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Original article

Physiological alteration and the expression of fibrogenesis-proinflammatory genes in hepatocellular carcinoma patients with chronic hepatitis C and onset metabolic syndrome: a preliminary report at Saiful Anwar General Hospital Malang

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Abstract

Hepatocellular carcinoma (HCC) is one type of cancer included in the fifth order of cancer that causes globally severe deaths. Liver dysfunction related to metabolic perturbation and chronic hepatitis C (CHC) may play a crucial role in inducing HCC incidence. Furthermore, the higher expression of potential fibrogenic and inflammation-related genes was hypothesized to contribute to the HCC progression. This study aims to describe the basic profile of classical serological markers and fibrogenesis-inflammation-related genes expression in the progression of HCC due to CHC and metabolic syndrome. Interestingly, the results of serological tests in the male gender group showed that the average fasting body glucose level was 86.14 mg/dL, total cholesterol 252 mg/dL, triglyceride 148.86 mg/dL, high-density lipoprotein-cholesterol (HDL-C) 19.43 mg/dL, and low-density lipoprotein-cholesterol (LDL-C) 186.67 mg/dL. On the other hand, in the female gender group, the average fasting blood glucose levels are 82.33 mg/dL, total cholesterol 338 mg/dL, triglyceride 234.67 mg/dL, HDL-C 18.33mg/dL, and LDL-C 190 mg/dL. All gender groups also showed out of range for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. Furthermore, males tend to have a higher expression level of fibrosis-related genes TGF-β1 (5.8 times) and NOX4 (1.2 times) than females. In addition, the expression of the proinflammatory cytokine TNF-α, is also known that two times higher in males compared to females. The study revealed that the physiological alteration related to metabolic imbalance and increased proinflammatory-fibrogenic genes expression may accelerate HCC development in subjects with CHC onset metabolic syndrome. Thus, the personalized observation of HCC patients with these symptoms may become a future concern to prevent the severity of this disease during clinical administration.

Keywords: Hepatocellular carcinoma, Chronic hepatitis C, Metabolic Syndrome, Fibrogenesis, Inflammation

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Introduction

Hepatocellular Carcinoma (HCC) is one type of cancer that was ranked fifth as the cancer-causing death in the world (Axley et al., 2018). HCC cases are often found in the Asian region, with a mortality rate of 72.4% (Goodarzi et al., 2019). The number of HCC cases in Indonesia is also the second-highest cancer incidence, with the percentage of sufferers around 12.4% of the 100,000 population (P2PTM Kementerian Kesehatan Republik Indonesia, 2019). From the high number of cases and the death rate from the HCC incident, the survival of patients is known to be only 4-8 months, and almost 60% of HCC patients are only detected when entering the final stage due to late diagnosis and poor prognosis (Loho et al., 2016; Tsuchiya, 2015). The low patient survival rate may be due to the lack of specificity and sensitivity of the initial diagnosis of HCC (Njei et al., 2015).

One of the causes of HCC is the Hepatitis C virus (HCV), whose infection can be accompanied by inflammation (Chronic Hepatitis C/CHC), steatosis, and fibrosis of the liver (Goossens & Hoshida, 2015). Chronic inflammation-mediated by HCV protein can trigger HCC through cell proliferation, energy metabolism, and apoptosis (Irshad et al., 2017). In this case, fibrosis and cirrhosis are also included in the dominant risk factors for the development of HCC because they are carcinogenic zones (Balogh et al., 2016; Irshad et al., 2017).

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Aside from chronic infection caused by Hepatitis C, pre-existing factors in the form of metabolic syndromes, such as hypertension, dyslipoproteinemia, and impaired fasting glucose, can also increase the HCC risk by 1.56 times (Welzel et al., 2011). Besides, obesity and diabetes mellitus type 2 (T2DM) also become the high-risk factors for liver cancer, pancreatic cancer, colorectal cancer, kidney cancer, endometrial cancer, bladder cancer, biliary tract cancer, breast cancer, and non-Hodgkin's lymphoma, as well as the independent risk factor of HCC that is correlated with non-alcoholic fatty liver disease (NAFLD) (Cohen & LeRoith, 2012). HCC develops from chronic liver injury, inflammation, and fibrosis (Baglieri et al., 2019). Consequently, the increase in free cholesterol level, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), which are the parameter of metabolic syndrome, also improves the cancer cell's proliferation during its progression (Mayengbam et al., 2021).

The HCC development phase involves the inflammation process so that the fibrosis and cirrhosis also associate various primary genes, such as tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF- β1), and NADPH Oxidase-*4* (NOX*4*). TNF-α is the proinflammatory cytokine involved in the tumor proliferation, invasion, and metastasis of cancer cells (Yang et al., 2019). Meanwhile, TGF- β1 performs as

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the central mediator in the fibrosis that activates NOX*4* to be the essential source of reactive oxygen species (ROS), resulting in the mitochondrial dysfunction and oxidative stress on the endoplasmic reticulum in the HCC case (Guo et al., 2013; Ivanov et al., 2013).

The development of HCC involves multiple processes ranging from chronic inflammation to fibrosis and cirrhosis. In this regard, tumor necrosis factor- α (TNF-α) acts as one of the major proinflammatory cytokines involved in tumor proliferation, invasion, and metastasis (Yang et al., 2019). Meanwhile, other genes such as transforming growth factor- β1 (TGF-β1) act as a central mediator of fibrosis and will activate NADPH Oxidase-4 (NOX4) as the primary source of Reactive Oxygen Species (ROS), which can cause mitochondrial dysfunction and oxidative stress on the endoplasmic reticulum (Guo et al., 2013; Ivanov et al., 2013).

Albeit studies on the HCC incidence in patients with chronic hepatitis C and fibrosis have been extensively carried out, little is known about the clinical information on HCC patients onset metabolic syndrome in collaboration with chronic hepatitis C. Therefore, further clinical investigation in this area could potentially provide additional clinical data for these specific cases and metabolic perturbation.

Methods

This study was carried out using the Quantitative Real-Time PCR method and standardized serological testing. The detail of the primer set for the RTqPCR test are as follows:

Table 1. The primer set of Real-Time Quantitative Polymerase Chain Reaction

Human Gene	Forward	Reverse
Beta-actin	AGCACTGTGTTGGCGTACAG	GGACTTCGAGCAAGAGATGG
TNF-*α*	GTTCCTCAGCCTCTTCTCCT	ACAACATGGGCTACAGGCTT
NOX4	GCAGGAGAACCAGGAGATTG	CACTGAGAAGTTGAGGGCATT
TGF-β1	AAGTGGACATCAACGGGTTC	GTCCTTGCGGAAGTCAATGT

The project was conducted in collaboration with Saiful Anwar General Hospital, Malang, East Java, Indonesia. The study has obtained ethical approval from the Institutional Review Board with the registration number: 400/133/K.3/302/2019. In this preliminary study, a total of 15 hepatocellular carcinoma patients with a history of chronic hepatitis C (CHC) and onset metabolic syndrome were willing to provide informed consent. Research respondents consisted of ten men and five women with several inclusion criteria, including the HCV load > 800,000 IU/mL, patients with onset metabolic syndrome, non-smoking, non-hypertension, non-diabetic, and non-alcoholic. The exclusion criteria were HCC patients with chemotherapy, diabetes, hypertension, alcoholic history, smoking, and special invasive administration. Moreover, the whole blood from the patients was used for the total RNA isolation (the NucleoSpin® protocol manual RNA Blood kit-catalog No. 740200 Macherey-Nagel, Duren, Germany), while the blood serum and used in serological testing. The reverse-transcription process was done by using the iScript™ protocol manual cDNA Synthesis Kit No. 1708890 Bio-Rad Laboratories. The collected data for genes expression was then analyzed by t-test statistical model with JMP software version 7 and presented in graphical form using the GraphPad Prism version 5 software.

Results

Baseline characteristics

Patients involved in this study were 15 individuals. The male patient group has an age range of 28-62 years, while the female patient group is in the 36-59 year age range. Based on the serological tests, the male gender group had a higher fasting blood glucose than the female even in the normal range. The serum level of total cholesterol, triglyceride, and LDL-C are higher in females than in males. In addition, the HDL-C levels were higher in male individuals than in the female. Baseline serological results and the two classical markers of liver injury in two groups of patients by gender are presented in Table 2 and Figure 1.

Table 2. Baseline characteristics

Parameters	Group	
	Male (n = 10)	**Female (n = 5)**
Age (yrs)	48.71 ± 1.19	44.25 ± 1.101
Fasting Blood Glucose (mg/dL)	86.14 ± 2.14	82.33 ± 3.64
Cholesterol (mg/dL)	252 ± 2.47	338 ± 4.17
Triglyceride (mg/dL)	148.86 ± 4.94	234.67 ± 6.29
HDL-C (mg/dL)	19.43 ± 8.12	18.33 ± 9.29
LDL-C (mg/dL)	186.67 ± 2.23	190 ± 1.22

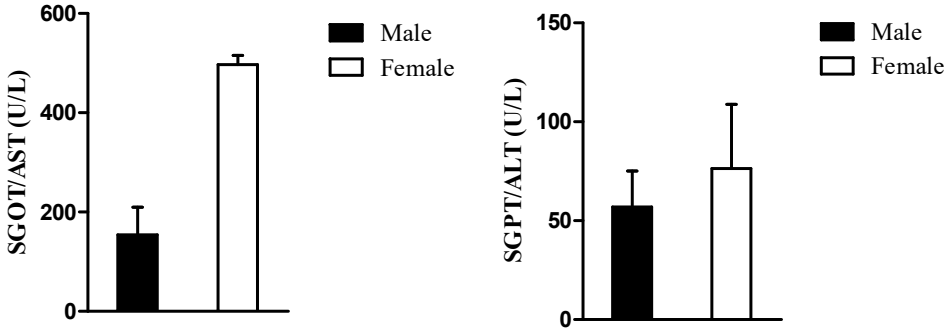

Figure 1. Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels in all HCC-CHC patients by gender group

The mean of AST level of male patients is 497 U/L, with the highest AST level of 1237 U/L. Meanwhile, the average AST level in male patients was 154 U/L, with the highest AST level being 237 U/L. From these two averages, the AST levels in both male and female patients have exceeded the normal AST level. Furthermore, the mean ALT level of female patients was 76.3 U/L, with the highest level being 137 U/L. The average ALT level obtained was 57 U/L in male patients, with the highest level being 79 U/L. Based on the analysis, the ALT levels have exceeded the normal ALT level.

Expression Levels of Fibrogenesis-related genes in HCC-CHC Patients

TGF-β1 and NOX4 gene expression in male patients was higher than in female patients. In male patients, the TGF-β1 gene was expressed 5.8 times higher than in female patients. Furthermore, in the NOX4 gene, the expression level in male patients was also 1.2 times higher than that of female patients. These results show a higher increase in fibrogenic activity in male patients characterized by high levels of expression of the TGF-β1 gene, which is a profibrogenic cytokine, and the NOX4 gene, which indicates increased oxidative stress (Fig. 2).

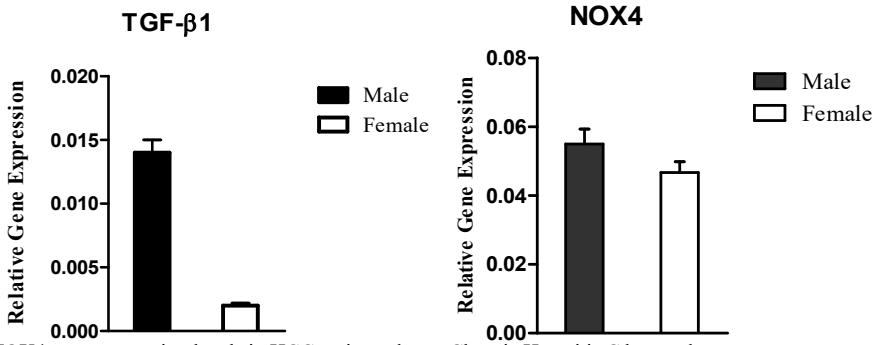

Figure 2. TGF-β1 and NOX4 gene expression levels in HCC patients due to Chronic Hepatitis C by gender

Expression Levels of TNF- α Pro-Inflammatory Cytokine Genes in HCC-CHC patients

Linear to the previous fibrogenic-related genes expression, the TNF-α gene in male patients was also expressed higher than in female patients.

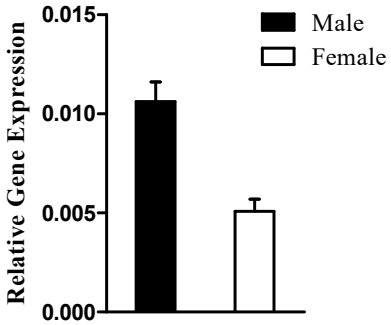

Figure 3. TNF- α gene expression levels of HCC patients due to Chronic Hepatitis C by gender group

In addition, it was found that in male patients, the TNF-α gene expression was increased by two times higher than in female patients (Figure 3). In line with the patients record, chronic hepatitis C has a significant correlation to the higher TNF-α gene expression. This cytokine plays a pivotal role in chronic inflammation and could contributes to fibrogenesis in HCC-CHC patients.

Discussion

Metabolic syndrome profiles in HCC patients due to chronic hepatitis C

The presence of metabolic syndrome in enhancing the HCC-CHC progression is correlated with its ability to worsen the metabolic complications caused by HCV infection from the glucose and lipid metabolism disorders (Chang, 2016; Chaudhari et al., 2021). The metabolic syndrome is characterized by the increase of fasting blood glucose (FBG), an increase of triglycerides,

decrease of HDL-C level, an increase of LDL-C, insulin resistance, dyslipidemia, obesity, and hypertension which also becomes the independent risk factor of the advanced liver fibrosis (Amihăesei & Chelaru, 2014; Chang et al., 2015). Among the baseline characteristics that covered some parameters, such as FBG, cholesterol, triglyceride, HDL-C, and LDL-C, only the FBG parameter that was within the normal level (80-110 mg/dL) in both men (86 ± 2.14 mg/dL) and women (82.33 ± 3.64 mg/dL) (Marbun & Mardiani, 2016). According to the FBG parameter, all HCC-CHC patients were not in the pre-diabetes and diabetes phase (Loftfield et al., 2016). Generally, the increase of FBG is closely related to the increase of liver cancer risk, by 77%, in the diabetes and pre-diabetes phases (Han et al., 2017). The correlation is induced by cancer cell proliferation caused by the increase of insulin-like growth factor-1 (IGF-1) in the hyperglycemia and hyperinsulinemia cases (Adachi et al., 2016; Han et al., 2017).

In addition, the triglycerides were also observed to increase, from the normal range (less than 150 mg/dL), in the male (148.86 ± 4.94 mg/dL) and female (234.67 ± 6.29 mg/dL) patients. This group of gender is categorized in the mild hypertriglyceridemia category (Lee & Siddiqui, 2022). In the HCC-CHC patients, the HCV infection can increase the accumulated triglycerides within the liver (Felmlee et al., 2013). The HCV infection also contributes to this accumulation by activating lipogenic gene transcription that facilitates the lipid synthesis in the patients (Fujino et al., 2010).The liver injury caused by HCV infection increases the triglycerides metabolism, accelerating the free fatty acid in the blood and generating hypertriglyceridemia (Qasim et al., 2020). It may occur due to the increase of ApoC-IV expression, which is a core-protein responsive element closely related to the increase of accumulated triglycerides in HCV patients (Felmlee et al., 2013). In addition, the presence of hepatic lipotoxicity caused by triglycerides accumulation in patients with metabolic syndrome may cause hepatocellular injury (Zhou & Sun, 2021).

The cholesterol level also increased from the normal range (< 100 mg/dL) in male (252 ± 2.47) and female (338 ± 4.17) patients. The high cholesterol level in HCC patients is correlated with the increase of liver tumorigenesis through steatohepatitis induction, hepatocyte division promoting, intracellular stress induction, and pro-inflammatory related genes, such as NF-kB and TNF-α (Simoni-Nieves et al., 2021; Wang et al., 2019). As an organ, the liver carries an essential role in cholesterol metabolism within the human body. Thus, liver damage in HCC-CHC patients disrupts the cholesterol balance (Zhou & Sun, 2021). Additionally, cholesterol induces the activation of hepatic stellate cells (HSCs), which contributes to the activation of fibrogenic-related genes, such as TGF-β in HSCs, directly or through mechanisms involving inflammatory cytokines (Sun et al., 2020; Tomita et al., 2014).

The high-density lipoprotein-cholesterol (HDL-C) levels in male (19.43 ± 8.12) and female (18.33 ± 9.29) patients are categorized as low (<40). Meanwhile, the low-density lipoprotein-cholesterol (LDL-C) in male patients (186.67 ± 2.23) is classified as high (160-189 mg/dL) and in female patients (190 ± 1.22) is categorized as very high (>190 mg/dL). The combination of low-HDL and high-LDL is closely related to the more aggressive HCC progression with a higher death rate (Akkiz et al., 2021). In the HCC-CHC, HDL-C, and LDL-C patients, free cholesterol is one of the parameters of the increasing cancer cell proliferation due to the interaction between HCV and SR-B1, as well as NPC1L1 and LDL, which are the cholesterol from HDL, LDL, and free cholesterol particles (Aizawa et al., 2015; Mayengbam et al., 2021).

The results from two gender groups showed that the female patients presented increasing cholesterol, triglyceride, and LDL-C at a higher level compared to the male patients. In relation to this finding, the correlation between metabolic syndrome and HCC risk factors is not significantly affected by the patients' gender (Chen et al., 2018). Besides, the baseline characteristics reveal the lipid metabolism disorders in the HCC-CHC patients, as they have high cholesterol, triglyceride, and LDL-C levels and low HDL-C levels, which have been observed to be beyond the normal level.

Liver injury marker profile (AST and ALT) in HCC patients due to chronic hepatitis C

AST and ALT levels are two types of aminotransferase group enzymes that are included in liver function tests (Liver Function Tests). They have the greatest clinical significance as an indicator of damage to the liver. (Hall & Cash, 2012; Zachariah et al., 2017). Based on the results of serological tests in the clinic, AST and ALT levels in female patients tend to be higher than in male patients. The normal range for AST levels for men is ≤ 37 U/L and ≤ 31 U/L for women. Meanwhile, the normal range for ALT levels for men is ≤ 40 U/L and ≤ 31 U/L for women. (Hann et al., 2012). Therefore, both male and female patients have AST and ALT levels that exceed normal limits from these results.

AST and ALT levels were found to be higher in patients with advanced liver fibrosis compared to patients in the early stages of the liver fibrosis process (Salum et al., 2018). In a person with generalized HCC, high levels of AST and ALT reflect damage to hepatocytes as a direct result of tumor growth or liver cell damage (Hasan et al., 2014). AST and ALT also show impaired hepatocytes in non-tumor (X. Liu et al., 2019). In addition, high ALT and AST values are known to indicate a risk of hepatosteatosis in patients with chronic hepatitis C infection (Lin et al., 2015).

HCV will cause chronic inflammation in hepatocytes resulting in regeneration and proliferation of hepatocytes which causes genetic instability (McGill, 2016). AST and ALT levels are associated with various pro-inflammatory and profibrogenic chemokines. AST and ALT levels will increase in the setting of acute HCV infection (Hajarizadeh et al., 2016).

Expression of fibrogenesis and proinflammatory-related genes in HCC patients with chronic hepatitis C

HCC is also called a gender-biased disease because HCC occurs more often in men than women in a ratio that ranges from 2:1 to 11:1 (Deng et al., 2015). The chance of a man at productive age suffering from HCC is 3-5 times greater than compared to women (Yan et al., 2017). The analysis showcases that the development of HCC starts from chronic inflammation and fibrosis due to exposure to the HCV as indicated by the gene expression levels of TNF-α, TGF-β1, and NOX4 in HCC patients exposed to HCV were more dominant in males.

When inflammation occurs, male produces TNF-α at much higher levels than female neutrophils (Aomatsu et al., 2013). This finding is in line with the results of research that has been done, which shows the level of TNF-α gene expression in men is much higher than in women. High levels of TNF-α in the body can increase tumor growth, invasion, and angiogenesis (Wang et al., 2019). TNF-α which acts as a pro-inflammatory cytokine will contribute to the formation of edema, blood coagulation and trigger oxidative stress in areas of inflammation and hepatocarcinogenesis. (Jang et al., 2014; Zelová & Hošek, 2013). Based on this, it can be seen that TNF-α is not produced in a healthy body. TNF-α can also stimulate the release of inflammatory cytokines and trigger the release of other fibrogenic factors such as interleukin-1 (IL-1), interleukin-6 (IL-6), and transforming growth factor-β. (Saleh et al., 2020).

In addition to the TNF- α gene, an increase in the expression level is also known to occur in the TGF-β1 gene. TGF-β1 gene expression levels in men tend to be significantly higher than in women. Men suffering from HCC will generally have higher levels of TGF-β1, cortisol, tumor-associated neutrophils (TAN) and tumor-associated macrophages (TAM) when compared to women. (Yan et al., 2017; Yang et al., 2012). TGF-β1 is secreted by M2 phenotype macrophages and is a profibrogenic cytokine that has increased expression in every fibrosis event (Liu et al., 2018). TGF-β1 also contributes to the death of hepatocyte cells, accumulated lipid, and production of reactive oxygen species (ROS) (Yang et al., 2014). The high level of TGF-β1 expression is directly proportional to the increasing HCC progression (Huang et al., 2018).

HCV infection that leads to the development of HCC can also increase the expression of other genes such as NOX4 due to the high expression of the profibrogenic cytokine TGF-β1 (Boudreau et al., 2009). TGF-β1 is initially stored in the extracellular matrix in the form of latent TGF- β1, then becomes active TGF-β1 and promotes ROS formation by inducing NOX4 (Okina et al., 2020). NOX4 produced will cause mitochondrial dysfunction, endoplasmic reticulum stress, and DNA damage due to oxidative stress (Ivanov et al., 2013; Pal et al., 2010). Due to oxidative stress, the general consequence is the inhibition of liver regeneration which leads to hepatocyte apoptosis, which encourages the activation of hepatic stellate cells (HSCs). HSC will proliferate into myofibroblasts, whereas the accumulation of these myofibroblasts will cause scarring of the liver. As a result, the stage of liver fibrosis will then develop into HCC (Jung et al., 2010; Lan et al., 2015; Sancho et al., 2012).

The expression levels of TNF- α, TGF-β1, and NOX4 genes are generally directly proportional to the increase in AST and ALT; however, this is not always the case in most cases (Mohy & Fouad, 2014). In the study results above, it appears that there is an increase in AST and ALT values that exceed normal limits in both male and female patients. Increased levels of TNF- α, TGF-β1, and NOX4 gene expression were accompanied by increased plasma ALT and AST levels (Zhao et al., 2020). AST and ALT levels also did not correlate with histological activity, viral replication and virological response in HCC patients who had previously been exposed to HCV (Gomaa et al., 2014).

The analysis showcased that, in cases of HCC due to HCV exposure, the gene expression levels of TNF-α, TGF-β1, and NOX4 in men tend to be higher when compared to women with AST and ALT values which also exceed normal limits. The metabolism imbalance is identified from the high cholesterol, triglyceride, and LDL-C level and the low HDL-C level, showing a more aggressive HCC progression with a higher possibility of death. It is also supported by the increase of TNF-α, TGF-β1, and NOX*4* gene expression, indicating the presence of liver inflammation and fibrosis. Therefore, profiling of HCC-CHC patients with a pre-existing factor of metabolic syndrome is essential to generate a clinical contribution in preventing the severity of this disease.

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References

Adachi, Y., Nojima, M., Mori, M., Matsunaga, Y., Akutsu, N., Sasaki, S., Endo, T., Kurozawa, Y., Wakai, K., Tamakoshi, A., & for JACC Study. (2016). Insulin-like growth factor-related components and the risk of liver cancer in a nested case-control study. *Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine*, *37*(11), 15125–15132. https://doi.org/10.1007/s13277-016-5360-z

Aizawa, Y., Seki, N., Nagano, T., & Abe, H. (2015). Chronic hepatitis C virus infection and lipoprotein metabolism. *World Journal of Gastroenterology*, *21*(36), 10299–10313. https://doi.org/10.3748/wjg.v21.i36.10299

Akkiz, H., Carr, B., Guerra, V., Donghia, R., Yalçın, K., U, Karaogullarindan, U., Altintas, E., Özakyol, A., Şimsek, H., Balaban, H.Y., Uyanikoglu, A., Ekin, N., & Delik, A. (2021). Plasma lipids, tumor parameters and survival in HCC patients with HBV and HCV. *Journal of Translational Science*, *7*(3). https://doi.org/10.15761/JTS.1000421

Amihăesei, I. C., & Chelaru, L. (2014). Metabolic syndrome a widespread threatening condition; risk factors, diagnostic criteria, therapeutic options, prevention and controversies: An overview. *Revista Medico-Chirurgicala a Societatii De Medici Si Naturalisti Din Iasi*, *118*(4), 896–900.

Aomatsu, M., Kato, T., Kasahara, E., & Kitagawa, S. (2013). Gender difference in tumor necrosis factor-α production in human neutrophils stimulated by lipopolysaccharide and interferon-γ. *Biochemical and Biophysical Research Communications*, *441*(1), 220–225. https://doi.org/10.1016/j.bbrc.2013.10.042

Axley, P., Ahmed, Z., Ravi, S., & Singal, A. K. (2018). Hepatitis C Virus and Hepatocellular Carcinoma: A Narrative Review.

Journal of Clinical and Translational Hepatology, *6*(2), 1–6. https://doi.org/10.14218/JCTH.2017.00067

Baghdady, I., Fouad, F., Sayed, M., Shoaib, A., Salah, Y., Elshayeb, E., Hasan, A. E., & others. (2014). Serum markers for the early detection of hepatocellular carcinoma in patients with chronic viral hepatitis C infection. *Menoufia Medical Journal*, *27*(3), 544.

Baglieri, J., Brenner, D., & Kisseleva, T. (2019). The Role of Fibrosis and Liver-Associated Fibroblasts in the Pathogenesis of Hepatocellular Carcinoma. *International Journal of Molecular Sciences*, *20*(7), 1723. https://doi.org/10.3390/ijms20071723

Balogh, J., Victor, D., Asham, E. H., Burroughs, S. G., Boktour, M., Saharia, A., Li, X., Ghobrial, M., & Monsour, H. (2016). Hepatocellular carcinoma: A review. *Journal of Hepatocellular Carcinoma*, *Volume 3*, 41–53. https://doi.org/10.2147/JHC.S61146

Boudreau, H. E., Emerson, S. U., Korzeniowska, A., Jendrysik, M. A., & Leto, T. L. (2009). Hepatitis C Virus (HCV) Proteins Induce NADPH Oxidase 4 Expression in a Transforming Growth Factor β-Dependent Manner: A New Contributor to HCV-Induced Oxidative Stress. *Journal of Virology*, *83*(24), 12934–12946. https://doi.org/10.1128/JVI.01059-09

Chang, M.-L. (2016). Metabolic alterations and hepatitis C: From bench to bedside. *World Journal of Gastroenterology*, *22*(4), 1461–1476. https://doi.org/10.3748/wjg.v22.i4.1461

Chang, M.-L., Hsu, C.-M., Tseng, J.-H., Tsou, Y.-K., Chen, S.-C., Shiau, S.-S., Yeh, C.-T., & Chiu, C.-T. (2015). Plasminogen activator inhibitor-1 is independently associated with non-alcoholic fatty liver disease whereas leptin and adiponectin vary between genders. *Journal of Gastroenterology and Hepatology*, *30*(2), 329–336. https://doi.org/10.1111/jgh.12705

Chaudhari, R., Fouda, S., Sainu, A., & Pappachan, J. M. (2021). Metabolic complications of hepatitis C virus infection. *World Journal of Gastroenterology*, *27*(13), 1267–1282. https://doi.org/10.3748/wjg.v27.i13.1267

Chen, Y., Li, X., Wu, S., Ye, W., & Lou, L. (2018). Metabolic syndrome and the incidence of hepatocellular carcinoma: A meta-analysis of cohort studies. *OncoTargets and Therapy*, *Volume 11*, 6277–6285. https://doi.org/10.2147/OTT.S154848

Cohen, D. H., & LeRoith, D. (2012). Obesity, type 2 diabetes, and cancer: The insulin and IGF connection. *Endocrine-Related Cancer*, *19*(5), F27–F45. https://doi.org/10.1530/ERC-11-0374

Deng, L., Yang, H., Tang, J., Lin, Z., Yin, A., Gao, Y., Wang, X., Jiang, R., & Sun, B. (2015). Inhibition of MTA1 by ERα contributes to protection hepatocellular carcinoma from tumor proliferation and metastasis. *Journal of Experimental & Clinical Cancer Research*, *34*(1), 128. https://doi.org/10.1186/s13046-015-0248-0

Fabregat, I., & Caballero-Díaz, D. (2018). Transforming Growth Factor-β-Induced Cell Plasticity in Liver Fibrosis and Hepatocarcinogenesis. *Frontiers in Oncology*, *8*. https://doi.org/10.3389/fonc.2018.00357

Felmlee, D. J., Hafirassou, M. L., Lefevre, M., Baumert, T. F., & Schuster, C. (2013). Hepatitis C virus, cholesterol and lipoproteins—Impact for the viral life cycle and pathogenesis of liver disease. *Viruses*, *5*(5), 1292–1324. https://doi.org/10.3390/v5051292

Fujino, T., Nakamuta, M., Yada, R., Aoyagi, Y., Yasutake, K., Kohjima, M., Fukuizumi, K., Yoshimoto, T., Harada, N., Yada, M., Kato, M., Kotoh, K., Taketomi, A., Maehara, Y., Nakashima, M., & Enjoji, M. (2010). Expression profile of lipid metabolism-associated genes in hepatitis C virus-infected human liver. *Hepatology Research: The Official Journal of the Japan Society of Hepatology*, *40*(9), 923–929. https://doi.org/10.1111/j.1872-034X.2010.00700.x

Gomaa, W. M., Ibrahim, M. A., & Shatat, M. E. (2014). Overexpression of cyclooxygenase-2 and transforming growth factor-beta 1 is an independent predictor of poor virological response to interferon therapy in chronic HCV genotype 4 patients. *Saudi Journal of Gastroenterology*, *20*(1), 59. https://doi.org/10.4103/1319-3767.126324

Goodarzi, E., Ghorat, F., Mosavi Jarrahi, A., Adineh, H. A., Sohrabivafa, M., & Khazaei, Z. (2019). Global incidence and mortality of liver cancers and its relationship with the human development index (HDI): An ecology study in 2018. *World Cancer Research Journal*, *6*(April 2019). https://doi.org/10.32113/wcrj_20194_1255

Goossens, N., & Hoshida, Y. (2015). Hepatitis C virus-induced hepatocellular carcinoma. *Clinical and Molecular Hepatology*, *21*(2), 105–114. https://doi.org/10.3350/cmh.2015.21.2.105

Loftfield, E., Freedman, N. D., Lai, G. Y., Weinstein, S. J., McGlynn, K. A., Taylor, P. R., Männistö, S., Albanes, D., & Stolzenberg-Solomon, R. Z. (2016). Higher Glucose and Insulin Levels Are Associated with Risk of Liver Cancer

Guo, Y., Zang, C., Li, Y., Yuan, L., Liu, Q., Zhang, L., & Li, S. (2013). Association Between *TGF-β1* Polymorphisms and Hepatocellular Carcinoma Risk: A Meta-Analysis. *Genetic Testing and Molecular Biomarkers*, *17*(11), 814–820. https://doi.org/10.1089/gtmb.2013.0268

Hajarizadeh, B., Lamoury, F. M., Feld, J. J., Amin, J., Keoshkerian, E., Matthews, G. V., Hellard, M., Dore, G. J., Lloyd, A. R., Grebely, J., & Applegate, T. L. (2016). Alanine aminotransferase, HCV RNA levels and pro-inflammatory and pro-fibrogenic cytokines/chemokines during acute hepatitis C virus infection. *Virology Journal*, *13*(1), 32. https://doi.org/10.1186/s12985-016-0482-x

Hall, P., & Cash, J. (2012). What is the real function of the liver "function" tests? *The Ulster Medical Journal*, *81*(1), 30–36.

Han, H., Zhang, T., Jin, Z., Guo, H., Wei, X., Liu, Y., Chen, Q., & He, J. (2017). Blood glucose concentration and risk of liver cancer: Systematic review and meta-analysis of prospective studies. *Oncotarget*, *8*(30), 50164–50173. https://doi.org/10.18632/oncotarget.16816

Hann, H.-W., Wan, S., Myers, R. E., Hann, R. S., Xing, J., Chen, B., & Yang, H. (2012). Comprehensive Analysis of Common Serum Liver Enzymes as Prospective Predictors of Hepatocellular Carcinoma in HBV Patients. *PLoS ONE*, *7*(10), e47687. https://doi.org/10.1371/journal.pone.0047687

Hasan, A., Salah, Y., Sayed, M., Shoaib, A., Baghdady, I., Elshayeb, E., & Fouad, F. (2014). Serum markers for the early detection of hepatocellular carcinoma in patients with chronic viral hepatitis C infection. *Menoufia Medical Journal*, *27*(3), 544. https://doi.org/10.4103/1110-2098.145509

Huang, J., Qiu, M., Wan, L., Wang, G., Huang, T., Chen, Z., Jiang, S., Li, X., Xie, L., & Cai, L. (2018). TGF-β1 Promotes Hepatocellular Carcinoma Invasion and Metastasis via ERK Pathway-Mediated FGFR4 Expression. *Cellular Physiology and Biochemistry*, *45*(4), 1690–1699. https://doi.org/10.1159/000487737

Irshad, M., Gupta, P., & Irshad, K. (2017). Molecular basis of hepatocellular carcinoma induced by hepatitis C virus infection. *World Journal of Hepatology*, *9*(36), 1305–1314. https://doi.org/10.4254/wjh.v9.i36.1305

Ivanov, A., Bartosch, B., Smirnova, O., Isaguliants, M., & Kochetkov, S. (2013). HCV and Oxidative Stress in the Liver. *Viruses*, *5*(2), 439–469. https://doi.org/10.3390/v5020439

Jang, M.-K., Kim, H. S., & Chung, Y.-H. (2014). Clinical aspects of tumor necrosis factor-α signaling in hepatocellular carcinoma. *Current Pharmaceutical Design*, *20*(17), 2799–2808. https://doi.org/10.2174/13816128113199990587

Jung, Y., Witek, R. P., Syn, W. K., Choi, S. S., Omenetti, A., Premont, R., Guy, C. D., & Diehl, A. M. (2010). Signals from dying hepatocytes trigger growth of liver progenitors. *Gut*, *59*(5), 655–665. https://doi.org/10.1136/gut.2009.204354

Lan, T., Kisseleva, T., & Brenner, D. A. (2015). Deficiency of NOX1 or NOX4 Prevents Liver Inflammation and Fibrosis in Mice through Inhibition of Hepatic Stellate Cell Activation. *PLOS ONE*, *10*(7), e0129743. https://doi.org/10.1371/journal.pone.0129743

Lee, Y., & Siddiqui, W. J. (2022). Cholesterol Levels. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK542294/

Lin, M.-S., Lin, H.-S., Chung, C.-M., Lin, Y.-S., Chen, M.-Y., Chen, P.-H., Hu, J.-H., Chou, W.-N., Huang, J.-C., & Huang, T.-J. (2015). Serum aminotransferase ratio is independently correlated with hepatosteatosis in patients with HCV: A cross-sectional observational study. *BMJ Open*, *5*(9), e008797. https://doi.org/10.1136/bmjopen-2015-008797

Liu, X., Meng, J., Xu, H., & Niu, J. (2019). Alpha-fetoprotein to transaminase ratio is related to higher diagnostic efficacy for hepatocellular carcinoma. *Medicine*, *98*(17), e15414. https://doi.org/10.1097/MD.0000000000015414

Liu, Z., Kuang, W., Zhou, Q., & Zhang, Y. (2018). TGF-β1 secreted by M2 phenotype macrophages enhances the stemness and migration of glioma□cells via the SMAD2/3 signalling pathway. *International Journal of Molecular Medicine*. https://doi.org/10.3892/ijmm.2018.3923

and Chronic Liver Disease Mortality among Men without a History of Diabetes. *Cancer Prevention Research (Philadelphia, Pa.)*, *9*(11), 866–874. https://doi.org/10.1158/1940-6207.CAPR-16-0141

Loho, I. M., Hasan, I., Lesmana, C. R. A., Dewiasty, E., & Gani, R. A. (2016). Hepatocellular Carcinoma in a Tertiary Referral Hospital in Indonesia: Lack of Improvement of One-Year Survival Rates between 1998-1999 and 2013-2014. *Asian Pacific Journal of Cancer Prevention*, *17*(4), 2165–2170. https://doi.org/10.7314/APJCP.2016.17.4.2165

Marbun, P. I. G., & Mardiani, T. H. (2016). Correlation Between Blood Glucose Level and Thinking Concentration. *Folia Medica Indonesiana*, *52*(3), 214–218.

Mayengbam, S. S., Singh, A., Pillai, A. D., & Bhat, M. K. (2021). Influence of cholesterol on cancer progression and therapy. *Translational Oncology*, *14*(6), 101043. https://doi.org/10.1016/j.tranon.2021.101043

McGill, M. R. (2016). The past and present of serum aminotransferases and the future of liver injury biomarkers. *EXCLI Journal; 15:Doc817; ISSN 1611-2156*. https://doi.org/10.17179/EXCLI2016-800

Mohy, A., & Fouad, A. (2014). Role of transforming growth factor-β1 in serum and −509 C>T promoter gene polymorphism in development of liver cirrhosis in Egyptian patients. *Meta Gene*, *2*, 631–637. https://doi.org/10.1016/j.mgene.2014.08.002

Njei, B., Rotman, Y., Ditah, I., & Lim, J. K. (2015). Emerging trends in hepatocellular carcinoma incidence and mortality: NJEI ET AL. *Hepatology*, *61*(1), 191–199. https://doi.org/10.1002/hep.27388

Okina, Y., Sato-Matsubara, M., Matsubara, T., Daikoku, A., Longato, L., Rombouts, K., Thanh Thuy, L. T., Ichikawa, H., Minamiyama, Y., Kadota, M., Fujii, H., Enomoto, M., Ikeda, K., Yoshizato, K., Pinzani, M., & Kawada, N. (2020). TGF-β1-driven reduction of cytoglobin leads to oxidative DNA damage in stellate cells during non-alcoholic steatohepatitis. *Journal of Hepatology*, *73*(4), 882–895. https://doi.org/10.1016/j.jhep.2020.03.051

P2PTM Kementerian Kesehatan Republik Indonesia. (2019, February 1). *Jenis Kanker Terbanyak pada Pria dan Wanita* [Goverment Website]. http://www.p2ptm.kemkes.go.id/kegiatan-p2ptm/pusat-/ini-jenis-kanker-terbanyak-pada-pria-dan-wanita

Pal, S., Polyak, S. J., Bano, N., Qiu, W. C., Carithers, R. L., Shuhart, M., Gretch, D. R., & Das, A. (2010). Hepatitis C virus induces oxidative stress, DNA damage and modulates the DNA repair enzyme NEIL1: HCV modulates NEIL1. *Journal of Gastroenterology and Hepatology*, *25*(3), 627–634. https://doi.org/10.1111/j.1440-1746.2009.06128.x

Qasim, S. F., Jami, A., Imran, P., Mushtaque, R., & Khan, R. N. (2020). Frequency of Metabolic Syndrome in Chronic Hepatitis C Patients: Findings From a Lower Middle Income Country. *Cureus*, *12*(12), e11975. https://doi.org/10.7759/cureus.11975

Saleh, A., Saed, A. M., & Mansour, M. (2020). Association of IL-10 and TNF-α polymorphisms with risk and aggressiveness of hepatocellular carcinoma in patients with HCV-related cirrhosis. *Egyptian Liver Journal*, *10*(1), 43. https://doi.org/10.1186/s43066-020-00052-w

Salum, G. M., Bader El Din, N. G., Ibrahim, M. K., Dawood, R. M., Farouk, S., & El Awady, M. K. (2018). Correlation Between TGF-β1 and c-MET Expression in HCV Genotype 4-Induced Liver Fibrosis. *Journal of Interferon & Cytokine Research*, *38*(12), 552–558. https://doi.org/10.1089/jir.2018.0096

Sancho, P., Mainez, J., Crosas-Molist, E., Roncero, C., Fernández-Rodriguez, C. M., Pinedo, F., Huber, H., Eferl, R., Mikulits, W., & Fabregat, I. (2012). NADPH Oxidase NOX4 Mediates Stellate Cell Activation and Hepatocyte Cell Death during Liver Fibrosis Development. *PLoS ONE*, *7*(9), e45285. https://doi.org/10.1371/journal.pone.0045285

Simoni-Nieves, A., Salas-Silva, S., Chávez-Rodríguez, L., Escobedo-Calvario, A., Desoteux, M., Bucio, L., Souza, V., Miranda-Labra, R. U., Muñoz-Espinosa, L. E., Coulouarn, C., Gutiérrez-Ruiz, M. C., Marquardt, J. U., & Gomez-Quiroz, L. E. (2021). The Consumption of Cholesterol-Enriched Diets Conditions the Development of a Subtype of HCC with High Aggressiveness and Poor Prognosis. *Cancers*, *13*(7), 1721. https://doi.org/10.3390/cancers13071721

Sun, X., Seidman, J. S., Zhao, P., Troutman, T. D., Spann, N. J., Que, X., Zhou, F., Liao, Z., Pasillas, M., Yang, X., Magida, J. A., Kisseleva, T., Brenner, D. A., Downes, M., Evans, R. M., Saltiel, A. R., Tsimikas, S., Glass, C. K., & Witztum, J. L. (2020). Neutralization of Oxidized Phospholipids Ameliorates Non-alcoholic Steatohepatitis. *Cell Metabolism*, *31*(1), 189-206.e8. https://doi.org/10.1016/j.cmet.2019.10.014

Tomita, K., Teratani, T., Suzuki, T., Shimizu, M., Sato, H., Narimatsu, K., Okada, Y., Kurihara, C., Irie, R., Yokoyama, H., Shimamura, K., Usui, S., Ebinuma, H., Saito, H., Watanabe, C., Komoto, S., Kawaguchi, A., Nagao, S., Sugiyama, K., … Hibi, T. (2014). Free cholesterol accumulation in hepatic stellate cells: Mechanism of liver fibrosis aggravation in non-alcoholic steatohepatitis in mice. *Hepatology*, *59*(1), 154–169. https://doi.org/10.1002/hep.26604

Tsuchiya, N. (2015). Biomarkers for the early diagnosis of hepatocellular carcinoma. *World Journal of Gastroenterology*, *21*(37), 10573. https://doi.org/10.3748/wjg.v21.i37.10573

Wang, M., Wu, M., & Yang, T. (2019). The synergistic effect of sorafenib and TNF-α inhibitor on hepatocellular carcinoma. *EBioMedicine*, *40*, 11–12. https://doi.org/10.1016/j.ebiom.2019.01.007

Wang, X., Tanaka, N., Hu, X., Kimura, T., Lu, Y., Jia, F., Sato, Y., Nakayama, J., Moriya, K., Koike, K., & Aoyama, T. (2019). Correction to: A high-cholesterol diet promotes steatohepatitis and liver tumorigenesis in HCV core gene transgenic mice. *Archives of Toxicology*, *93*(6), 1727–1728. https://doi.org/10.1007/s00204-019-02491-w

Welzel, T. M., Graubard, B. I., Zeuzem, S., El-Serag, H. B., Davila, J. A., & McGlynn, K. A. (2011). Metabolic syndrome increases the risk of primary liver cancer in the United States: A study in the SEER-medicare database. *Hepatology*, *54*(2), 463–471. https://doi.org/10.1002/hep.24397

Yan, C., Yang, Q., & Gong, Z. (2017). Tumor-Associated Neutrophils and Macrophages Promote Gender Disparity in Hepatocellular Carcinoma in Zebrafish. *Cancer Research*, *77*(6), 1395–1407. https://doi.org/10.1158/0008-5472.CAN-16-2200

Yang, F., Wei, K., Qin, Z., Shao, C., Shu, Y., & Shen, H. (2019). Association between TNF-α-308G/A polymorphism and esophageal cancer risk: An updated meta-analysis and trial sequential analysis. *Journal of Cancer*, *10*(5), 1086–1096. https://doi.org/10.7150/jca.29390

Yang, L., Roh, Y. S., Song, J., Zhang, B., Liu, C., Loomba, R., & Seki, E. (2014). Transforming growth factor beta signaling in hepatocytes participates in steatohepatitis through regulation of cell death and lipid metabolism in mice. *Hepatology (Baltimore, Md.)*, *59*(2), 483–495. https://doi.org/10.1002/hep.26698

Yang, W., Lu, Y., Xu, Y., Xu, L., Zheng, W., Wu, Y., Li, L., & Shen, P. (2012). Estrogen Represses Hepatocellular Carcinoma (HCC) Growth via Inhibiting Alternative Activation of Tumor-associated Macrophages (TAMs)*. *Journal of Biological Chemistry*, *287*(48), 40140–40149. https://doi.org/10.1074/jbc.M112.348763

Zachariah, S. K., Paul, V., Mathews, K. S., Gopinath, J., M., C. T., & Rajeeve, S. (2017). Hepatic transaminases as predictors of liver injury in abdominal trauma. *International Surgery Journal*, *5*(1), 181. https://doi.org/10.18203/2349-2902.isj20175891

Zelová, H., & Hošek, J. (2013). TNF-α signalling and inflammation: Interactions between old acquaintances. *Inflammation Research: Official Journal of the European Histamine Research Society … [et Al.]*, *62*(7), 641–651. https://doi.org/10.1007/s00011-013-0633-0

Zhao, S., Jiang, J., Jing, Y., Liu, W., Yang, X., Hou, X., Gao, L., & Wei, L. (2020). The concentration of tumor necrosis factor-α determines its protective or damaging effect on liver injury by regulating Yap activity. *Cell Death & Disease*, *11*(1), 70. https://doi.org/10.1038/s41419-020-2264-z

Zhou, F., & Sun, X. (2021). Cholesterol Metabolism: A Double-Edged Sword in Hepatocellular Carcinoma. *Frontiers in*

Cell and Developmental Biology, *9*, 762828. https://doi.org/10.3389/fcell.2021.762828