Alpha-1 Acid Glycoprotein Cut-off Value as Diagnostic Biomarker in Hepatocelular Carcinoma with Liver Cirrhosis

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ABSTRACT

Background: *Hepatocellular carcinoma (HCC) remains a major problem throughout the world, especially in diagnostic and therapeutic management. Previous studies stated that alpha-1 acid glycoprotein (AAG) was a potensial biomarker in diagnostic of HCC. This study assessed the best cut-off value of AAG as a diagnostic biomarker of HCC with liver cirrhosis.*

Method: *This was a cross-sectional, diagnostic study, conducted from January to October 2013 in Cipto Mangunkusumo Hospital. The subjects were HCC with liver cirrhosis patients and as control were liver cirrhosis patients. Abdominal ultrasonography (USG), abdominal 3 phase contrast* computerized tomography (*CT) scan, and liver biopsy, if necessary, were done. All patients were having AAG examination, then the result was analyzed using receiver operating characteristic (ROC) curve and assessment of some cut-off values was done.*

Results: *There were 25 HCC with liver cirrhosis patients and 37 liver cirrhosis patients as control included in this study. HCC with liver cirrhosis patients were 92% male and 8% were female, over 50 years old (72%). HBV infection was the most common etiology and most of the patients had multiple nodules in the liver (80%). ROC curve showed the area under the curve (AUC) was 81.44%.*

Conclusion: *The best cut-off value of AAG to be aware of HCC with liver cirrhosis was 61 mg/dL and as a diagnostic was 136 mg/dL.*

Keywords: *hepatocelullar carcinoma, alpha-1 acid glycoprotein, cut-off*

ABSTRAK

Latar belakang: *Karsinoma hepatoselular (KHS) masih menjadi masalah utama di seluruh dunia, terutama dalam hal diagnosis dan penatalaksanaannya. Penelitian terdahulu menyatakan bahwa alfa-1 asam glikoprotein (AAG) merupakan biomarker potensial dalam mendiagnosis KHS. Penelitian ini mengkaji nilai titik potong terbaik AAG sebagai biomarker diagnostik KHS dengan sirosis hati.*

Metode: *Penelitian ini merupakan studi diagnostik dengan desain potong lintang yang dilakukan dari bulan Januari hingga Oktober 2013 di Rumah Sakit Cipto Mangunkusumo, Jakarta. Subjek penelitian ini adalah pasien KHS dengan sirosis hati, sebagai kontrol pasien sirosis hati saja. Pemeriksaan pencitraan yang dilakukan adalah ultrasonografi (USG) abdomen,* computerized tomography (*CT) scan abdomen dengan kontras 3 fase, dan bila perlu dilakukan biopsi hati. Pemeriksaan AAG dilakukan pada semua pasien, kemudian dianalisis*

dengan receiver operating characteristic (ROC) dan dikaji beberapa titik potongnya.

Hasil: *Sebanyak 25 pasien KHS dengan sirosis hati dan 37 pasien sirosis hati sebagai kontrol terlibat dalam penelitian ini. Dari seluruh pasien KHS dengan sirosis hati, 92% laki-laki, 8% perempuan, sebagian besar berusia diatas 50 tahun (72%). Etiologi terbanyak adalah infeksi HBV (56%) dan sebagian besar dijumpai nodul multipel (80%). Dengan menggunakan ROC, didapatkan area under the curve (AUC) sebesar 81,44%.*

Simpulan: *Titik potong terbaik AAG untuk mulai mewaspadai KHS dengan sirosis hati adalah 61 mg/dL dan untuk diagnostik adalah 136 mg/dL.*

Kata kunci: *karsinoma hepatoselular, alfa 1-asam glikoprotein, nilai titik potong*

INTRODUCTION

Hepatocelular carcinoma (HCC) is one of the most common malignancies in the world. At present, HCC is the world fifth most common solid cancer.[1,2,3] According to the World Health Organization (WHO) in 2002, it was around 564,000 new cases of HCC per year globally.[4] In 2010, 650,000 people in the world died every year due to HCC.[3]

HCC is the top three causes of cancer death in Asia Pacific region because the high rate of chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections.[5] Some countries such as China and Taiwan, are still having high incidence with male predilection although doing immunization program. Moreover, there was high incidence of HBV-related HCC among Asia Pacific immigrant (especially from China and Taiwan) in Australia.[6] In Indonesia, some centers in big cities such as Jakarta, Surabaya, or Bandung have their own HCC prevalence data; however national HCC prevalence data has not been available yet. Out-patient visit data in hepatolobiliary clinic, Department of Internal Medicine, Cipto Mangunkusumo Hospital in 2001 showed prevalence of HCC in out-patient visit was 7.3% (60 from 819 visits) in a year and most of them are in terminal stage.[7]

According to Asia Pacific Association for the Study of the Liver (APASL) 2010 guidelines, we need abdominal ultrasonography (USG) and 3 phases contrast abdominal computerized tomography (CT) examinations to diagnose HCC.[8] In daily clinical practice, the increase of alpha feto-protein (AFP) to greater than 200 ng/mL also supports the diagnosis of HCC. If both imaging and AFP examination are uncertain, we need to do liver biopsy to establish the diagnosis of HCC. APASL 2010 guidelines cannot be widely applied, especially in Indonesia. It is just suitable for big centers having CT scan machine and radiologists. Also, it is not affordable in low income society due to the high cost. To date, AFP and other diagnostic modalities (abdominal USG, CT scan, etc) are still being widely used as biomarker to support diagnostic surveillance and prognostic indicator in HCC patients.[9-13] However, some studies showed the accuracy of AFP in diagnosing HCC was not as good as Des-gamma carboxyprothrombin (DCP) dan Lens culinaris agglutinin-reaction of alpha feto-protein (AFP L-3).[14,15] Researchers are looking for better HCC diagnostical biomarker. There were some potential biomarkers such as alpha-1 acid glycoprotein (AAG). A study by Chio LF and Oon CJ, AAG increased in 80% HCC patients, higher than cirrhotic patients (20%) and chronic hepatitis patients (5.7%).[16] AAG had good sensitivity and accuracy in diagnosing HCC. The combination of AFP and AAG resulted better sensitivity and accuracy compared with AFP examination alone.[17]

AAG is not an invasive examination and the result can soon be obtained. The examination can be performed using usual technique in common laboratories. Compared with AFP examination, the AAG examination is cheaper with better sensitivity and accuracy. This study evaluated the best diagnostic cut off value of AAG in diagnosing HCC patients with liver cirrhosis, since majority (80%) of HCC patients had liver cirrhosis prior to HCC and one third of liver cirrhosis patients developed HCC in their life.[18] It was just a small portion of patients got HCC without having liver cirrhosis.

METHOD

This was a cross sectional diagnostic study conducted at Division of Hepatobiliary, Department of Internal Medicine, Cipto Mangunkusumo Hospital in January to October 2013. The study group consisted of HCC patients having liver cirrhosis and the control group consisted of liver cirrhosis patients without HCC in the medicine ward of General Hospital Dr. Cipto Mangunkusumo and Persahabatan Hospital, Jakarta. All subjects were taken consecutively until achieving sample size requirement with inclusion criteria as

follow: 1) Patient diagnosed with HCC with liver cirrhosis based on anamnesis, physical examination, laboratoryoratory examination and abdominal USG, abdominal CT with 3 phases contrast (liver biopsy was done if needed); 2) Patient diagnosed with liver cirrhosis without nodule based on anamnesis, physical examination, laboratoryoratory examination and abdominal USG; 3) First visit patient and have not been treated for HCC; 4) 17 years old and above; 5) willing to participate in the study. Exclusion criteria were: 1) Having other malignancy diseases such as colon tumor, lung tumor, pancreatic tumor, etc; 2) HCC patients without liver cirrhosis; 3) Having acute infection diseases, such as pneumonia, colitis, acute hepatitis, sepsis or HIV; 4) Undergoing corticosteroid treatment; 5) Having surgery or any trauma recently; 6) Having cardiac myocard infarct recently. Minimal sample size was 46 subjects.

AAG examination result of all subjects were presented in the receiver operating characteristic (ROC) with area under the curve (AUC) and a table consisting several cut-off AAG value with each sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR). We then assessed the best AAG cut-off value to be aware of HCC and to diagnose HCC with liver cirrhosis.

Blood sample for AAG examination was collected from each subject by using 1 yellow cap serum separating tube. The 6 cc of blood sample was put in the room temperature for 30-45 minutes until it clotted. Sample was directly sent to the laboratory to be centrifuged (3000 rpm) for 15 minutes duration. Serum then was separated and put into 2 cups, 0.3 cc each (1 for sample, 1 for reserve). The cup then was labeled with subject's name, date, type of examination prior to AAG examination.

RESULTS

Twenty five HCC patients with liver cirrhosis and 37 liver cirrhosis patients were included within the study period. All patients were 30 years and above, with the tendency of increasing in the number of patient as the age increased. Seventy two percent of HCC patients with liver cirrhosis were 50 years old and above. Most of the patients in each group had ascites and decreased serum albumin. The most common etiology in each group was chronic hepatitis B infection, 56% and 67.6% respectively (Table 1). According to abdominal USG and 3 phases contrast abdominal CT, most of the HCC patients with liver cirrhosis had multiple nodules in both liver lobes (80%).

Table 1. Basic characteristic of patient

Variable	HCC + cirrhosis (study group) n (%)	Cirrhosis (control group) n (%)
Sex		
Male	23 (92)	21 (56.7)
Female	2 (8)	16 (43.3)
Age (year)	55.62 (± 11.92)*	55,56 (± 12.62)*
< 30	0 (0)	0 (0)
30 – 40	3 (12)	3 (8.1)
40 – 50	4 (16)	10 (27.1)
50 – 60	9 (36)	11 (29.7)
> 60	9 (36)	13 (35.2)
Physical examination		
Ascites	15 (60)	20 (54.1)
Laboratory examination		
Albumin (g/dL)	2.95 (± 0.56)*	2.87 (± 0.37)*
Total bilirubin (mg/dL)	1.87 (± 0.96)*	1.54 (± 0.66)*
HBsAg positive	14 (56)	25 (67.6)
Anti HCV positive	5 (20)	8 (21.6)
HBsAg & anti HCV negative	6 (24)	4 (10.8)
Total	25 (100)	37 (100)

HCC: hepatocellular carcinoma; HCV: hepatitis c virus *mean, SD

AAG result of each group was presented in Table 2 and Figure 1.

Table 2. Median of AAG examination result in study group and control group

Variable	Study group median (min-max)	Control group median (min-max)
Alpha-1 acid glyco-protein (mg/dL)	124,7 (48,1-291,4)	59,0 (19,0-218,5)

AAG: alpha-1 acid glycoprotein

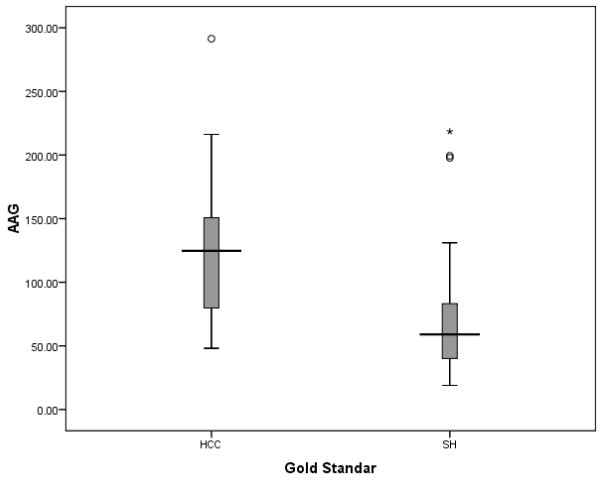

Figure 1. Distribution of alpha-1 acid glycoprotein (AAG) examination result in study group and control group

Using ROC, the AUC for AAG examination to diagnose HCC was 81.44% with standard error 0.0548 and 95% confidence interval (CI) 0.70-0.92 (Figure 2).

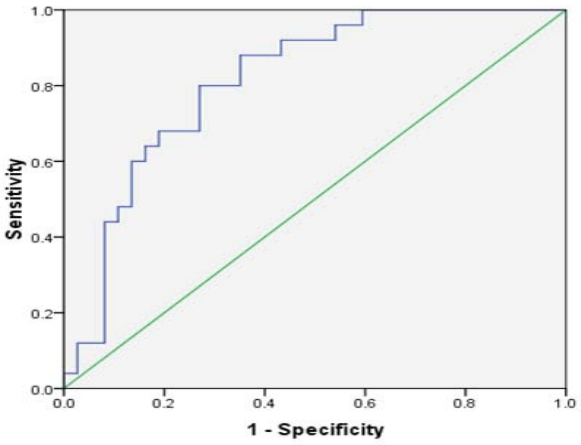

Figure 2. ROC of alpha-1 acid glycoprotein (AAG) result in study group

To get the best cut-off value of AAG, we assessed the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR) from each cut-off (Table 3).

DISCUSSION

There were 92% male in study group. Incidence in male was 8 times greater than female. Similar result was reported by Lehman et. al. from Egypt with 80% male[19] This result is different with the incidence of HCC in Asia Pacific, Europe, and America generally, with male to female ratio was 3 : 1.[1,5] Ajayi et al from Nigeria also reported male to female ratio in HCC was 2.31 : 1. In Japan, however, the incidence of HCC in male was almost the same with female.[5,20]

The incidence of HCC increased since 40 years old and above, mostly in patients older than 50 years old (72%). Mean age of the HCC patients with cirrhosis was 55.62 ± 11.92 years old. This finding was in coherent with data in Asia Pacific and Africa region showing highly increased of HCC incidence started 40 years old and above, although there is a tendency recently of HCC incidence in younger age group of patients (40 to 60 years old).[1,19,20]

In this study, there was a different in the composition of male and female between the two groups, but the mean age of each group was not highly different. The mean age in the study group was 55.62 ± 11.92 years old. Physical and laboratoryoratory examination of both groups showed most of the patient had poor liver function. According to Child-Pugh score, most patients in this study were Child-Pugh score B, having ascitesin each group (605 and 54.1%) and decreased serum albumin (less than 3 g/dL). Most common etiology in this study was chronic hepatitis B virus infection (56%), and chronic hepatitis C virus infection was just 20%. There was various etiology of HCC in each country in the same region.[21]

Chronic hepatitis B virus infection was the most common etiology of HCC in China, Taiwan, Korea, Thailand, India, Vietnam, Turkey, and Myanmar (50-85%). Especially in China, most off HCC etiology was chronic hepatitis B virus infection with odds ratio 12.45, and the odds ratio of chronic hepatitis C virus infection was just 4.28.[3,5] Moreover, the incidence of hepatitis B related HCC increased linearly from 1960 to 2005 in Australia in immigrant population originating

Table 3. Sensitivity, specificity, PPV, NPV, PLR, NLR of each AAG cut-off

AAG cut-off (mg/dL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR
20.7	100.0	2.78	40.98	100.00	1.03	0.00
30.8	100.0	11.11	43.10	100.00	1.13	0.00
40.1	100.0	25.00	47.16	100.00	1.33	0.00
54.3	96.0	44.44	53.33	94.11	1.73	0.09
61.3	92.0	58.33	59.00	91.00	2.21	0.14
74.3	88.0	66.67	57.89	87.50	2.64	0.18
78.6	80.0	73.0	64.66	84.37	2.88	0.28
82.6	72.0	72.22	64.28	79.41	2.59	0.39
93.8	68.0	80.56	70.83	78.94	3.49	0.39
106.1	64.0	83.33	72.72	77.50	3.84	0.43
117.5	56.0	86.11	72.22	72.72	4.03	0.51
129.6	48.0	88.89	73.33	70.21	4.32	0.58
136.2	44.0	91.67	79.00	71.00	5.28	0.61
146.7	32.0	91.67	72.72	66.66	3.84	0.74
175.1	24.0	91.67	66.66	64.15	2.88	0.83
190.6	16.0	91.67	57.14	61.81	1.92	0.92
207.1	12.0	97.22	75.00	62.06	4.32	0.90
291.4	4.0	100.00	100.00	60.65		0.96

AAG: alpha-1 acid glycoprotein; PPV: positive predictive value; NPV: negative predictive value; PLR: positive likelihood ratio; NLR: negative likelihood ratio

from Asia Pacific region.[6] In Japan and Pakistan, chronic hepatitis C virus infection was the most common etiology of HCC, ranging from 45% to 80%. In Japan, 79% of HCC patients were due to chronic hepatitis C virus infection and only 11% were due to chronic hepatitis B virus infection.[5] In Europe, the most common etiology of HCC, as reported in Germany, Austria, Sewdia, Belgia, England, was neither chronic hepatitis B infection nor chronic hepatitis C infection (50-80%).[21] Only Greek reported chronic hepatitis B virus infection as the most common etiology of HCC (55%). Italy and Spain reported chronic hepatitis C virus infection as the most common etiology of HCC (40-50%).[21]

In America, almost the same with Europe region, most of the HCC cases were not due to chronic hepatitis B or C virus infection (65%). This was due to the low prevalence of chronic hepatitis B and C virus infection in that region, high rate of alcohol consumption, and increased prevalence of overweight and obesity which was associated with liver cirrhosis in America and Europe.[22,23] The low prevalence of chronic hepatitis B virus infection in Europe and America was due to the success of universal vaccination program in child and adult.[24] And the low prevalence of chronic hepatitis C virus infection was due to good preventing program, early diagnosis, and accessibility to the good standard treatment of hepatitis C.[24,25] The good socio-economic level and insurance system also contributed to this result. In contrast, culture in the community with high alcoholic consumption, high prevalence of obesity, overweight and hypertension contributed as the etiology of HCC in that region.

The distribution of AAG laboratory examination in each groups were not normal. In the study group, there was 1 subject having result above other subjects' result. In the control group, there were 2 subjects having extremely high result above others'. Although totally there were 3 extreme AAG examination results, we did not take them out and still included them in the statistics calculation. We assumed that there was no laboratory error and it was a reflection of a true AAG laboratory examination result.

The median of AAG laboratory examination result in the study group was 124.7 mg/dL, which was above normal. The median of AAG laboratory examination result in the control group was within normal reference value, 59.0 mg/dL. This result showed that most subjects in the study group had AAG laboratory examination result above normal. This was in coherent with the previous study showing around 80% HCC patients had increased of AAG and only 20% liver cirrhosis patients had increased of AAG. Majority of subjects in the study group had multiple nodules (80%). One subject had 2 nodules (4%), 4 four subjects had single nodules (16%) with diameter greater than 5 cm. Using Barcelona Clinic Liver Cancer (BCLC) criteria, most subjects were in the intermediate stadium (B) or higher. Therefore, the treatment more on palliative therapy.[24]

One of the parameter to assess the suitability of an examination to be used as a diagnostic examination is the AUC value of the ROC. Theoritically, the AUC value is between 50% and 100%. Based on AUC, Sopiyudin stratified 5 levels of interpretation in clarifying the diagnostic power of an examination as follow: very weak (AUC 50 - 60%), weak (60% - 70%), intermediated (70% - 80%), good (80% - 90%), very good (90% - 100%).[25] The AUC in our study was 81.44%, showing that AAG laboratory examination was statistically categorized as a good examination for diagnostic examination of HCC in liver cirrhosis.

We evaluated the best AAG laboratory examination cut-off to diagnose HCC in liver cirrhosis. The first criterion was high specificity value; then we looked for the PPV, LPR, and sensitivity. Based on several cut-off values (Table 5), the specificity was good started from 136.2 mg/dL until 291.4 mg/dL (91.67% until 100%). Within that values, the best PPV and LPR was at 136.2 mg/dL (79% and 5.28, respectively). The sensitivity value was also the best within that range, which was 44.0%. Therefore, the best AAG value in diagnosing HCC in patients with liver cirrhosis was 136.2 mg/dL. To make it easier in daily clinical practice, it was rounded to 136 mg/dL. The daily clinical application of this study was if a liver cirrhosis patient with liver nodule had AAG laboratory examination < 136 mg/dL, the patient had 91.67% probability having no HCC. But, if the AAG laboratory examination was ≥ 136 mg/dL, the patient had 79% probability having HCC. To increase the confidence in diagnosing HCC in liver cirrhosis, we can use 291 mg/dL as the cut-off. If the AAG was > 291 mg/dL, the patient had very high probability of having HCC with 100% specificity.

We also evaluated the possibility using AAG laboratory examination to be aware of HCC in liver cirrhosis. In our study, the finding of liver nodule through abdominal USG in liver cirrhosis patient let us aware of HCC possibility. Other method to be aware of HCC in liver cirrhosis patient was checking AAG in liver cirrhosis patients. If the result was above the cut-off value, abdominal USG should be done to look for liver nodule.

From the various cut-off values to be aware of HCC in liver cirrhosis (Table 5), the sensitivity was good between 78.6 mg/dL and 20.7 mg/dL (80% and 100%, respectively). Within the range, 61.3 mg/dl had a quite good sensitivity value (92%), but the specificity, PPV, LPR values were just 58.33%, 59%, and 2.21, respectively). The sensitivity value at 78.6 mg/dl was lower then previous (80%), but the specificity, PPV, LPR values were better (72.22%, 64% and 2.88, respectively). In comparison of those two cut-offs, 61.3 mg/dL was the best cut-off since we needed greater sensitivity (> 90%) to be aware of a disease (HCC in liver cirrhosis) although it had lower specificity, PPV, LPR values. To make it simpler in daily clinical practice, the best cut-off value to be aware of HCC in liver cirrhosis was 61 mg/dL. In clinical application, for a cirrhosis patient with liver nodule having AAG less than 61 mg/dL, the liver nodule highly probable (91%) was not HCC (NPV in 61 mg/dL was 91%); therefore, the nodule can be managed as non HCC nodule in liver cirrhosis. However, if the AAG was greater than 61 mg/dL, 92% HCC nodule would be diagnosed at that cut-off and 59% was true HCC. Hence, other additional examinations should be performed to ensured the HCC, such as AFP.

The AAG examination can be used to be aware of and diagnose HCC in liver cirrhosis patients with liver nodule, especially in the patients who are not able to undergo 3 phases contrast abdominal CT or liver biopsy. The AAG might also serves as alternative biomarker examination of AFP with lower cost, faster gained result, and simpler examination which can be done in ordinary laboratory.

CONCLUSION

The best cut-off value of AAG to be aware of HCC in liver cirrhosis was 61 mg/dL and the best cut-off value of AAG to diagnose HCC in liver cirrhosis was 136 mg/dL. AAG can be used as an alternative biomarker to be aware of and to diagnose HCC in liver cirrhosis with liver nodule greater than 5 cm, no infection, no other malignancies, not recently got heart attack or any surgical procedures. Further studies in evaluating AAG as diagnostic biomarker in HCC patients without liver cirrhosis, HCC patients with different size of nodules, and HCC patients with different etiologies should be performed.

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