VOLUME-7, ISSUE-10, OCTOBER-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Medicine

DIFFERENCES OF 25-HYDROXYVITAMIN D SERUM LEVEL BETWEEN LIVER CIRRHOSIS AND HEPATOCELLULAR CARCINOMA PATIENTS IN HAJI ADAM MALIK GENERAL HOSPITAL MEDAN

Handoko Halim — Department Of Clinical Pathology, Faculty Of Medicine, Universitas Sumatera Utara / Haji Adam Malik General Hospital Medan

Ratna Akbari Ganie* — Department Of Clinical Pathology, Faculty Of Medicine, Universitas Sumatera Utara / Haji Adam Malik General Hospital Medan *Corresponding Author

Gontar Alamsyah Siregar — Department Of Internal Medicine, Faculty Of Medicine, Universitas Sumatera Utara / Haji Adam Malik General Hospital Medan

ABSTRACT Liver cirrhosis is the third largest cause of death after cardiovascular and cancer. HCC is a liver malignancy that requires early diagnosis. Biological effects of vitamin D include the effects of anti-proliferation, anti-angiogenesis, pro-differentiation, anti-inflammatory, pro-apoptosis, and immunity regulation. Previous studies reported that chronic liver patients had low levels of 25-hydroxyvitamin D. The aim of this study is to differ 25-hydroxyvitamin D serum level between liver cirrhosis and hepatocellular carcinoma patients. Cross-sectional study of 32 consecutive admitted patients in Haji Adam Malik General Hospital Medan from December 2017 to March 2018. Patients were classified as liver cirrhosis and hepatocellular carcinoma patients. Full blood count, hemostasis and liver function test were done. AFP and 25-hydroxyvitamin were measured. Univariate analysis and bivariate were done with SPSS version 24. There was no significant difference between 25-hydroxyvitamin D serum level on liver cirrhosis and hepatocellular carcinoma patients (p = 0.052).

KEYWORDS : liver cirrhosis, hepatocellular carcinoma, AFP, 25-hydroxyvitamin D

INTRODUCTION

According to WHO data in 2012, liver cirrhosis in Indonesia reached 52.7 for men and 16.6 for women per 100,000 population. Liver cirrhosis is the third leading cause of death in patients aged 45-46 years (after cardiovascular disease and cancer). The results of research in Indonesia stated that about 40-50% of the most common causes of liver cirrhosis are hepatitis B virus, 30-40% are caused by hepatitis C virus and 10-20% of the causes are unknown.[1-2]

Stiphany et al obtained 103 patients of cirrhosis patients, among of them were 71 men (68.9%) and 32 women (31.1%) at Pirngadi Hospital Medan during 2010-2011.[3]

Hepatocellular carcinoma (HCC) is the 6th most common cancer in the world and the second largest cause of cancer mortality and the third largest cause of mortality in the Asia-Pacific region.[4]

According to Globocan (2012) data, HCC ranks the 5th highest estimated number of cancer incidence rates in Indonesia for both sexes, with a total of 18,121 incidents from 299,673 events. The mortality rate for both sexes is 17,175.[5]

Rasyid et al reported 367 (76%) of the total HCC patients with comorbid disease HCC cases at Adam Malik Hospital Medan; there were 232 (63%) HCC patients comorbid with liver cirrhosis, 91 (25%) HCC patients comorbid with hepatitis B and 44 (12%) HCC patients comorbid with hepatitis C. Whereas another 116 people (24%) were unrelated altogether with liver cirrhosis, HBV or HCV.[6]

There are several risk factors for HCC, such as chronic HBV, HCV, alcoholic or non-alcoholic cirrhosis and exposure to environmental toxins such as aflatoxin.[4,7]

HCC is usually diagnosed at advanced stage, and often has a poor prognosis with limited treatment options. Early diagnosis of HCC plays a role in improving appropriate treatment. AFP marker and ultrasound examination are often used as diagnostic tools. AFP serum is one of the markers that has been commonly used in screening and diagnosis of HCC. However, AFP false positive value is 40% in diagnosing early HCC. For this reason, new biochemical markers for HCC are still important in the world.[7]

Most HCC is preceded by cirrhosis, therefore cirrhosis is considered as a pre-cancerous condition and requires early detection or continuous screening.[8]

Vitamin D deficiency is associated with a variety of skeletal and non-skeletal chronic diseases. Vitamin D deficiency has long been reported in various chronic liver diseases, especially in stages of cirrhosis. A study by Lange et al, 2013 showed that vitamin D deficiency is associated with the occurrence of hepatocellular carcinoma, but the relationship is still unclear until now.[9-10]

There are limited literatures that discuss level differences of 25 (OH) vitamin D in patients with liver cirrhosis and hepatocellular carcinoma. In this study, researchers tried to show the differences between 25 (OH) vitamin D levels and serum AFP levels in liver cirrhosis and hepatocellular carcinoma.

METHODS

The study was conducted in the Clinical Pathology Department of the Faculty of Medicine, Universitas Sumatera Utara / RSUP Haji Adam Malik Medan in collaboration with the Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara from December 2017 to March 2018.

The study was conducted with a cross sectional approach. This research subjects on every patient who came to Haji Adam Malik General Hospital Medan and met the inclusion criterias. They were given explanation of the purpose of the study and the benefits of this study. Malignancy (except hepatocellular carcinoma), acute infectious diseases, corticosteroid treatment, surgery or vitamin D supplementation were excluded in this study. 25(OH) vitamin D and AFP were measured from patients' blood serum.

Other hematology, liver function and hemostasis laboratory parameters such as leukocytes (WBC), hemoglobin (Hb), hematocrit (Ht), platelets, PT, INR, APTT, TT, fibrinogen, D-dimer, ALT, AST, ALP, GGT, albumin, globulin, total protein, total bilirubin, indirect bilirubin and direct bilirubin were carried out in this study.

AFP was examinated by using ARCHITECT in a two-step immunoassay for measuring quantitative AFP in serum, ARCHITECT 25-OH Vitamin D examination is a one step delayed competitive quantitative immunoassay that determine the presence of vitamin

D in human serum and plasma. Both AFP and 25-OH Vitamin D were examined using chemiluminescent microparticle immunoassay technology according to the manufacturer's instructions.

Independent t-test was used to compare AFP levels and vitamin D levels in patients with cirrhosis and HCC. Independent t-test was used to compare quantitative variables between two independent groups if the data is normally distributed and Mann-Whitney U test if it is not normally distributed. Fisher's exact and kolmogorov-smirnov test were used to compare qualitative variables between 2 or more groups. p-value is considered significant if the p value is ≤ 0.05 and very significant if the value is p ≤ 0.01.

Data entry and statistical analysis in this study were performed by using SPSS (Statistical Product and Service Solution) version 24.0

RESULTS

This study was attended by 32 patients who had fulfilled the inclusion criterias. A total of 25 patients (78.2%) were male. The mean age in this study was 54.28 years. 18 patients have liver disorders caused by non-B, non-C hepatitis (56.3%) followed by 12 people with hepatitis B (37.5%) and 2 people with hepatitis C (6.3%). The median value of serum AFP was 20.92 (0.665 - 6794.43) ng / mL. The mean of 25-OH vitamin D was 20.45 ± 8.87 ng / mL.

TABLE – 1 Relationship between HCC and Liver Cirrhosis Characteristics Data

	HCC	Liver Cirrhosis	Total	P	PR (IK 95%)
Gender Man Woman	 15(46.9%) 2 (6.3%)	 10 (31.3%) 5 (15.6%)	 25 (100%) 7 (100%)	0.21[a]	2.1 (0.62 – 7.07)
Age (years)	51.06 ± 11.02	57.93 ± 10.37		0.08[b]	
Hepatitis marker Hepatitis B Hepatitis C Non-B, non-C	 9 (75%) 2 (100%) 6 (33,3%)	 3 (25%) 0 (0%) 12 (66,7%)	 12 (100%) 2 (100%) 18 (100%)	0.083[c]	

[a] fisher's exact test
[b] independent t-test
[c] kolmogorov smirnov test

There was no significant relationship between sex, age, markers of hepatitis with HCC and liver cirrhosis. (Table 1)

There were four parameters that were significantly related to the HCC group and liver cirrhosis from bivariate analysis. They were INR, ALT, AST, and GGT (Table 2).

In table 3, there was significant difference in AFP tumor marker levels between HCC and liver cirrhosis groups. Serum AFP level was significantly higher in the HCC group. There was no significant difference of 25-hydroxyvitamin D serum level between liver cirrhosis and hepatocellular carcinoma patients (p = 0.052).

TABLE 2. Comparison of Hematological Laboratory Parameters, Liver Function, Hemostasis between HCC Patients and Liver Cirrhosis

	HCC n = 17	Liver Cirrhosis n = 15	p
Hb (g/dl)	10.38 ± 2.36	10.79 ± 2.13	0.611[a]
Ht (%) WBC (sel/mm3)	30.71 ± 7.44 11233 ± 5707	31.93 ± 5.80 11286 ± 5776	0.610[a] 0.98[a]
Trombosit (sel/mm3)	235000 (59000 – 681000)	229000 (47000 – 412000)	0.462[b]
PT (second) INR	20.5 (11.4 – 80.0) 1.4 (0.79 – 6.0)	16.2 (11 – 30.7) 1.11 (0.78 – 2.26)	0.051[b] 0.041*[b]
APTT (second) TT (second) Fibrinogen (mg/dL)	39.3 (22.2 -120.0) 22.6 (15.5 - 80.0) 150 (20 - 375)	36.3 (26.0 – 48.5) 23.5 (16.0 – 34.0) 175 (80 - 825)	0.777[b] 0.955[b] 0.473[b]
D-dimer (ng/mL)	950 (115 – 5402)	635 (100 – 9885)	0.706[b]
ALT (U/L)	59 (13 – 363)	26 (9 - 121)	0.033*[b]
AST (U/L)	137 (42 – 766)	46 (14 – 203)	<0.001*[b]
ALP (U/L)	180 (57 – 1007)	113 (52 – 653)	0.450b
GGT (U/L)	279.29 ± 161.64	150.33 ± 102.22	0.011*a
Albumin (mg/dl)	2.3 ± 0.61	2.5 ± 0.60	0.343a
Globulin (mg/dL) Total Protein (mg/dL) Total bilirubin (mg/dl)	3.51 ± 0.79 5.8 (4.4 – 8.7) 3.1 (0.4 – 11.6)	3.52 ± 1.43 6.0 (4.0 – 7.8) 2.8 (0.4 – 16.6)	0.972a 0.865b 0.88b
Bilirubin direk (mg/dl)	1.8 (0.2 – 7.1)	1.40 (0.2 – 12.2)	0.81b

*p<0.05
[a] Mann-Whitney U Test
[b] Independent T-Test

TABLE 3. Comparison of Mean Serum AFP and 25 (OH) Vitamin D Levels between Patients with HCC and Liver Cirrhosis

	HCC n = 12	Liver Cirrhosis n = 20	p
AFP (ng/mL)	2000 (13.93 – 6794)	2.49 (0.65 – 130.36)	<0.001*[a]
25(OH)D (ng/mL)	17.61 ± 7.72	23.67 ± 9.23	0.052[b]

*p<0.05
[a] *Mann-Whitney-U Test*
[b] *Independent T-Test*

DISCUSSION

This study showed a significant INR difference between the HCC and liver cirrhosis groups (p = 0.041). The same thing was also presented by Gopal's research that showed differences between the HCC group and the liver cirrhosis group 1.2 (1.1-1.5) vs 1.2 (1.1-1.4) with p = 0.001. While in other studies conducted by Sameea et al, no significant difference was found between INR levels in the HCC group and liver cirrhosis (p = 0.225). Coagulopathy in chronic liver disease such as HCC and liver cirrhosis involves procoagulants and anticoagulants. One of the changes that occurs is the reduced synthesis of procoagulant factors such as Factors II, V, VII, X, XI, XII, XIII and fibrinogen which can cause bleeding risk in patients with chronic liver disease. In addition, hypercoagubility can also occur due to a decrease in the synthesis of anticoagulant factors such as anti-thrombin, protein C, protein S as well as fibrinolytics such as plasminogen.[11-13]

There was a significant difference in ALT levels between the HCC and liver cirrhosis groups. This study is also in accordance with study conducted by Zahra et al, Li et al, where ALT levels differed between groups of liver cirrhosis and HCC. Serum AST increases due to damage to hepatocytes into the circulation and its activity is recognized to detect liver disease. Serum ALT level is an independent determinant good for intervention and advanced management to reduce mortality, especially HCC caused by endemic viruses. Periodic ALT examination is important in patient with liver cirrhosis because ALT is a precursor marker of HCC.[14-16]

There were significant differences in AST levels between the HCC group and liver cirrhosis (p <0.001). This research is also in line with the research by Hann et al, Ishizuka et al, where AST is a potential parameter that shows the presence of liver cirrhosis. Even in Nishikawa's study, AST parameter is also a parameter that contribute to overall survival in multivariate analysis.[16-18]

There was significant difference of GGT levels between the HCC group and liver cirrhosis (p = 0.011). Studies show that elevated GGT serum levels are independently associated with a risk of solid malignancy and have a poor overall survival. This is supported by previous studies such as in the research of Xie et al, Hammad et al and Dong et al, where GGT abnormalities were associated with severity, postoperative outcome and prognosis of chronic liver disease.[8,19-20]

In this study, there was significant difference in AFP level between HCC and liver cirrhosis groups. This is supported by previous studies, such as Chang et al where there were significant differences between the HCC and liver cirrhosis groups followed at diagnosis, 3, 6, 9 and 12 months later. Hammad et al also found significant differences in AFP levels between the HCC group, liver cirrhosis and HCV infection. Mohammed et al also found differences in AFP levels known for the diagnosis of HCC, but their usefulness is not recommended by current guidelines because of their low sensitivity and specificity. The diagnostic algorithms recommended by AASLD and EASL depend on contrast enhanced imaging tools (eg. CT-scan) for diagnosis of HCC. However, by increasing the cut-off by 400 ng / mL AFP specificity increased. AFP annual screening in the McMahon et al study in Alaska in detecting HCC at an early stage can improve survival rates. AFP level can be affected by the activity of liver disease so it also increases in patients with elevated ALT levels even with the absence of HCC. HCC usually occurs in the liver that has been damaged by several conditions such as liver cirrhosis, chronic hepatitis (HBV infection, HCV). In addition, HCC has a wide variety, where some patients experience an increasing AFP, resulting in low marker sensitivity.[8,21-26]

Vitamin D which is a fat-soluble sterol derivative is mainly synthesized in the liver. In its active form, 1,25 (OH) D, it has an immune system modulation function seen in innate and acquired immunity. Vitamin D increases innate defense and modulates lymphocyte activation from Th1 (pro-inflammatory) to Th2 (anti-inflammatory). Researchers believe that lack of ultraviolet light exposure from the sun is a major cause of vitamin D insufficiency. In addition, vitamin D can be affected by insufficient sun exposure (indoor work, excessive use of closed clothing, air pollution), physiological factors (dark skin, obesity, pregnancy, aging), low vitamin D intake (absence or low vitamin D intake, lactose intolerance, low socioeconomic status), specific polymorphisms in various enzymes, binding proteins and vitamin D receptors.[27-29]

In chronic liver disease, decreased vitamin D levels are associated with both malnutrition and low sun exposure to UV rays. In addition, liver disease was also characterized by low intestinal vitamin D absorption and low levels of vitamin D-binding globulins which function to transfer hormones to liver and kidneys to be activated.[30-31]

There was no significant difference in 25 (OH) D levels between HCC and liver cirrhosis groups. This is supported by previous study such as by Paternostro et al that showed no differences between vitamin D levels between the HCC group and liver cirrhosis. But deficiency of 25 (OH) D had a high mortality impact in groups with 25 (OH) D deficiency . Almeida Borges et al. explained that there was no relationship between vitamin D deficiency and histological severity. In this study, levels of 25 (OH) D in the HCC group had a lower propensity level compared to the liver cirrhosis group. While in other studies such as Finkelmeier, Mohammed and Stokes showed significant differences between various stages of liver cirrhosis. In liver cirrhosis patients with hepatocyte dysfunction to synthesize, there is a tendency of low free form and total vitamin D levels. In this research, it was revealed that vitamin D might be a non-invasive marker and could predict adverse events [25 (OH) D <10 ng / mL) but not accurate. Hammad et al found that there were significant differences in levels of 25 (OH) D in the HCC, liver cirrhosis and HCV infected groups. Trepo et al reported that low levels of 25 (OH) D were associated with massive liver damage and mortality in alcoholic liver disease. In a prospective cohort study of 23 centers of 10 European countries conducted by Fedirko et al, there was an inverse relationship between serum 25 (OH) D levels and the incidence of HCC.[9,22,30,32-37]

CONCLUSION

There was no significant difference in 25-hydroxyvitamin D level between patients with HCC and liver cirrhosis. Further study is needed to prove the role of 25 (OH) D in HCC and liver cirrhosis knowing that the big role of 25-hydroxyvitamin D, the precursor of 1,25-hydroxyvitamin D as an immunomodulator.

REFERENCES:

1. WHO. Age-standardized death rates, liver cirrhosis (15+). 2012. Global Health Observatory data repository.
2. Nurdjanah, S. Sirosis Hati. Dalam : S Setiati, et al. Buku Ajar Ilmu Penyakit Dalam. ed. 6. Jakarta : InternaPublishing, 2014:1978-83.
3. Stiphany, Hiswani and Jemadi. Karakteristik Penderita Sirosis Hati Rawat Inap di RSUD dr. Pirngadi Medan Tahun 2010-2011. portalgaruda. http://download. portalgaruda. org/article.php?article=51529&val=4108
4. McGlynn, KA, Petrick, JL and WT. Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability. Clin Liver Dis. 2015;19(2):223-38.
5. Ferlay, J, et al. GLOBOCAN 2012 v1.0. Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. http://globocan.iarc.fr, accessed on 12/December/2016.
6. Rasyid, A. Temuan Ultrasonografi Kanker Hati Hepato Selular (Hepatoma). 2, Medan. Majalah Kedokteran Nusantara. 2006;39:104-8.
7. Youssef, E.M.I., Ali, H.A.A., Ewieda, G.H., et al. 25-Hydroxy Vitamin D: Novel Biomarker for the Diagnosis of Hepatocellular Carcinoma. Research In Cancer and Tumor. 2014;3(1): 19 -25.
8. Hammad, LN, et al. Circulating IL-6, IL-17 and vitamin D in hepatocellular carcinoma: Potential biomarkers for a more favorable prognosis? J Immunotoxicol. 2013:Early online;1-7.
9. Trepo, E, et al. Marked 25-hydroxyvitamin D deficiency is associated with poor prognosis in patients with alcoholic liver disease. J Hepatol. 2013:59;344-50.
10. Lange, CM, et al. Genetic analyses reveal a role for vitamin D insufficiency in HCV-associated hepatocellular carcinoma development. PLoS ONE. 2013;8(5):e64053.
11. Gopal P, Yopp CC, Waljee AK, Chiang J, Nehra M, Kandunoori P and Singal AG. Factors that affect accuracy of α-fetoprotein test in detection of hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol. 2014;12(5):870-7.
12. Sameea EA, Zakareya T, Metwaly K, Youssef AAR, Kamal HM, and Abdalla WM. Serum soluble CD25 in hepatocellular carcinoma, shall we BE able to change the natural history? International Journal of Medicine and Medical Research. 2017;3(2):10-5.
13. Khoury T, Ayman AR, Cohen J, Daher S, Shmuel C and Mizrahi M. The Complex Role of Anticoagulation in Cirrhosis: An Updated Review of Where We Are and Where We Are Going . Digestion. 2016;93:149–59.
14. Zahra MK, Attia TE, Ahmad AY and Othman MA. Serum Galectin-3 Levels in Patients with Hepatocellular Carcinoma, Liver Cirrhosis and Chronic Viral Hepatitis. The Egyptian Journal of Hospital Medicine. 2018;70(1):132-9.
15. Li B, Li B, Guo T, Sun Z, Li X, Li X, et al. The Clinical Values of Serum Markers in the Early Prediction of Hepatocellular Carcinoma. BioMed Research International. 2017; 5358615.
16. Hann HW, Wan S, Myers RE, Hann RS, Xing J, Chen B, et al. Comprehensive Analysis of Common Serum Liver Enzymes as Prospective Predictors of Hepatocellular Carcinoma in HBV Patients. PLos One. 2012;7(10):e47687.
17. Ishizuka M, Kubota K, Kita J, Shimoda M, Kato M, Mori S, et al. Aspartate aminotransferase-to-platelet ratio index is associated with liver cirrhosis in patients undergoing surgery for hepatocellular carcinoma. J Surg Res. 2015;14(1):63-8.
18. Nishikawa H, Kita R, Kimura T, Endo M, Ohara Y, Sakamoto A, et al. Proposal of the performance status combined Japan Integrated Staging system in hepatocellular carcinoma complicated with cirrhosis. International Journal Of Oncology. 2015;46:2371-9.
19. Xie W, Cao Y, Xu M, Wang J, Zhou C, Yang X, et al. Prognostic Significance of Elevated Cholestatic Enzymes for Fibrosis and Hepatocellular Carcinoma in Hospital Discharged Chronic Viral Hepatitis Patients. Scientific Reports. 2017;7:10289.
20. Dong ZR, Zou J, Sun D, Shi GM, Ke AW, Cai JB, et al. Preoperative Albumin-Bilirubin Score for Postoperative Solitary Hepatocellular Carcinoma within the Milan Criteria and Child-Pugh A Cirrhosis. J Cancer. 2017;8(18):3862-7.
21. Mohammed MA, Omar NM, Mohammed SA and Deiab AG. The Significance of Vitamin D Receptor Gene Polymorphisms for Susceptibility to Hepatocellular Carcinoma in Subjects Infected with Hepatitis C Virus. Gastroenterol Hepatol Open Access. 2017;7(4):00246.
22. Mohammed, H.A.A., Yang, J.D., Giama, N.H., et al. 2017. Factors Influencing Surveillance for Hepatocellular Carcinoma in Patients with Liver Cirrhosis. Liver Cancer. 2017;6:126-36.
23. Bellissimo F, Pinzone MR, Cacopardo B and Nunnari G. Diagnostic and therapeutic management of hepatocellular carcinoma. World J Gastroenterol. 2015;21(42):12003-21.
24. Fateen W and Ryder SD. Screening for hepatocellular carcinoma: patient selection and perspectives. Journal of Hepatocellular Carcinoma. 2017;4:71–9.
25. McMahon BJ, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, et al. Screening for Hepatocellular Carcinoma in Alaska Natives Infected With Chronic Hepatitis B: A 16-Year Population-Based Study. Hepatology. 2000;32:842-46.
26. Lou J, Zhang L, Lv S, Zhang C and Jiang S. Biomarkers for Hepatocellular Carcinoma. Biomark Cancer. 2017;9:1-9.
27. Pincus, MR and Abraham, NZ. Interpreting Laboratory Results. Dalam: RA McPherson and MR Pincus. Henry's Clinical Diagnosis and Management By Laboratory Methods. St. Louis : Elsevier, 2017;23:84-101.
28. Anty R, Tonohouan M, Ferrari-Panaia P, Piche T, Pariente A, Anstee QM et al. Low Levels of 25-Hydroxy Vitamin D are Independently Associated with the Risk of Bacterial Infection in Cirrhotic Patients. Clin Transl Gastroenterol. 2014;5(5):e56.
29. Chen EQ, Shi Y and Tang H. New insight of vitamin D in chronic liver diseases. Hepatobiliary Pancreat Dis Int. 2014;13(6):580-5.
30. Stokes CS, Krawczyk M, Reichel C, Lammert F and Grunhage F. Vitamin D deficiency is associated with mortality in patients with advanced liver cirrhosis. Eur J Clin Invest. 2014;44(2):176–83.
31. Konstantakis, C., Tselekouni, P., Kalafateli, M., et al. Vitamin D deficiency in patients with liver cirrhosis. Annals of Gastroenterology. 2016;29:297-306.
32. Paternostro R, Wagner D, Reiberger T. Low 25-OH-vitaminD levels reflect hepatic dysfunction andare associated with mortality in patientswith liver cirrhosis. Wien Klin Wochenschr.2017;129:8–15.
33. Borges PS, Guimaraes VM, Farias JLR, et al. Absence of Relationship between Serum Vitamin D Levels with Chronic Hepatitis C. J Gastroenterol Hepatol Res. 2016;5(2).
34. Finkelmeier, F, et al. Severe 25-hydroxyvitamin D deficiency identifies a poor prognosis in patients with hepatocellular carcinoma – a prospective cohort study. Aliment Pharmacol Ther. 2014;39:1204-12.
35. Finkelmeier F, Bettinger D, Koberle V, Schultheiß M, Zeuzem S, Kronenberger B, et al.

Single measurement of hemoglobin predicts outcome of HCC patients. Med Oncol. 2014;31:806.
36. Hammad, LN, et al. Circulating IL-6, IL-17 and vitamin D in hepatocellular carcinoma: Potential biomarkers for a more favorable prognosis? J Immunotoxicol. 2013:Early online;1-7.
37. Fedirko V, Duarte-Salles T, Bamia C, et al. Prediagnostic Circulating Vitamin D Levels and Risk of Hepatocellular Carcinoma in European Populations: A Nested Case-Control Study. Hepatology.2014;60:1222-30.