs, there's a potential to prevent the progression of these infections, subsequently mitigating the risk of HCC incidence. This approach involves the regulation of numerous oncogenic miRNAs, such as miR125b and miR489-3p, both of which serve as viable biomarkers for HCC (Elemeery et al., 2017). In this study, a noticeable elevation in mRNA125b gene expression was observed in HCV individuals, while gene expression of mRNA489-3p exhibited a marked reduction. Correspondingly, serum levels of miR125b were heightened in chronic HCV genotype 1 infection in contrast to NAFLD individuals. thus, hepatic miR-125b expression remained consistent among chronic HCV infection and NAFLD individuals) Choudhuri et al., 2016). Our investigation revealed a noteworthy augmentation in miR-125b expression within HCV replicon cells and in the serum of HCV infection patients. This miR-125b upregulation was consistently seen in human samples obtained from infected individuals. (Bala et al.,

2012). HCV-induced cirrhosis and HCV-associated hepatocellular carcinoma (Giray et al., 2014), MiR-125b is extensively expressed in peripheral blood mononuclear cells, notably monocytes and macrophages (Chaudhuri et al., 2011), and its expression has been reported to be negatively related to improved treatment result of chronic HCV infection (His et al., 2014). Elevated levels of miR-125 b were observed to be positively associated with HCV infection both in replicon cells and sera from HCV-infected individuals. Furthermore, miR-125b inhibition demonstrated a decrease in HCV gene expression. IL-6 signal transducer and transcription 3 activator (STAT3) pathway was identified as a contributor to the induction of miR-125b gene expression. It was noted that inhibiting STAT3 through RNA or inhibitors led to a reduction in HCV replication. The results showed that miR-125b expression was increased by treatment with recombinant human IL-6 (rhIL-6) in Huh7 cells. Because the miR-125b promoter sequence was predicted to contain two STAT3 transcription factor binding sites, the activated STAT3 may bind to the miR-125b promoter and enhance its expression. Nishitsuji et al., (2013) Demonstrated that STAT3 could bind the miR-125b promoter and regulate its expression in HCV replicon cells. In addition, HCV infection can induce inflammatory cytokine responses and enhance IL-6 production. Elevated plasma and serum levels of IL-6 have been reported in HCV-infected individuals (Sandler et al.2011), and it was also found for the first time that IL-6 could increase the promoter activity and expression of miR-125b. On the other hand, exogenous miR-125b could induce STAT3 protein levels, STAT3 phosphorylation, and HCV non-structural protein expression and could repress PSMB9 protein levels in an HCV replicon cell line. Previous studies have indicated that HCV triggers the formation of reactive oxygen species (ROS), leading to DNA damage and constitutive STAT3 activation (Machida et al., 2006). reported that miR-125b expression is increased by oxidative stress. Therefore, STAT3-induced miR-125b expression might occur via triggering of the oxidative stress pathway by HCV. In addition, (Manca et al., 2011) found that both STAT3 siRNAs and inhibitors could decrease HCV replication in Con1 replicon cells. STAT3 inhibition markedly reduced HCV replication, possibly through interaction with essential host cell factors or partly and indirectly through miR-125b expression. (Nishitsuji et al., 2013) Reported that an important role for miR-125b expression during HCV infection in replicon cells and clinical samples. The serum level of miR-125b was significantly elevated in HCV-infected patients. On the other hand, both the promoter activity and expression of miR-1- 125b were increased in HCV replicon cells in vitro, whereas the miR-125b inhibitor reduced HCV expression levels. Our results also indicate that the IL-6/STAT3 pathway plays an inducible role in miR-125b expression. Taken together, we suggest that knockdown of STAT3 or miR-125b could be a promising strategy for anti-HCV therapy. Notably, this study found a clear relationship between elevated plasma miR-125b expression and advanced liver fibrosis in HCV-infected individuals who had not received therapy (Zaltron et al., 2012). In primary hepatic stellate cell cultures, suppressing MiR-125b decreased the expression of profibrogenic genes. (You et al., 2018).

In the present study, significantly down-regulated in the expression of miR- HCV induced miR489-3p. According to (You et al., 2018). miR-489-3p decreased cancer cell nvasive ability in HCC. Furthermore, MMP7 was identified as a downstream molecule of miR-489-3p that may have mediated the biological roles of miR-489-3p in HCC. Chen et al. (2013) disclosed that miR489-3p suppressed the expression of MMP7 in HCC cells. The levels of MMP7 in HCC tissues were negatively correlated with the expressions of miR-489. Moreover, we found that miR 489-3p could directly interact with the 3′-UTR of MMP7. These experiments suggest that MMP7 is a downstream molecule of miR 489-3p. Furthermore, we found that restoration of MMP7 could abrogate the anti-metastatic effects of miR-489 on HCC cell migration and invasion. These suggest that miR 489-3p inhibits the migration and invasion of HCC cells, possibly by targeting MMP7.

(Chen et al.,.2013) demonstrates that miR489-3p expression is significantly decreased in HCC. The low level of miR489-3p correlates with adverse clinical parameters of HCC patients and shortened survival. And miR489-3p inhibits the metastasis of HCC cells. Furthermore, MMP7 is a direct target of miR489-3p in HCC. Altogether, miR489-3p exerts its inhibitory effects on HCC metastasis, at least in part, by targeting MMP7. Finally, miR-489-3p was considerably down-regulated in liver fibrosis compared to controls.

5. CONCLUSIONS

MiRNA125b expression was dramatically elevated in HCV patients, but the miRNA489-3p expression was significantly reduced, suggesting that miRNA125b and miRNA489-3p expression, in addition to other biochemical parameters, may be employed as helpful indicators for chronic HCV diagnosis.

6. REFERENCES

1. Alazzawy, M. A. 2018. Role of Interleukin-28B in clearance of HCV in acute and chronic hepatitis patients in Kirkuk city. Kurdistan Journal of Applied Research, 146-149.
2. Ashraf-Uz-Zaman, M., Begum, B. A., Asad, H. B., Moutoshi, S. S., Nasiruddin, M. 2010. Biochemical parameters in common viral hepatitis. Journal of medicine, 11(1), 42-45.
3. Bala, S., Tilahun, Y., Taha, O., Alao, H., Kodys, K., Catalano, D. 2012. Increased microRNA-155 expression in the serum and peripheral monocytes in chronic HCV infection. J Transl Med.; 10:151-179.
4. Bleta, Gella . 1990. Report on the symposium "drug effect in clinical chemistry methods. Eur J Clin Chem clin Biochem .34:385-386.
5. Cazanave SC et al. 2011. A role for miR-296 in the regulation of lipoapoptosis by targeting PUMA. Journal of Lipid Research.;52,8:1517-1525
6. Chaudhuri, AA, So, AY., Sinha, N., Gibson, WS., Taganov, KD., O'Connell, RM., Baltimore, D. 2016. MicroRNA-125b potentiates macrophage activation. J Immunol.;187:5062–5068.
7. Chen, HY., Han, ZB., Fan, JW., Xia, J., Wu, JY., Qiu, GQ. 2003. miR-203 expression predicts outcome after liver transplantation for hepatocellular carcinoma in cirrhotic liver. Med Oncol. 29:1859–65.
8. Chen, CJ., Yang, HI., Iloeje, UH. 2019. Hepatitis B REVEAL-HBV Study Group. Hepatitis B virus DNA levels and outcomes in chronic hepatitis B. Hepatology; 49:S72-79.
9. Chen, M., Li, Q., Li, CJ., Wang, SQ., Xie .2013. promotes hepatocellular carcinoma cell migration and invasion through beta-catenin/MMP7 signaling pathway Mol Cancer, 12 , p. 157-167.
10. Dufour, D., Lott, J., Nolte, F. 2000. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. Clin Chem 2000; 46:2050-68.
11. Elemeery , M.N. 2017. Validation of a serum microRNA panel as biomarkers for early diagnosis of hepatocellular carcinoma

post-hepatitis C infection in Egyptian patients. World Journal of Gastroenterology.;23(21):3864-3871.
12. Emokpae, M. A., Adejumol, B. G., Abdu, A., Sadiq, N. M. 2013. 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, 54(6), 426-437.
13. Gupta, E., Bajpai, M., Choudhary, A. 2014. Hepatitis C virus: Screening, diagnosis, and interpretation of laboratory assays. Asian journal of transfusion science, 8(1), 19-33.
14. Hong D.S., Kang Y.-K., Borad M., Sachdev J., Ejadi S., Lim H.Y., Brenner A.J., Park K., Lee J.-L., Kim T.-Y., et al. 2020. Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. Br. J. Cancer.;122:1630–1637. doi: 10.1038/s41416-020-0802-1
15. Hsi, E., Huang, CF., Dai, CY., Juo, SH., Chou, WW., Huang, JF. 2014. Peripheral blood mononuclear cells microRNA predicts treatment outcome of hepatitis C virus genotype 1 infection. Antiviral Res. 42(9);105-135.
16. Hussein, R. H. 2016. Comparison in Some Biochemical and Hematological Tests Between Chronic Hepatitis B and C. Ibn AL-Haitham Journal for Pure and Applied Science, 24(1):505-539.
17. Hyder, M. A., Hasan, M., Mohieldein, A. H. 2013. Comparative levels of ALT, AST, ALP and GGT in liver associated diseases. European journal of experimental biology, 3(2), 280-284.
18. John, D. 1982. laboratory mothed for estimation of alkaline phosphatase 9th Ed 81.
19. Johns Hopkins Medicine. 2022. Johns Hopkins Medicine; Health Library: Liver Cancer (Hepatocellular Carcinoma); Aug 23; 4: 855-936.
20. Kalra A, Yetiskul E, Wehrle CJ, Tuma F.2023 Physiology, Liver. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing
21. Kanwal, F., Hoang T., Kramer, J.R. 2011. Increasing prevalence of HCC and cirrhosis in patients with chronic hepatitis C virus infection. Gastroenterology. 140:1182 e1–1188 e1.
22. Machida, K., Cheng, KT., Lai, CK., Jeng, KS., Sung, V.M., Lai, M.M. 2006 Hepatitis C virus triggers mitochondrial permeability transition with production of reactive oxygen species, leading to DNA damage and STAT3 activation. Journal of virology.; 80:7199–7207.
23. Manca, S., Magrelli, A., Cialfi, S., Lefort, K., Ambra, R., Alimandi, M., Biolcati, G., Uccelletti, D., Palleschi, C., Screpanti, I., Melino, G., Salvatore, M. 2011. Oxidative stress activation of miR-125b is part of the molecular switch for Hailey- Hailey disease manifestation. Exp Dermatol ; 20:932–937.
24. Masetti, Chiara, Lionetti, Raffaella, Lupo, Marinella, Siciliano, Massimo, Giannelli, Valerio ,Ponziani, Francesca Romana, Teti, Elisabetta, Dell'Unto, Chiara , Francioso, S., Brega, Arianna, Montalbano, Marzia, Visco Comandini, Ubaldo, Taibi, Chiara, Galati, Giovanni, Vespasiani-Gentilucci, Umberto, Picardi, Antonio,Andreoni, Massimo, Pompili, Maurizio, Pellicelli, Adriano,Angelico, Mario. Lack of reduction of serum alpha fetoprotein during treatment with direct antiviral agents predicts hepatocellular carcinoma development in a large cohort of patients with hcv-related cirrhosis. Journal of Viral Hepatitis 25, 4. DOI:10.1111/jvh.12982
25. Min, Du., Shanshan Zhang, Lin, Xiao., Yanyan, Xu., Peiyi, Liu., Yuhan, Tang., Sheng, Wei., Mingyou, Xing., Xiaoping Miaoand Ping, Yao. 2016.The Relationship between Serum Bilirubin and Elevated Fibrotic Indices among HBV Carriers: A Cross-Sectional Study of a Chinese Population. Int J Mol Sci. Dec; 17(12): 2057-3390.
26. Murray. 1984. Report on the symposium “drug effect in clinical chemistry methods for Estimation of AST. Eur J Clin Chem clin Biochem . 34:1112-1116.
27. Murray. 1984. Report on the symposium “drug effect in clinical chemistry methods for Estimation of ALT. Eur J Clin Chem clin Biochem . 34:1088-1090.
28. Nishitsuji, H., Funami, K., Shimizu, Y., Ujino, S., Sugiyama, K., Seya, T., Takaku , H., Shimotohno, K. 2013. Hepatitis C virus infection induces inflammatory cytokines and chemokines mediated by the cross talk between hepatocytes and stellate cells. Journal of virology.; 87:8169–8178.
29. Pagana, KD., Pagana, TJ., Pagana, TN. 2019. Mosby’s Diagnostic & Laboratory Test .14th ed. St. Louis, Mo: Elsevier; 61(5):233-247.
30. Sandler, NG., Koh, C., Roque, A., Eccleston, JL., Siegel, R.B., Demino, M., Kleiner, DE., Deeks, SG., Liang, T.J., Heller, T., Douek, D.C. 2011.Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. Gastroenterology; 141:1220–1230, 1230 e1221–1223.
31. Shah, N. J., Mousa, O. Y., Syed, K., John, S. 2020. Acute On Chronic Liver Failure. StatPearls, Sci Rep 6(1):1-14.
32. Shenouda SK, Alahari SK. 2009. MicroRNA function in cancer: oncogene or a tumor suppressor? Cancer Metastasis Rev. Dec;28 ,3-4,:369-78. doi: 10.1007/s10555-009-9188-5.
33. Shrivastava, S., Ray, R.B. 2014. Hepatitis C virus infection, autophagy and innate immune response. In: Hayat M.A., editor. vol. 3. Academic Press, Elsevier; pp. 164–190.
34. Thrift. A.P., El-Serag, H.B., Kanwal, F. 2017.Global epidemiology and burden of HCV infection and HCV-related disease. Nature Reviews. Gastroenterology & Hepatology.;14(2):122-132.
35. WHO, Hepatitis C. 2020? Retrieved from https://www.who.int/news-room/fact-sheets/detail/hepatitis-c, (27 /1/2021).
36. You, K., Li, S.Y., Gong, J., Fang, J.H., Zhang, C., Zhang, M. 2018. MicroRNA-125b promotes hepatic stellate cell activation and liver fibrosis by activating RhoA signaling. Mol Ther Nucleic Acids ;12:57-66.
37. Young, I.S., Woodside, J.V. 2001. Antioxidants in health and disease. J. Clin. Pathol. ;54:176–86.
38. Zaltron ,S., Spinetti, A., Biasi, L., Baiguera, C., Castelli, F. 2012. Chronic HCV infection: Epidemiological and clinical relevance. BMC Infect Dis.;12 Suppl 2. S2. doi: 10.1186/1471-2334.
39. Zhang, Z., Chen, J., He, Y., Zhan, X., Zhao, R., Huang, Y., Xu, H., Zhu, Z., Liu, Q. 2020. miR-125b inhibits hepatitis B virus expression in vitro through targeting of the SCNN1A gene. Arch virol. 159:3335–3343.