Differences Between Alpha-Fetoprotein (AFP) and Prothrombine Induced by Vitamin K Absence or antagonist II (PIVKA-II) Values as Early Detection Method for Hepatocellular Carcinoma (HCC) and Cirrhosis

Bayu Eka Nugraha, Nugraha Setiawan*, Hani Susianti**, Bogi Pratomo****

[*]Department of Internal Medicine, Faculty of Medicine,
Brawijaya University/Dr. Saiful Anwar Hospital, Malang
[**]Department of Clinical Pathology, Faculty of Medicine,
Brawijaya University/Dr. Saiful Anwar Hospital, Malang
[***]Division of Gastroentero-hepatology, Department of Internal Medicine, Faculty of Medicine
Brawijaya University/Dr. Saiful Anwar Hospital, Malang

Corresponding author:
Bogi Pratomo. Division of Gastroentero-hepatology, Department of Internal Medicine, Dr. Saiful Anwar Hospital. Jl. Jaksa Agung Suprapto No. 2 Malang Indonesia. Phone/facsimile: +62-341-348265. E-mail: bogi.pratomo@yahoo.com

ABSTRACT

Background: *Hepatocellular carcinoma is a common cancer worldwide and has a high mortality. Biomarkers could theoretically help to detect the disease at an earlier stage before symptoms occur and improve the treatment outcomes. The first biomarker found was alpha-fetoprotein/AFP (not very accurate and 30-40% of HCC may be missed). Prothrombin induced by vitamin K absence or antagonist II (PIVKA-II) can be used as an early detection method to diagnose hepatocellular carcinoma (HCC).*

Method: *A cross sectional study on in-patients or out-patients at Dr. Saiful Anwar Malang Hospital from July 2016 to October 2016.*

Results: *The p value ($p > 0.05$) obtained using Kolmogorov-Smirnov was 0.166 for diagnosis of HCC and 0.147 for the diagnosis of hepatic cirrhosis. The p value ($p > 0.05$) obtained using Shapiro-Wilk was 0.103 for diagnosis of HCC and 0.087 for the diagnosis of cirrhosis. Comparative test using the LSD method showed PIVKA-II serum levels in HCC as compared to hepatic cirrhosis as significant with a p-value less than 0.05 ($p < 0.05$), that is 0.025. However comparative test using the Tukey HSD method showed that the results obtained were not significant. According to the PIVKA-II cut off value, the sensitivity and specificity to detect cirrhosis and HCC was as large as 100%. According to the AFP cut off value, the sensitivity to detect cirrhosis and HCC was 93.3% and the specificity was 76.92%.*

Conclusion: *Both PIVKA-II and AFP can be used to detect cirrhosis and HCC. However PIVKA-II exhibited better sensitivity and specificity in the detection of cirrhosis and HCC.*

Keywords: *Hepatocellular carcinoma, cirrhosis, alpha-fetoprotein (AFP), prothrombin induced by vitamin K absence or antagonist II (PIVKA-II)*

ABSTRAK

Latar belakang: *Karsinoma sel hati (KSH) merupakan kanker dengan prevalensi yang tinggi di seluruh dunia dengan tingkat mortalitas tinggi. Secara teori, biomarker dapat digunakan untuk mendeteksi KSH pada fase*

awal sebelum terjadi gejala sehingga dapat memberikan prognosis terapi yang lebih baik. Biomarker pertama yang ditemukan adalah alpha-fetoprotein/AFP (dengan tingkat akurasi yang lebih rendah, sekitar 30-40% KSH dapat terlewatkan). Biomarker prothrombin induced by vitamin K absence or antagonist II (PIVKA-II) dapat digunakan sebagai metode deteksi awal untuk mendiagnosis KSH.

Metode: *Studi cross sectional pada pasien rawat inap dan rawat jalan di RSU Dr. Saiful Anwar Malang mulai bulan Juli 2016 hingga Oktober 2016.*

Hasil: *Nilai p ($p > 0.05$) sebesar 0.166 didapatkan dari pengujian Kolmogorov Smirnov untuk diagnosis KSH dan 0.147 untuk diagnosis sirosis. Nilai p value ($p > 0.05$) menggunakan uji Saphiro-Wilk didapatkan sebesar 0.103 untuk diagnosis KSH dan 0.087 untuk diagnosis sirosis. Pengujian perbandingan menggunakan metode LSD menunjukkan level serum PIVKA II pada KSH memiliki perbedaan yang signifikan dibandingkan dengan sirosis nilai $p < 0.05$ yakni sebesar 0.025. Namun uji perbandingan menggunakan Tukey HSD menunjukkan bahwa hasil yang didapatkan tidak signifikan. Terkait nilai ambang batas PIVKA II, didapatkan sensitivitas dan spesifisitas dalam mendeteksi SH dan KSH hingga 100%. Sedangkan AFP didapatkan sensitivitas sebesar 93.3% dan spesifisitas sebesar 76.92% dalam mendeteksi sirosis dan KSH*

Simpulan: *PIVKA-II maupun AFP dapat digunakan untuk mendeteksi sirosis dan KSH. Namun PIVKA II memberikan sensitivitas dan spesifisitas yang lebih baik dalam mendeteksi sirosis dan KSH.*

Kata kunci: *karsinoma sel hati, sirosis hepatis, alpha-fetoprotein (AFP), prothrombin induced by vitamin K absence or antagonist II (PIVKA II)*

INTRODUCTION

Hepatocellular carcinoma is recently becoming worldwide health problem with increasing incidence in the last 5 years. According to WHO report in 2008, HCC caused 600,000 deaths each year in 2004. Hepatocellular carcinoma is the 5th most prevalent cancer in the world, with 500,000 new cases each year. It is the 3rd cause of cancer related death after lung and gastric cancer. The incidence of HCC has specific geographical distribution, in which it is more commonly found in Asia, West Pacific, and South-East Africa and it is more rarely found in region of America, Europe, and Oceania.[1,2] Hepatocellular carcinoma is the 8th most prevalent cancer in Indonesia, especially in Dharmais Cancer Hospital, from 2010 until 2013. New cases and cancer related death of HCC are continuously increasing.[3] The burden of the disease certainly gives great impact on public health, productivity, life expectancy, social, and economic aspects of society.[4] Patient often visit the doctor in the late course of disease because early detection of hepatocellular carcinoma is still problematic.

Biomarkers could theoretically help to detect the disease at an earlier stage before symptoms occur and improve the treatment outcomes. The first biomarker found was alpha-fetoprotein (AFP) and it is currently used as part of the HCC surveillance recommended in many countries. However AFP is not very accurate and 30-40% of HCCs may be missed.[5] Although serum alpha-fetoprotein (AFP) is often elevated in patients with HCC, its sensitivity and specificity were estimated at 41% to 65% and 80% to 94%, respectively, in one study.[6] It is generally accepted that serum levels greater than 500 μ/L in high-risk patients are diagnostic for HCC. However, negative values do not rule out HCC. AFP also may be elevated in patients with chronic liver disease in the absence of cancer (especially with inflammation), in pregnancy, tumors of gonadal origin, and a variety of other malignancies. Because of the limitations of serum AFP measurements, several other serum markers of HCC used alone or in combination with AFP have been evaluated.[7]

Des-gamma-carboxy-prothrombin (DCP), which is also known as protein induced by vitamin K absence or antagonist II (PIVKA-II), is the form of abnormal prothrombin. In normal liver function, prothrombin precursor undergoes post translation carboxylation (adding of carboxylic acid group) by the enzyme of gamma glutamyl carboxylase before it is finally released into peripheral circulation. Carboxylase depends on vitamin K. This enzyme is not found in the case of HCC, which causes abnormal prothrombin to be secreted in circulation.[4] The aims of this study was investigating the role DCP/PIVKA-II as a marker in early detection method of diagnosing HCC. Early detection of HCC will bring the treatment to be given earlier which may give a better prognosis for the patient.

METHOD

This was an analytical cross-sectional study which was carried on inpatient and outpatient clinic of Dr. Saiful Anwar Hospital, Malang. The study has been reviewed and accepted by the Ethical Committee Board of Dr. Saiful Anwar Hospital, Malang.

All HCC and hepatic cirrhosis patients, that came to outpatient clinic or had been hospitalized, from July 2016 until October 2016 in Dr. Saiful Anwar Hospital, Malang, were recruited to the study. The eligible subjects from study period were 28 patients.

Inclusion criteria for sample selection were male/female with established diagnosis of hepatocellular carcinoma or hepatic cirrhosis. The diagnosis was confirmed from history taking, physical examination, and additional examination such as imaging examination, biological marker examination, etc. Exclusion criteria included male/female with hepatocellular carcinoma or hepatic cirrhosis was got treatment for his/her disease

Analysis of PIVKA and AFP was done parametrically using ANOVA. Assumption of normality and assumption of homogeneity of variance are the two assumptions that became the basis of ANOVA. Test for normality assumption was performed using Saphiro Wilk test. Normality of data was fulfilled if p-value of the test was > 0.05. If the assumption of normality was not achieved, in which the p-value was < 0.05, the analysis of hypothesis could be performed non-parametrically. The research analyzed the comparison between AFP and PIVKA measurement in HCC.

RESULTS

Results showed different values of sensitivity and specificity of AFP and PIVKA in the detection of hepatic cirrhosis and hepatocellular carcinoma. AFP had sensitivity about 93.33% and specificity about 76.92% in the detection of hepatic cirrhosis and hepatocellular carcinoma. According to the *Konsensus Nasional Penatalaksanaan Karsinoma Sel Hati*, AFP has diagnostic value with cut-off point of >200 ng/mL and the cut-off point for PIVKA II is > 40 mAU/mL. Cut off value of AFP is described in Table 1. Table 2 describe the cut off value of PIVKA-II in detecting hepatic cirrhosis and hepatocellular carcinoma, with 100% sensitivity and specificity.

Table 1. Contigency table for alpha-fetoprotein (AFP) in patient with hepatocellular carcinoma (HCC) and hepatic cirrhosis (HC)

Diagnosis	Alpha-fetoprotein (AFP)	
	Hepatocellular carcinoma (HCC)	Hepatic cirrhosis (HC)
Hepatocellular carcinoma (HCC)	14	3
Hepatic cirrhosis (CC)	1	10
Total	15	13

Sensitivity: 14/15 x 100% = 93.33%; specificity: 10/13 x 100% = 76.92%; positive predictive value (PPV) = 14/17 x 100% = 82.35%; negative predictive value (NPV) = 10/11 x 100% = 90.91%; Accuracy = 85.71%; positive likelihood ratio (LR+)= 4.04; negative likelihood ratio (LR-)= 0.09

Table 2. Contigency table for PIVKA-II in patient with hepatocellular carcinoma (HCC) and hepatic cirrhosis (HC)

Diagnosis	Prothrombin Induced by Vitamin K Absence or antagonist II (PIVKA-II)	
	Hepatocellular carcinoma (HCC)	Hepatic cirrhosis (HC)
Hepatocellular carcinoma (HCC)	17	0
Hepatic cirrhosis (HC)	0	11
Total	17	11

Sensitivity: 17/17 x 100% = 100%; specificity: 11/11 x 100% = 100%; positive predictive value (PPV): 17/17 x 100% = 100%; negative predictive value (NPV) = 11/11 x 100% = 100%; Accuracy = 100%; positive likelihood ratio (LR+)= ~ ; negative Likelihood ratio (LR-)= 0

DISCUSSION

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver.[8] The global burden of this cancer in 2012 was an all-time high with 14 million cases and it is predicted to grow to 22 million cases over the next two decades.[9] Liver cancers have the seventh highest age-adjusted incidence rate in the world, with 0.8 million cases diagnosed for the year of 2012.[10] Its most common etiological factor in the world is hepatitis B virus (HBV) infection. The development of cirrhosis is also associated with high risk for developing HCC, with the most common risk factors including alcohol, viral hepatitis such as hepatitis C virus (HCV) infection, and non-alcoholic fatty liver disease (NAFLD). Due to its high prevalence, HCC carries a significant economic burden on society, especially in East Asian countries where HBV infection is endemic. HCC is the third most common cause of cancer-related death in the world and the seventh most common cause in the United State (US). Surveillance programs have also been implemented to screen for HCC in high-risk individuals, which is more cost effective than the treatment of HCC.[9,10]

The initial approach in the management of HCC is to determine if either surgical resection or liver transplantation is feasible. Since the majority of HCC cases develop in cirrhotic patients, surgical interventions

can become challenging and the treatment has been directed toward liver transplantation. Certainly, prevention of cancer development seems to be the appropriate strategy to solve the problem regarding shortage of donor organs. Hence, understanding the epidemiology, etiology, and pathogenesis of this economically burdening cancer is of prime significance for hepatologist and oncologist.[11]

Hepatocellular carcinoma is more common among males with male and female ratio worldwide is 2.4.[12] The most common age at presentation is usually between 30 and 50 years.[13] HCC is predominant in Asian countries including China, Mongolia, Southeast Asia, Sub-Saharan, Western and Eastern Africa. The prevalence of HCC in developed countries of the world is lower, except Japan, Italy, and France.[11]

In the previous guideline, groups were specified for which surveillance was likely to be cost-effective because the hepatocellular carcinoma (HCC) incidence was high enough. New data on defining HCC risk have emerged for hepatitis B virus, hepatitis C virus, and autoimmune hepatitis. Surveillance is deemed cost-effective if the expected HCC risk exceeds 1.5% per year in patients with hepatitis C and 0.2% per year in patients with hepatitis B. Analysis of recent studies show that alpha-fetoprotein determination lacks adequate sensitivity and specificity for effective surveillance (and for diagnosis).[14]

Alpha-fetoprotein is one of glycoprotein, synthesized by hepatocyte and saccus vitelinus. It is usually found in the serum of fetus. In 2 weeks after delivery, AFP in serum is mostly undetectable. In normal person, AFP is only found in relatively small amount (< 25 ng/mL). When the hepatocyte changed to be a malignant cell, it will produce AFP. The concentration of AFP in the serum will increase as a tumor marker, although it isn't always found in all hepatocellular carcinoma. Concentration of AFP increase in 50-90% of hepatocellular carcinoma patient. Increase of AFP > 200 ng/mL in patient with hepatic cirrhosis which is accompanied by mass at hepatic region, increase the possibility of hepatocellular carcinoma. Alkaline phosphatase can be used as screening and diagnostic measurement, and also monitoring after treatment.[1]

The absence of effective treatment in the late stage of HCC favors the early detection and also screening procedure especially in high risk population. Recently, early diagnosis of HCC becomes an important strategy in the management of HCC. Not all imaging technique can detect liver cancer immediately after the development of tumor. In some cases, oncologists use alpha-fetoprotein (AFP) as a marker for liver cancer beside imaging technique. Des-gamma-carboxy prothrombin (DCP), which is also known as protein induced by vitamin K absence or antagonist II (PIVKA-II), is recently used in the early detection of hepatocellular carcinoma, because as a non-functional prothrombin, PIVKA-II is produced by liver in the condition of vitamin K deficiency as in HCC.[15]

PIVKA-II is a more sensitive and specific marker for early detection of HCC than AFP, because in the case of HCC, the abnormal liver tissue produces higher concentration of non-functional prothrombin precursor which certainly will influence the production of PIVKA-II. It was also suggested in some studies that PIVKA-II has property to differentiate HCC histopathologically and it has a good diagnostic validity to evaluate portal vein thrombosis. Those properties are useful in differentiating early and late stage of HCC. Direct correlation between PIVKA-II concentration and tumor size, makes this marker became new attractive marker for research in early diagnosis and survival prediction of HCC. Besides, the high sensitivity and specificity of PIVKA-II can be beneficial in the screening process of high-risk population and also early stage diagnosis of HCC, therefore patients can still be given curative treatment.[4,15]

This study revealed 100% sensitivity and specificity of PIVKA in detecting HCC, compared with only 93.33% sensitivity and 76.92% specificity of AFP. Our results are consistent with those from Marrero et al and Durazo et al, in which they found that PIVKA-II was more sensitive than AFP in differentiating HCC from patient with hepatic cirrhosis or chronic hepatitis, with sensitivity of 89% and specificity of 87%.[16,17]

Clinical significance of tumor markers in HCC diagnosis remains controversial. The potential roles played by tumor markers, tend to be underrated in Western reports, although it is considered of greater value in Eastern setting. Recently, a combination of AFP and PIVKA-II levels has been recommended for diagnosing HCC malignancy in Japan.[18] Limitations of the study include small sample size of and long term follow up of the participants was not taken. In Future a study can be done with larger sample size.

CONCLUSION

PIVKA and AFP examinations both can be applied in detecting hepatocellular carcinoma and hepatic cirrhosis. PIVKA has some advantages, compared to

AFP, in detecting HCC and hepatic cirrhosis. Cut off value of PIVKA gave 100% sensitivity and specificity in detecting hepatic cirrhosis and HCC, while cut off value of AFP gave 93.3% sensitivity and 76.92% specificity.

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