Journal of Clinical & Cellular Immunology

Eldin et al., J Clin Cell Immunol 2016, 7:3
http://dx.doi.org/10.4172/2155-9899.1000433

Research Article Open Access

Evaluation of the Diagnostic Performance of Soluble Human Leukocyte Antigen-G versus α-Fetoprotein for Hepatitis C Virus-Induced Hepatocellular Carcinoma

Amel Mahmoud Kamal Eldin[1*], Manal Mohamed Saber[1*], Aliaa Higazi[1], Amal Kamal Helmy[2], Hala Ibrahem Mohamed[3] and Asmaa Ahmed Aly[1]

[1]*Department of Clinical Pathology, Faculty of Medicine, Minia University, Minia, Egypt*

[2]*Department of Internal Medicine, Faculty of Medicine, Minia University, Minia, Egypt*

[3]*Department of Tropical Medicine, Faculty of Medicine, Minia University, Minia, Egypt*

[*]**Corresponding authors:** Amel Mahmoud Kamal Eldin, Department of Clinical Pathology, Faculty of Medicine, Minia University, Minia, Egypt, Tel: +201019748497; E-mail: amlkoktail@yahoo.com

Manal Mohamed Saber, Department of Clinical Pathology, Minia University, Minia, Egypt, Tel: +201019748497; E-mail: Manal.Saber@mu.edu.eg

Received date: March 18, 2016; **Accepted date:** June 17, 2016; **Published date:** June 28, 2016

Copyright: © 2016 Eldin AMK, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Background: Hepatocellular carcinoma (HCC) is the leading cause of death in HCV-related liver diseases and the third most common cause of cancer-related mortality worldwide. A reliable serum marker for early diagnosis of HCC is currently lacking.

Objectives: The aim of this study was to identify the serum levels of soluble Human leukocyte antigen-G (sHLA-G) in HCC and liver cirrhosis (LC) patients. As well, we aimed to estimate the diagnostic performance of sHLA-G for HCC by comparing its levels with the levels of Alpha fetoprotein (AFP).

Subjects and methods: The study included 100 subjects divided into: 25 apparently healthy volunteers who served as healthy control subjects (group I), and 50 patients with untreated HCC on top of liver cirrhosis (group II) in addition to 25 cirrhotic patients (group III). HCC group was divided into two subgroups, 25 patients with AFP levels ≤ 200 ng/ml and 25 patients with AFP levels >200 ng/ml. All subjects were subjected to routine laboratory investigations plus detection of serum levels of both AFP and sHLA-G by Enzyme Immune Assay (EIA).

Results: Blind parallel detection was conducted for sHLA-G and AFP. AFP was highly significantly increased in HCC and LC patients when compared to healthy controls (p=0.001). Also, there was a highly significant increase in AFP level in HCC patients when compared to cirrhotic ones (p=0.001). sHLA-G was highly significantly increased in HCC patients when compared to both healthy controls and cirrhotic patients (p=0.001). sHLA-G was not significantly increased in cirrhotic patients when compared to healthy controls (p=0.001). There was a significant strong positive correlations between sHLA-G and AFP in HCC patients (p<0.001) while weak negative correlation between sHLA-G and AFP was detected in cirrhotic group. The area under the receiver operating characteristic curve (ROC) was used to evaluate the diagnostic efficacies of both markers. The superiority of sHLA-G over AFP was more profound in the diagnosis of HCC [AUC: 0.993], in discriminating HCC from LC patients [AUC: 0.992] as well as in the diagnosis of HCC patients with AFP levels ≤ 200 ng/ml or in discriminating this HCC subgroup from LC patients [AUC: 0.986 and 0.984 respectively].

Conclusion: sHLA-G was found to be superior over AFP because of its higher AUC than that of AFP. As well, sHLA-G has better diagnostic performance with higher sensitivity and specificity than AFP. According to our data, sHLA-G could serve as a new efficient marker for early diagnosis of HCV-related HCC patients and to discriminate HCC from LC patients. Thus, measuring sHLA-G levels can help to reduce both false negative and false positive rates of AFP. Moreover, sHLA-G could have predictive value for tumorogenesis in HCV-related LC patients and could be used along with other tumor markers for early detection of malignant transformation.

Keywords: Hepatocellular carcinoma (HCC); Liver cirrhosis (LC); Soluble HLA-G (sHLA-G); Alpha feto protein (AFP)

Introduction

Hepatocellular carcinoma (HCC) is considered one of the most frequent neoplasms worldwide. It is the major complication and the main cause of death in people with liver cirrhosis (LC). HCC is the fifth tumor in prevalence in men and the ninth in women as stated by the American Cancer society report in 2012 [1,2]. HCC usually presents late in an advanced stage of the disease because of the deficient of early symptoms as well as lack of reliable early diagnostic markers. Therefore, a lot of patients miss the opportunity of early therapeutic interventions and thus have a very bad prognosis and so far poor survival. Accordingly, better early diagnostic modalities are urgently required [2].

Alpha fetoprotein (AFP) is still considered the conventional marker in the diagnosis of HCC. However, AFP has poor sensitivity and specificity as its level may increase in patients with other liver diseases including acute hepatitis, chronic active hepatitis or LC [2]. Moreover,

the false negative rate of AFP levels in diagnosing HCC is high. This is because its level may not be elevated enough in approximately one third of HCC patients especially in the very early stages of the disease and even in patients with advanced HCC or in small sized HCC focal lesions [3,4]. Also, the positive rate of AFP in HCC is only 60–80% plus the false-positive AFP results during pregnancy as well as embryonic and certain gastrointestinal tumors [5]. Accordingly, it is obvious that yet there is no reliable marker for the early diagnosis of HCC. Therefore, more studies should be considered to improve the early diagnostic rate of HCC including the combined detection of several serum markers.

Human leukocyte antigen G (HLA-G) was first described as a key molecule inducing materno-fetal tolerance, tumor escape, and allograft transplantation acceptance. HLA-G molecules belong to the non classical major histocompatibility complex-class Ib molecules [6]. Different studies have revealed HLA-G antigens and soluble (secreted) HLA-G (sHLA-G) in various human carcinomas involving endometrial adenocarcinoma, colorectal cancer, renal cell carcinoma, gastrointestinal cancer, carcinomas of the lung, breast, ovary in addition to hematolymphoid malignancies [7,8]. Consequently, HLA-G may serve as a tumor marker for the prediction of certain types of cancers. This may be attributed to the possible role of HLA-G in tumor development by suppressing immune regulation within the tumor micro environment [9]. Additionally, HLA-G was found to correlate with various malignant clinic-pathological parameters as in lymphoma, ovarian and gastric carcinomas [8]. Therefore, HLA-G could have clinical value in the diagnosis of HCC.

This study was designed to elucidate the significance of sHLA-G in HCC and in patients with LC as having major risk for development of HCC in the Egyptian community. We also evaluated the relations between sHLA-G and AFP which is till now the conventional marker that has been used as a reference for early diagnosis of HCC.

Subjects and Methods

Subjects

This study was carried out in the Clinical Pathology Department, Internal Medicine Department and Tropical Medicine Department, Faculty of Medicine, El-Minia University Hospital. A total of 100 subjects were enrolled in this study. They were including 25 apparently healthy volunteers who served as a control group (Group I). Their ages ranged from 35 to 52 years old with mean ± Standard Deviation (SD) of 42 ± 9 years. They had normal liver and renal laboratory tests and were all negative for the markers of hepatitis viruses A, B and C as well as HIV antibodies. As well, they were free by clinical examination with no liver diseases and no malignancies. Additionally, the study was including 50 HCC patients (Group II). Their ages ranged from 38 to 57 years old with mean ± SD of 47 ± 9 years. The HCC patients were diagnosed on basis of either histopathological findings or by dynamic contrast enhanced CT and AFP. AASLD's practice guidelines proposed a single radiologic hallmark method positive for HCC to be sufficient for diagnosis regarding nodules larger than 2 cm and nodules between 1-2 cm. [10,11]. This group was further subdivided into 2 subgroups according to the levels of AFP. These 2 subgroups were incorporating Group IIa which comprised HCC patients with AFP levels less than 200 ng/ml and Group IIb which comprised HCC patients with AFP levels more than or equal to 200 ng/ml [12]. As well, the study involved 25 cirrhotic patients (Group III). Their ages ranged from 45 to 65 years old with mean ± SD of 47 ± 9 years. They were diagnosed by physical examination, laboratory investigations, imaging tests and liver biopsy in some patients [2]. Patients in Group II and III were proved to be positive for HCV- RNA by real time (RT) PCR and negative for hepatitis B surface antigen virus by enzyme immune assay (EIA). Patients with other malignancies or other infectious diseases were excluded from the study. Thorough history questionnaires were filled for all subjects plus full clinical examination. In addition, informed written consents were signed from all subjects.

Blood sampling

Blood samples were withdrawn from all subjects under complete sterile condition for assessment of Complete Blood Count (CBC), liver function tests, prothrombin time (PT) and concentration (PC), AFP levels as well as sHLA-G concentrations. For CBC, 2 ml of venus blood were collected in an EDTA tubes and CBCs were performed immediately. About 2 ml of blood were collected in citrated tubes (3.2% trisodium citrate) for estimation of PT and PC. The last 5 ml of blood were evacuated in plain tubes and expressed serum was used immediately for determination of liver function tests. The remaining serum was stored at -70°C for further evaluation of AFP and sHLA-G.

Laboratory methods

CBC was carried out using automated cell counter Sysmex KX-21N (TAO Medical incorporation, Japan). PC and PT were measured by STAGO COMPACT CT Coagulation Analyzer (Diamond Diagnostics, USA). Furthermore, liver function tests (AST, ALT, Alkaline phosphatase, bilirubin, total protein and albumin) were detected using fully automated clinical chemistry auto-analyzer system Konelab 20i (Thermo Electron Incorporation, Finland).

Serum AFP levels were determined by EIA kit according to the manufacturer's instructions using anti-AFP antibodies for quantitative detection of human AFP (TECO diagnostics, California, USA). As well, sHLA-G concentrations were evaluated with a commercially available EIA kit (USCAN life Science Inc., Houston, TX, USA) according to the manufacturer's instructions.

Statistical analysis

All collected data were analyzed statistically using statistical package for social sciences (SPSS) program version 17.0 (SPSS Inc., Chicago, IL, USA). The quantitative data were presented as mean ± SD while the qualitative variables were described as number and percentage. Student t-test was used to compare results between groups as regards quantitative data. Chi-square test was used to compare qualitative variables between groups. p-values equal to or less than 0.05 are statistically significant. One Way Anova test was used for comparison of parametric quantitative data between more than two groups. Correlation was performed by using Pearson correlation coefficient of (r). Moreover, ROC curve was used to evaluate the diagnostic performance of both AFP and sHLA-G in HCC.

Results

Demographic features and laboratory results of enrolled subjects

The demographic characteristics of the participants involved in this study are summarized in Table 1. The subjects in the involved groups were age matched with no significant difference when the three groups

Citation: Eldin AMK, Saber MM, Higazi A, Helmy AK, Mohamed HI, et al. (2016) Evaluation of the Diagnostic Performance of Soluble Human Leukocyte Antigen-G versus α-Fetoprotein for Hepatitis C Virus-Induced Hepatocellular Carcinoma. J Clin Cell Immunol 7: 433. doi: 10.4172/2155-9899.1000433

were compared to each other's (p=0.336, 0.321 and 0.975). There was a male predominance in both (HC) healthy control and HCC groups. However, the ♀/♂ ratio within LC patients was 48%/52% (Figure 1).

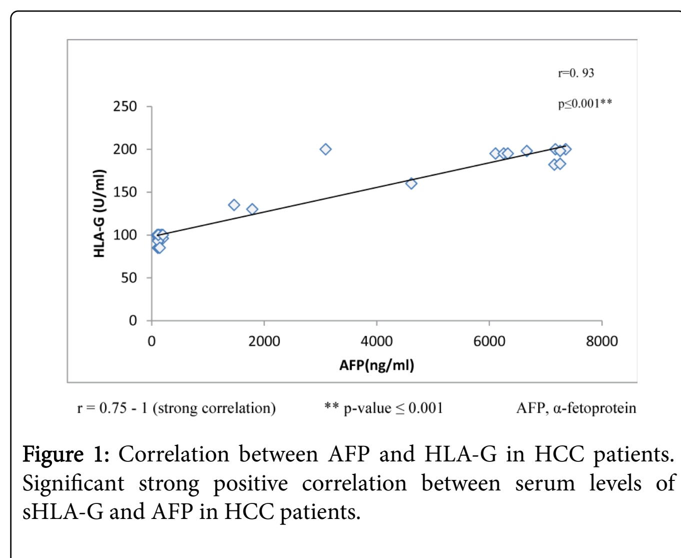


Figure 1: Correlation between AFP and HLA-G in HCC patients. Significant strong positive correlation between serum levels of sHLA-G and AFP in HCC patients.

There was a highly statistical significant increase in both ALT and AST levels in HCC and LC groups when compared to HC group (p<0.001). As well, there was a significant increase in both aminotransferases in HCC group when compared to LC group (p<0.001 and 0.006 respectively) (Table 1). Additionally, there was a highly significant increase in total and direct bilirubin in HCC group and in LC group (p<0.001) when compared to HC group. On the other hand, there was no significant difference in total bilirubin when HCC group was compared to LC group (p=0.20 and 0.4 respectively). Also, there was a highly significant decrease in albumin concentration in HCC group and in LC group (p<0.001) when compared to HC group but there was no significant difference between HCC group in comparison to LC one (p=0.08). Furthermore, there was a highly significant decrease in PC in HCC group and in LC group (p<0.001) when compared to HC group. Contrary, there was no significant difference between HCC group and LC group (p=0.10) as regards PC (Table 1).

Regarding CBC parameters, firstly, there was significant decrease in TLC in HCC and in LC groups when compared to HC group (p<0.001). Though, when HCC group was compared to LC group, there was no significant difference (p=0.54). Secondly, there was a highly significant decrease in platelets count in HCC group and in LC group in compare to HC group (p<0.001) then when HCC group was compared to LC group, there was no significant difference (p=0.34). Finally, there was a highly significant decrease in Hb levels when comparing both HCC and LC groups with HC group (p<0.001). However, when HCC group was compared to LC group, there was no significant difference (p=0.64) (Table 1).

Levels of AFP and sHLA-G according to the study groups

Serum levels of AFP and sHLA-G were significantly increased in patients with HCC than in patients with LC or in HC group (p<0.001). Likewise, serum levels of AFP were significantly increased in patients with LC when compared to HC group (p<0.001). However, there was a difference in the serum levels of sHLA-G within LC patients when compared to HC group but did not reach a statistical significance (p=0.07). Moreover, sHLA-G levels were significantly increased in HCC subgroup IIb when compared to HCC subgroup IIa (p<0.001). The serum levels of both AFP and sHLA-G in different studied groups are shown in Table 2.

Variables	Groups			p-value		
	Healthy control (N=25)	HCC (N=50)	LC (N=25)	I vs II	I vs III	II vs III
Age (years) Mean ± SD	44.28 ± 5.02	45.52 ± 5.27	45.56 ± 2.91	0.336	0.321	0.975
Gender Male/female (%)	60%	60%	48%	_		
ALT(U/L) Mean ± SD	16.32 ± 7.04	61.52 ± 20.59	33.17 ± 13.79	<0.001**	<0.001**	<0.001**
AST(U/L) Mean ± SD	18.32 ± 5.89	91.29 ± 37.47	63.59 ± 28.04	<0.001**	<0.001**	0.006*
Total bilirubin (mg/dl) Mean ± SD	0.37 ± 0.2	2.10 ± 1.16	1.81 ± 0.78	<0.001**	<0.001**	0.2
direct bilirubin (mg/dl) Mean ± SD	0.17 ± 0.08	0.78 ± 0.48	0.68 ± 0.36	<0.001**	<0.001**	0.4
Albumin (g/L) Mean ± SD	4.45 ± 0.50	2.97 ± 0.64	2.51 ± 0.49	<0.001**	<0.001**	0.08
PC (%)	95 ± 6	56 ± 18	49 ± 14	_		
Platelets (1 × 10^3/μL) Mean ± SD	254.79 ± 79.27	100.73 ± 39.28	90.45 ± 34.86	<0.001**	<0.001**	0.34
TLC (1 × 10^3/μL) Mean ± SD	7.51 ± 2.04	3.90 ± 1.98	5.55 ± 1.90	<0.001**	<0.001**	0.54
Hb (g/dl) Mean ± SD	13.1 ± 1.80	10.5 ± 2.7	9.4 ± 1.7	<0.001**	<0.001**	0.64

N, number; * p-value ≤ 0.05; ** p-value ≤ 0.001 HCC: Hepatocellular Carcinoma; LC: Liver Cirrhosis; ALT: Alanine Transaminase; AST: Aspartate Transaminase; PC: Prothrombin Concentration; TLC: Total Leucocytes Count; Hb: Hemoglobin.

Table 1: Comparison between patients and control groups regarding demographic and laboratory data.

Citation: Eldin AMK, Saber MM, Higazi A, Helmy AK, Mohamed HI, et al. (2016) Evaluation of the Diagnostic Performance of Soluble Human Leukocyte Antigen-G versus α-Fetoprotein for Hepatitis C Virus-Induced Hepatocellular Carcinoma. J Clin Cell Immunol 7: 433. doi: 10.4172/2155-9899.1000433

Variable	Group				p-value		
	Healthy controls (N=25)	Group II HCC (N=50)		LC (N=25)	I vs II	I vs III	II vs III
AFP (ng/ml) Mean ± SD	4.6 ± 1.5	1484.9 ± 1415		161.6 ± 15.8	< 0.001**	< 0.001**	< 0.001**
		IIa HCC é AFP<200 (N=25)	**IIb HCC é AFP ≥ 200 (N=25)**				
		141.5 ± 35.4	5692 ± 2095		< 0.001**		
sHLA-G (U/ml) Mean ± SD	14.2 ± 16.8	136.2 ± 55.7		26.5 ± 7.8	< 0.001**	0.07	< 0.001**
		IIa HCC é AFP<200 (N=25)	**IIb HCC é AFP ≥ 200 (N=25)**				
		96.9 ± 4.9	183.6 ± 24.3		< 0.001**		

N: Number; ** p-value ≤ 0.001; HCC: Hepatocellular Carcinoma; LC: Liver Cirrhosis; AFP: α-Fetoprotein

Table 2: Comparison between patients and control groups as regards AFP and sHLA-G.

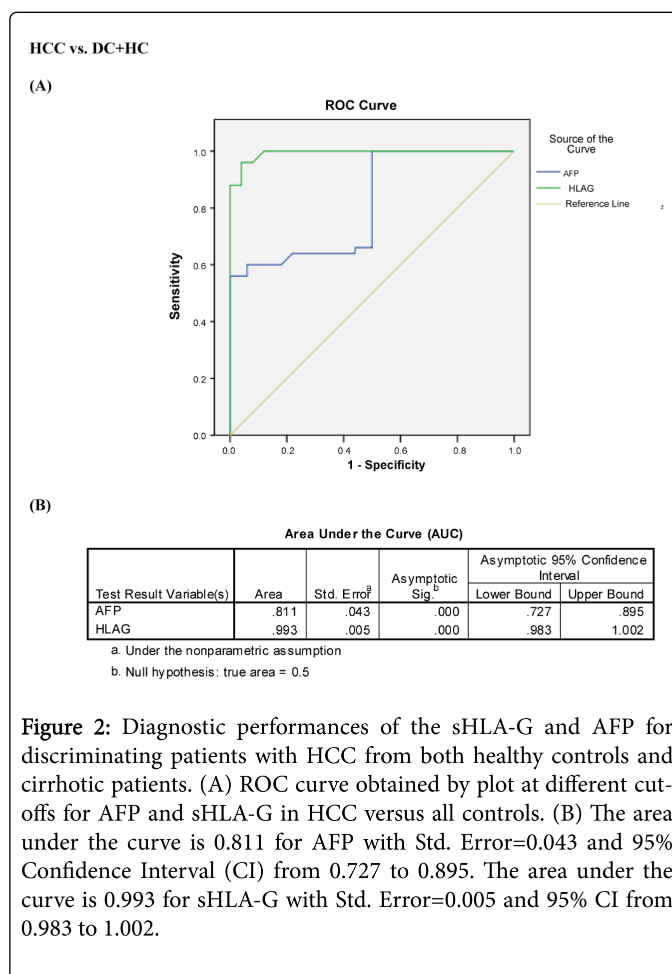
Variables	sHLA-G	
	r	p-value
ALT (U/L)	0.37	<0.001**
AST (U/L)	0.27	<0.001**
Total bilirubin (mg/dl)	0.24	<0.001**
Direct bilirubin (mg/dl)	0.16	<0.001**
Albumin (g/dl)	-0.14	<0.001**
PC (%)	-0.25	<0.001**
Platelets (1x103/μL)	-0.15	0.002*
TLC (1x103/μL)	0.24	<0.001**
Hb (g/dl)	-0.01	<0.001**

r=0.75-1 (strong correlation), r=0.5-0.74 (moderate correlation), r=0.25-0.49 (fair correlation), r=0.1-0.24 (weak correlation); ** p-value ≤ 0.001; HCC: Hepatocellular Carcinoma; LC: Liver Cirrhosis; ALT: Alanine Transaminase; AST: Aspartate Transaminase; PC: Prothrombin Concentration; TLC: Total Leucocytes Count; Hb: Hemoglobin

Table 3: Correlation between sHLA-G and laboratory findings in HCC patients.

Correlation of sHLA-G levels with serum AFP and other laboratory data in HCC group

To assess the role of sHLA-G expression in HCC, we correlated between serum levels of sHLA-G and AFP as well as other laboratory parameters in HCC group. Serum sHLA-G correlation with AFP was strong positive and highly statistically significant (r=0.93, p<0.001). As well, the correlations between serum sHLA-G and other laboratory findings in HCC group were shown in Table 3. Regarding the correlation between serum sHLA-G levels with liver function tests as well as CBC parameters, we found that serum sHLA-G had a highly statistical significant positive correlation with ALT, AST, total bilirubin, direct bilirubin and with TLC (p<0.001). On the other hand, serum sHLA-G had a highly statistical significant negative correlation with albumin, PC and with Hb (p<0.002) in addition to statistical significant negative correlation with platelets count (p=0.002).

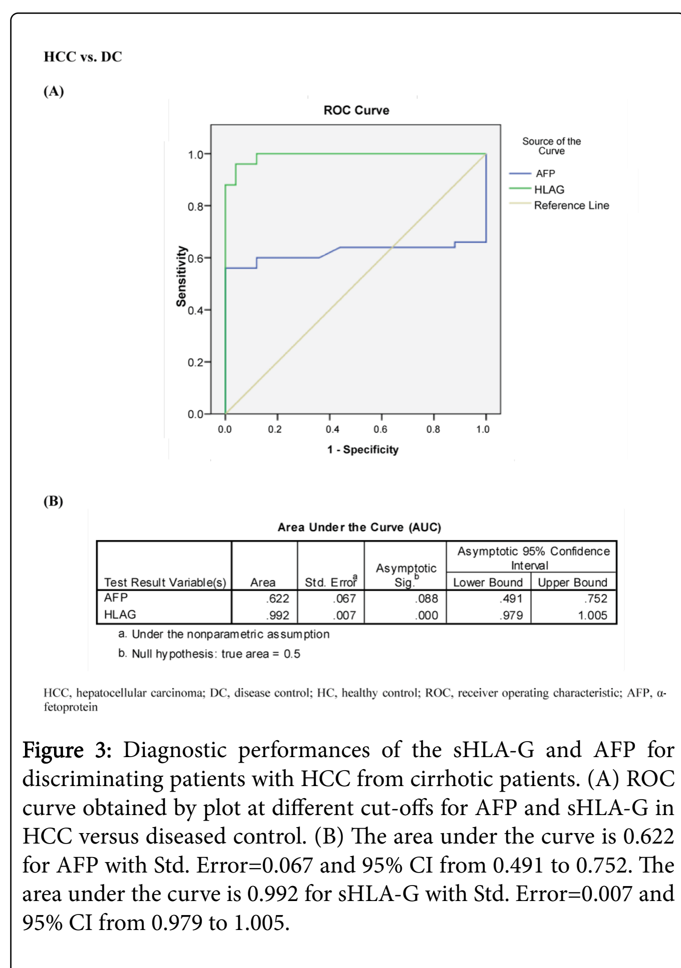


Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval Lower Bound	Upper Bound
AFP	.811	.043	.000	.727	.895
HLAG	.993	.005	.000	.983	1.002

Figure 2: Diagnostic performances of the sHLA-G and AFP for discriminating patients with HCC from both healthy controls and cirrhotic patients. (A) ROC curve obtained by plot at different cut-offs for AFP and sHLA-G in HCC versus all controls. (B) The area under the curve is 0.811 for AFP with Std. Error=0.043 and 95% Confidence Interval (CI) from 0.727 to 0.895. The area under the curve is 0.993 for sHLA-G with Std. Error=0.005 and 95% CI from 0.983 to 1.002.

Diagnostic performance of serum sHLA-G versus AFP for HCC patients

To determine cut-off levels that balanced the false-positive and the false-negative rates with the best positive predictive value, ROC analysis was performed for AFP and sHLA-G. ROC curves of sHLA-G and AFP for discriminating patients with HCC from all non HCC subjects were shown in (Figure 2). The AUC value of sHLA-G was 0.993 [95% confidence interval (CI)=0.983–1.002, p=0.000]. Additionally, AFP showed an AUC value of 0.811 (95% CI=0.727–0.895, p=0.000), and there was a significant difference between the AUC values of sHLA-G and AFP (p<0.001) (Figure 2). ROC curves of sHLA-G and AFP for discriminating patients with HCC from those with LC were shown in (Figure 3). The AUC value of sHLA-G was 0.992 [95% CI=0.979–1.005, p=0.0001]. Also, AFP showed an AUC value of 0.622 (95% CI=0.491–0.752, p=0.088) (Figure 3) and there was a significant difference between the AUC values of sHLA-G and AFP (p<0.001).



Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
AFP	.622	.067	.088	.491	.752
HLAG	.992	.007	.000	.979	1.005

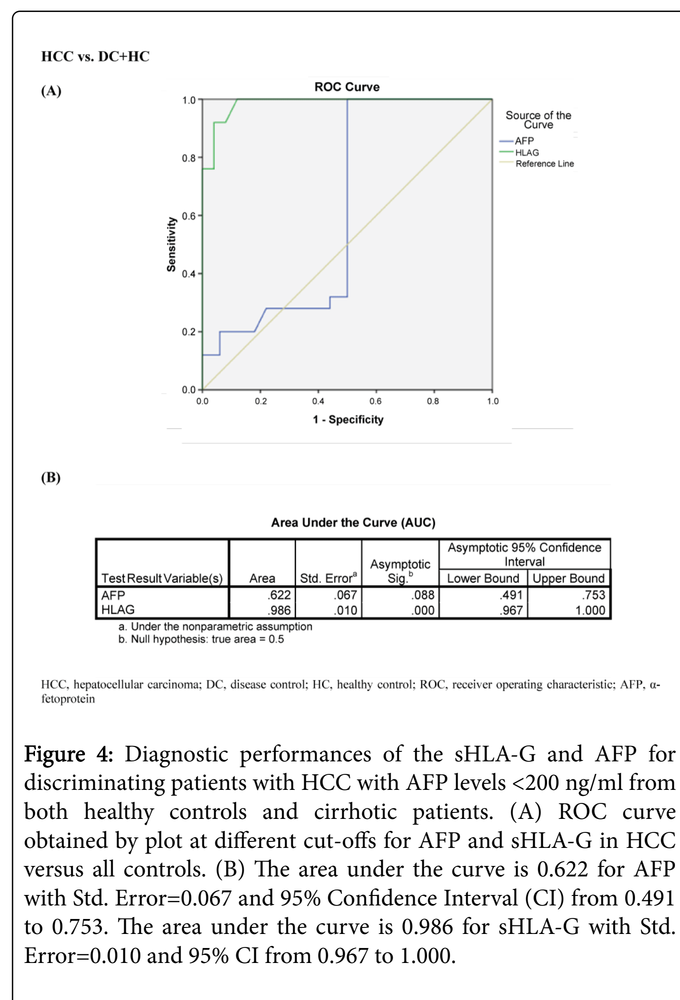
Figure 3: Diagnostic performances of the sHLA-G and AFP for discriminating patients with HCC from cirrhotic patients. (A) ROC curve obtained by plot at different cut-offs for AFP and sHLA-G in HCC versus diseased control. (B) The area under the curve is 0.622 for AFP with Std. Error=0.067 and 95% CI from 0.491 to 0.752. The area under the curve is 0.992 for sHLA-G with Std. Error=0.007 and 95% CI from 0.979 to 1.005.

These ROC curves indicated that a sHLA-G value of 44 U/mL yielded the best sensitivity and specificity for differentiating patients with HCC from those without HCC as a whole (HC plus diseased controls (DC)) as well as from LC (Table 4). For AFP, the best cut-off value that yielded the maximum sensitivity and specificity for differentiating patients with HCC from those without HCC as a whole (HC plus diseased controls (DC)) and from DC only was 171 ng/mL (Table 4). Moreover, based on these ROC defined cut-off values, the sensitivity and specificity of sHLA-G was 96.7% and 96% respectively when the comparison was versus both all non HCC patients and LC. The PPV of sHLA-G versus all controls and DC was 96% and 98% respectively As well, the sensitivity, specificity and PPV of AFP was 60%, 86% and 81% respectively when performed to HC plus DC while when versus LC group the results were 60%, 72% and 88% respectively (Table 4).

Variable	Cut-Off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
HCC vs. DC+HC					
AFP	171	60%	86%	81%	31.80%
sHLA-G	44	96.70%	96%	96%	4.16%
HCC vs. DC					
AFP	171	60%	72%	88%	48.80%
sHLA-G	44	96.70%	96%	98%	7.70%

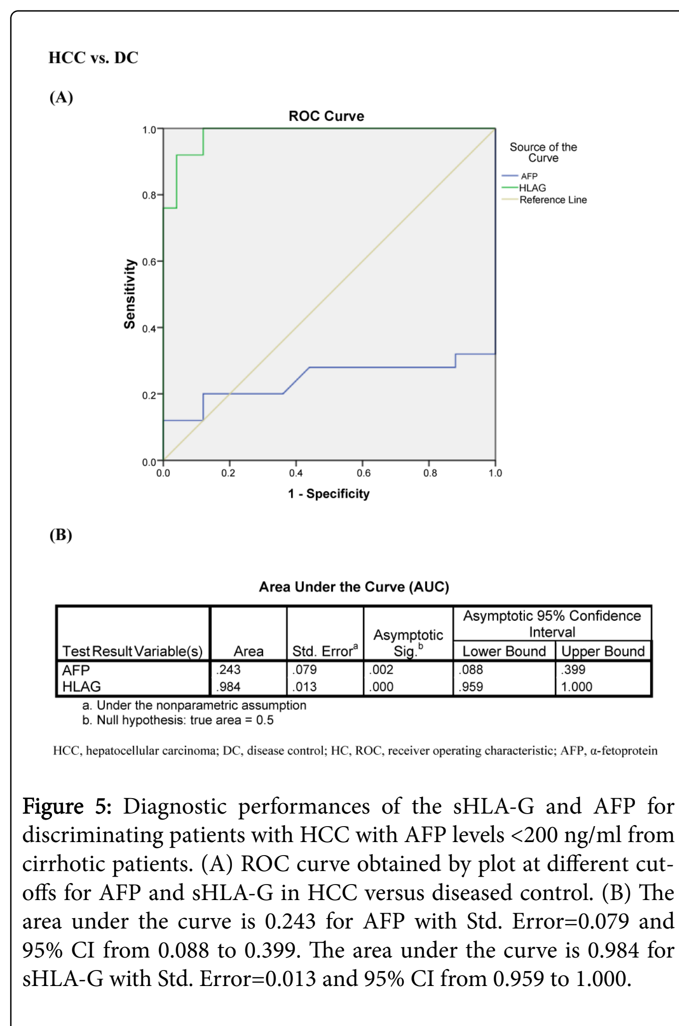
HCC: Hepatocellular Carcinoma; DC: Disease Control; HC: Healthy Control; AFP: α-Fetoprotein; PPV: Positive Predictive Value; NPV: Negative Predictive Value

Table 4: Results of measurement of AFP or sHLA-G in the diagnosis of HC.



Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
AFP	.622	.067	.088	.491	.753
HLAG	.986	.010	.000	.967	1.000

Figure 4: Diagnostic performances of the sHLA-G and AFP for discriminating patients with HCC with AFP levels <200 ng/ml from both healthy controls and cirrhotic patients. (A) ROC curve obtained by plot at different cut-offs for AFP and sHLA-G in HCC versus all controls. (B) The area under the curve is 0.622 for AFP with Std. Error=0.067 and 95% Confidence Interval (CI) from 0.491 to 0.753. The area under the curve is 0.986 for sHLA-G with Std. Error=0.010 and 95% CI from 0.967 to 1.000.



Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
AFP	.243	.079	.002	.088	.399
HLAG	.984	.013	.000	.959	1.000

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

HCC, hepatocellular carcinoma; DC, disease control; HC, ROC, receiver operating characteristic; AFP, α-fetoprotein

Figure 5: Diagnostic performances of the sHLA-G and AFP for discriminating patients with HCC with AFP levels <200 ng/ml from cirrhotic patients. (A) ROC curve obtained by plot at different cut-offs for AFP and sHLA-G in HCC versus diseased control. (B) The area under the curve is 0.243 for AFP with Std. Error=0.079 and 95% CI from 0.088 to 0.399. The area under the curve is 0.984 for sHLA-G with Std. Error=0.013 and 95% CI from 0.959 to 1.000.

Furthermore, the HCC group was subdivided into 2 subgroups according to the concentration of AFP in their sera. These subgroup were comprising those HCC patients who had AFP levels <200 ng/ml as well as those with AFP levels ≥ 200 ng/ml. ROC curves of sHLA-G and AFP for differentiating patients with HCC (AFP<200 ng/ml) from all controls (HC+DC) were analyzed (Figure 4). The AUC value of sHLA-G was 0.986 [95% CI=0.967-1.00, p=0.000]. In addition, AFP showed an AUC value of 0.622 (95% CI=0.491-0.753, p=0.088) and there was a significant difference between the AUC values of sHLA-G and AFP (p<0.001) (Figure 4). When the analysis were versus LC group (Figure 5A), the AUC value for sHLA-G was 0.984 [95% CI=0.959-1.00, p=0.000] and for AFP was 0.243 (95% CI=0.088-0.399, p=0.002) (Figure 5B). There was a significant difference between the AUC values of sHLA-G and AFP (p<0.001).

Sensitivity and specificity of sHLA-G for differentiating HCC from all controls and LC were 92% and 96% respectively when applying a cut-off level of 44 U/ml, which was the cut-off with the maximal sum of sensitivity and specificity (Table 5). Similarly, sensitivity of AFP for distinguishing these patients from all control and LC patients was 20%. The AFP specificity was 86% when the analysis was versus diseased plus healthy controls and 72% when versus LC group only. The cut-off level of 171 ng/ml was the one that showed the maximum value of sensitivity plus specificity (Table 5).

Variable	Cut-Off value	Sensitivity (%)	Specificity (%)
HCC vs. (DC+HC)			
AFP	171	20%	86%
sHLA-G	44	92%	96%
HCC vs. DC			
AFP	171	20%	72%
sHLA-G	44	92%	96%

Table 5: Results of measurement of AFP or sHLA-G in the diagnosis of HCC with AFP levels <200 ng/ml.

Discussion

HCV infection is a major health problem in the Egyptian community that may evolve to LC and HCC. LC is considered the end stage of a variety of chronic liver diseases. The major complication of LC is HCC which representing an increased cause of mortality [13]. Hepatic carcinogenesis is a multistep process mainly associated with persistent infection with hepatitis B virus or hepatitis C virus [14]. Behnke et al. stated a 20-fold increased risk of HCC in patients with HCV infection compared to those without infection [15].

The malignant transformation from LC to HCC is usually asymptomatic so diagnosis is always late and therefore mortality rate increased. The diagnosis of HCC is often based on screening and surveillance strategies whose mainstays are the measurement of the levels of AFP [16]. AFP is still one of the most important indicators in the diagnosis of HCC [17]. The present study showed that AFP was significantly increased in HCC and LC patients when compared to healthy control subjects (p<0.001). Also, there was a significant increase in the levels of AFP in HCC patients when compared to LC patients (p<0.001). These results were in agreement with Mukozu et al. and Sterling et al. [18,19]. Moreover, measurement of AFP levels in different studies had proved that there is a strong correlation between AFP values, tumor dimensions, as well as microvascular invasion. As well, all well-known predictors of HCC recurrence such as greater tumor size, bilobar involvement, massive or diffuse types, portal vein thrombosis, and low survival rate tend to have high AFP concentration [20,21].

However, AFP levels may increase in patient with acute hepatitis, chronic active hepatitis or liver cirrhosis [17]. Also, AFP has multiple limitations when applied to the detection of small tumors and varies significantly in the presence of benign or nonmalignant liver nodules [22,23]. Likewise, it was reported that only 10-20% of patients in early stages of HCC present with abnormal AFP serum levels [24]. Thus, establishing a more reliable early marker for diagnosing HCC either as a single one or in combination with AFP is mandatory for the diagnosis of HCC at early stages and hence better prognosis.

It has been demonstrated that HLA-G plays an important role in mediating immune tolerance. HLA-G may down regulate the function of immune system cells in many tumors via its tolerogenic properties thus helps cancers to escape from host immunity. Moreover, HLA-G expression may be induced by particular viruses as it is known that incompletely understood immune mechanisms have been associated with impaired viral clearance. Therefore, it is important to shed more light on HLA-G role in different cancers and viruses infections [25].

sHLA-G provokes apoptosis in CD8+ T lymphocytes and natural killer (NK) cells. This is because of binding of sHLA-G to CD8 which is mediated through Fas/FasL-dependent mechanism [26]. Many studies had demonstrated the level of sHLA-G in the serum of various tumors but few studies had reported its levels in HCC [25-28]. In the current study, we provided more insights into the possible role of sHLA-G as a diagnostic marker for HCC on top of HCV infection. In addition, we investigated the relation between the levels of the conventional marker AFP and sHLA-G in the sera from HCC patients.

In our study, sHLA-G was significantly increased in HCC patients when compared to healthy control subjects and LC patients (p<0.001). These results were similar to those reported by Wang et al. who studied sHLA-G in HCC patients (N=36), cirrhotic patients (N=25) and apparently healthy controls (N=25) [8]. The same results were obtained by Park et al. and Lin et al. who designed their study on HBV-induced HCC [28,29]. Also, these results are similar to those in patients with breast and ovarian cancer along with a variety of other malignant lesions, where sHLA-G levels were increased in comparison to healthy controls as stated by Rebmann, et al. and Singer et al. [30,31]. Furthermore, Yan et al. studied the membrane bound HLA-G protein expression in HCC patients. They reported that high HLA-G expression was associated with reduced survival and increased recurrence of HCC. Besides, they reported that HLA-G expression was associated with HCC prognosis, especially in early-stage of the disease, with high expression associated with shortened overall survival and increased tumor recurrence [32]. As well, Wang et al. demonstrated that patients with HLA-G-positive tumors had shorter survival rates than patients with HLA-G negative tumors [8].

Additionally, in our study, there was no significant difference in sHLA-G levels between LC patients and healthy controls (p-value=0.07). These results were in agreement with those reported by Wang et al. which provided definite evidence of HLA-G expression in HCC specimens but not in LC tissue. They attributed their data to the studies which supposed that sHLA-G is more frequently present in malignant lesions than in benign lesions and healthy individuals [8]. According to these data, sHLA-G may be more efficient marker in minimizing the false positive rate of AFP. As a result, concurrent measurement of both AFP and sHLA-G concentrations in serum could be beneficial. However, Shi et al. found that sHLA-G levels were increased in patients with acute and chronic hepatitis B in comparison to healthy controls and subjects with resolved HBV infection. In their study, AUC values of sHLA-G for differentiating acute and chronic hepatitis B from healthy controls were 1.000 and 0.993, respectively [33]. Therefore, in order to achieve clinical application, we need to verify the accuracy of this marker by testing a larger number of samples and by performing multicentric studies.

The current study demonstrated significant fair positive correlation between sHLA-G with ALT and AST in HCC as well as significant weak positive correlation between sHLA-G with TLC and with total and direct bilirubin in HCC patients. Moreover, there was a significantly negative weak correlation between sHLA-G with albumin, PC, platelets count and HB concentration in HCC patients. In addition, a strong significant positive correlation was detected between serum sHLA-G and AFP in HCC patients (r=0.93, p<0.001). Thus, sHLA-G could be considered as associating with the severity of HCV infections and HCC. In contrary, Park et al. reported that there was no correlation between the levels of sHLA-G with both AFP and aminotransferases [29]. The difference may be because the study of Park et al. was performed on HBV-induced HCC which differs from our study which was conducted on HCV-induced HCC [29]. Additionally, these results differed from the reported data by Wang et al. who showed that there was no association between sHLA-G and AFP in HCC patients [8]. HLA-G expression was reported as showing positive correlations with higher histological grade in addition to clinical stage in colorectal cancer, gastric cancer, esophageal carcinomas as well as many other malignancies which reflect that HLA-G expression is a highly specific indicator for malignant transformation not benign conditions as reviewed by Dias et al. [34]. Likewise, in our study there was insignificant weak negative correlation between serum sHLA-G and AFP in LC patients (r=-0.11, p=0.352) [data not shown]. This strongly verifies the concept that concluded in Dias et al. as well as detailed in other studies [34-37].

The disease controls included both diseased subjects with LC and apparently healthy controls to evaluate the different diagnostic performance between LC and HCC in the HCV infection. In our data, the AUC of sHLA-G for distinguishing HCC from all controls (healthy plus cirrhotic subjects) was 0.993 and was higher than that of AFP (AUC=0.811) with a cut-off value for sHLA-G=44 U/ml. Sensitivity and specificity of sHLA-G for differentiating HCC from all controls were 96%. The AUC value of sHLA-G for detecting HCC from LC group was also as high as 0.992 and which was as well higher than that of AFP (AUC=0.622) and with cut-off value for sHLA-G=44 U/ml. Sensitivity and specificity of sHLA-G for differentiating HCC from LC were 96% too. These findings are similar and even better than a previous study done by Park et al. who reported that the diagnostic performance of sHLA-G was superior to that of AFP in diagnosing HCC [29]. However, Park et al. performed their analysis on HBV-related LC and HCC. Thus, there is etiological difference of the enrolled subjects [29]. Despite, HBV and HCV infections are the major risk factors for HCC and both are viral infections several studies have indicated that AFP values in HBV-related HCC differ from values of HCV-related HCC, which might be related to their different clinical manifestations and mechanisms of carcinogenesis [38-41].

To the best of our knowledge, there are no published articles that used the ROC curve to investigate the diagnostic performance of sHLA-G for HCV- induced HCC detection in comparison with AFP. There are several studies concerned about the diagnostic performance of various markers other than sHLA-G with AFP in both HBV and HCV-induced HCC. These markers comprising des-gamma-carboxy prothrombin (DCP), alpha-L-fucosidase (AFU), thymidine kinase (TK1), vascular endothelial growth factor (VEGF) plus many others [18,42-44]. Most of these studies reported an upper hand in the diagnostic value of these markers over AFP. According to our data, measuring the serum level of sHLA-G could be a valuable diagnostic tool for diagnosing HCV-related HCC as well as distinguishing it from HCV-related LC. Hence, studies with a large scale of subjects could be supportive for more exploration of the clinical value of sHLA-G. Most previous studies concerning about the association between HLA-G and HCC were executed on a small number of subjects. That is because HLA-G expression was mostly examined in HCC tissues.

AFP levels ≥ 200 ng/ml is considered diagnostic for HCC. However, an elevated percentage of HCC patients show serum AFP levels <200 ng/ml [12]. Division of Gastroenterology and Hepatology, St Louis University Liver Center, St Louis University School of Medicine, USA, Eldad S. Bialecki, Division of Gastroenterology and Hepatology, Saint Louis University Liver Center, Saint Louis University School of Medicine, USA. Corresponding author. In the current study, 50% of HCC patients were having AFP concentrations <200 ng/ml.

Consequently, we confined the HCC subjects to those having HCC plus AFP levels <200 ng/ml and those having HCC plus AFP levels ≥ 200 ng/ml. In the current study, the AUC of sHLA-G for distinguishing HCC with lower levels of AFP from all controls was 0.989 and was higher than that of AFP (AUC=0.622) with a cut-off value for sHLA-G=44 U/ml. Sensitivity and specificity of sHLA-G for differentiating this HCC subgroup from all controls were 92% and 96% respectively. Also, the AUC of sHLA-G for detecting HCC with lower levels of AFP from LC group was higher than that of AFP (AUC=0.984 and 0.243 respectively) and with cut-off value for sHLA-G=44 U/ml. Similarly, sensitivity and specificity of sHLA-G for differentiating this HCC subgroup from LC were 92% and 96% respectively. Again, sHLA-G showed better diagnostic performance for diagnosing HCC with AFP levels <200 ng/ml.

All over, the superiority of sHLA-G to AFP in differentiating between HCC and liver cirrhosis as well as in identifying HCC with AFP levels <200 ng/ml is obvious in our study. These findings are vital because AFP levels are increased in approximately 11–47% of subjects with liver cirrhosis and its false-negative results are reported in 30–40% of HCC patients [45]. Therefore, finding an improved diagnostic marker for HCC with enhanced diagnostic performance containing higher specificity as well as higher sensitivity commands a critical priority. To validate this marker, future studies on a larger sample size are required. Additionally, follow up studies of wide range of chronic HCV-induced liver diseases which will help to explore the exact nature of HLA-G roles in the progression as well as the survival rate of HCC. The relationship between sHLA-G and clinicopathologic staging of HCV-related HCC has not yet been extensively studied and should be focused in further researches. As well, it has been reported that HLA-G could be a marker of susceptibility to chemotherapy in ovarian cancer and a lot of other tumor cells [46,47]. Thus, more broad research on sHLA-G expression in HCV-induced HCC would be helpful to facilitate the use of sHLA-G as a prospective therapy for HCC plus monitoring chemotherapy.

Conclusion

sHLA-G was significantly increased in HCC patients but not in LC patients, while AFP was significantly increased in both HCC and LC patients. Having higher AUC than that of AFP, sHLA-G has better diagnostic performance with higher sensitivity and specificity than AFP. According to our data, sHLA-G could serve as a new efficient marker for early diagnosis of HCV-related HCC and to discriminate HCC from LC with the ability to identify HCC with low levels of AFP. Thus using sHLA-G could help to reduce both the false negative and positive rates of AFP. Moreover, sHLA-G could have a predictive value for malignant transformation in HCV-related LC patients either alone or with other tumor markers.

References

1. Ma L, Chua MS, Andrisani O, So S (2014) Epigenetics in hepatocellular carcinoma: an update and future therapy perspectives. World J Gastroenterol 20: 333-345.
2. Cui Z, Yu X, Guo L, Wei Y, Zheng Sh, et al. (2013) Combined Analysis of Serum Alpha-Fetoprotein and MAGE-A3-Specific Cytotoxic T Lymphocytes in Peripheral Blood for Diagnosis of Hepatocellular Carcinoma. Dis Markers 35: 915-923.
3. Jirun P, Zhang G, Kim HK, Ha SA, Zhongtian J, et al. (2011) Clinical utility of alpha fetoprotein and HCCR-, alone or in combination, in patients with chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Dis Markers 30: 307-315.
4. Kuromatsu R, Tanaka M, Tanikawa K (1993) Serum alpha-fetoprotein and lens culinaris agglutinin-reactive fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. Liver 13: 177-182.
5. Zhao YJ, Ju Q, Li GC (2013) Tumor markers for hepatocellular carcinoma. Mol Clin Oncol 1: 593-598.
6. Zekri AR, Hafez MM, Mohamed NI, Hassan ZK, El-Sayed MH, et al. (2007) Hepatitis B virus (HBV) genotypes in Egyptian pediatric cancer patients with acute and chronic active HBV infection. Virol J 4: 74.
7. González A, Rebmann V, LeMaoult J, Horn PA, Carosella ED, et al. (2012) The immunosuppressive molecule HLA-G and its clinical implications. Crit Rev Clin Lab Sci 49: 63-84.
8. Wang Y, Ye Z, Meng XQ, Zheng SS (2011) Expression of HLA-G in patients with hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int 10: 158-163.
9. Rouas-Freiss N, Marchal RE, Kirszenbaum M, Dausset J, Carosella ED (1997) The alpha1 domain of HLA-G1 and HLA-G2 inhibits cytotoxicity induced by natural killer cells: is HLA-G the public ligand for natural killer cell inhibitory receptors? Proc Natl Acad Sci U S A 94: 5249-5254.
10. Bruix J, Sherman M; American Association for the Study of Liver Diseases (2011) Management of hepatocellular carcinoma: an update. Hepatology 53: 1020-1022.
11. Bruix J, Sherman M (2005) Management of hepatocellular carcinoma. Hepatology 42: 1208-1236.
12. Arrieta O, Cacho B, Morales-Espinosa D, Ruelas-Villavicencio A, Flores-Estrada D, et al. (2007) The progressive elevation of alpha fetoprotein for the diagnosis of hepatocellular carcinoma in patients with liver cirrhosis. BMC Cancer 7: 28-36.
13. Schuppan D, Afdhal NH (2008) Liver cirrhosis. Lancet 371: 838-851.
14. Tornesello ML, Buonaguro L, Tatangelo F, Botti G, Izzo F, et al. (2013) Mutations in TP53, CTNNB1 and PIK3CA genes in hepatocellular carcinoma associated with hepatitis B and hepatitis C virus infections. Genomics 102: 74-83.
15. Behnke MK, Reimers M, Fisher R (2012) The expression of embryonic liver development genes in hepatitis C induced cirrhosis and hepatocellular carcinoma. Cancers; 4: 945-968.
16. Cheng CH, Yu MC, Wu TH, Lee CF, Chan KM, et al. (2012) Surgical resection of centrally located large hepatocellular carcinoma. Chang Gung Med J; 35: 178-191.
17. Sari R, Yildirim B, Sevinc A, Bahceci F, Hilmioglu F (2001) The importance of serum and ascitic fluid alpha-fetoprotein, carcinoembryonic antigen, CA 19-9, and CA 15-3 levels in differential diagnosis of ascites etiology. Hepatogastroenterology; 48: 1616-1621.
18. Mukozu T, Nagai H, Matsui D, Kanekawa T, Sumino Y (2013) Serum VEGF as a tumor marker in patients with HCV-related liver cirrhosis and hepatocellular carcinoma. Anticancer Res 33: 1013-1021.
19. Sterling RK, Wright EC, Morgan TR, Seeff LB, Hoefs JC, et al. (2012) Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. Am J Gastroenterol 107: 64-74.
20. Merani S, Majno P, Kneteman NM, Berney T, Morel P, et al. (2011) The impact of waiting list alpha-fetoprotein changes on the outcome of liver transplant for hepatocellular carcinoma. J Hepatol 55: 814-819.
21. Lai Q, Merli M, Ginanni Corradini S, Mennini G, Gentili F, et al. (2009) Predictive factors of recurrence of hepatocellular carcinoma after liver transplantation: a multivariate analysis. Transplant Proc 41: 1306-1309.
22. Daniele B, Bencivenga A, Megna AS, Tinessa V (2004) Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma. Gastroenterology 127: S108-112.
23. Singer G, Rebmann V, Chen YC, Liu HT, Ali SZ, et al. (2003) HLA-G is a potential tumor marker in malignant ascites. Clin Cancer Res 9: 4460-4464.
24. Yamashita T, Forgues M, Wang W, Kim JW, Ye Q, et al. (2008) EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. Cancer Res 68: 1451-1461.
25. De Oliveira Crispim JC, Silva TG (2012) Upregulation of soluble and membrane-bound human leukocyte antigen G expression is primarily

observed in the milder histopathological stages of chronic hepatitis C virus infection. Hum Immunol 73: 258-262.

26. Contini P, Ghio M, Merlo A, Poggi A, Indiveri F, et al. (2005) Apoptosis of antigen-specific T lymphocytes upon the engagement of CD8 by soluble HLA class I molecules is Fas ligand/Fas mediated: Evidence for the involvement of p56lck, calcium calmodulin kinase II, and Calcium-independent protein kinase C signaling pathways and for NF-kappaB and NF-AT nuclear translocation. J Immunol 175: 7244-7254.
27. Rouas-Freiss N, Moreau P, LeMaoult J, Carosella ED (2014) The dual role of HLA-G in cancer. J Immunol Res 2014: 359748.
28. Lin A, Chen HX, Zhu CC, Zhang X, Xu HH, et al. (2010) Aberrant human leucocyte antigen-G expression and its clinical relevance in hepatocellular carcinoma. J Cell Mol Med 14: 2162-2171.
29. Park Y, Park Y, Lim HS, Kim YS, Hong DJ, et al. (2012) Soluble human leukocyte antigen-G expression in hepatitis B virus infection and hepatocellular carcinoma. Tissue Antigens 79: 97-103.
30. Rebmann V, Regel J, Stolke D, Grosse-Wilde H (2003) Secretion of sHLA-G molecules in malignancies. Semin Cancer Biol 13: 371-377.
31. Singer G, Rebmann V, Chen YC, Liu HT, Ali SZ, et al. (2003) HLA-G is a potential tumor marker in malignant ascites. Clin Cancer Res 9: 4460-4464.
32. Liu Z, Ling K, Wu X, Cao J, Liu B, et al. (2009) Reduced expression of cenp-e in human hepatocellular carcinoma. J Exp Clin Cancer Res 28: 156.
33. Shi WW, Lin A, Xu DP, Bao WG, Zhang JG, et al. (2011) Plasma soluble human leukocyte antigen-G expression is a potential clinical biomarker in patients with hepatitis B virus infection. Hum Immunol 72: 1068-1073.
34. Dias FC, Castelli EC, Collares CV, Moreau P, Donadi EA (2015) The Role of HLA-G Molecule and HLA-G Gene Polymorphisms in Tumors, Viral Hepatitis, and Parasitic Diseases. Front Immunol 6: 9.
35. Ye SR, Yang H, Li K, Dong DD, Lin XM, et al. (2007) Human leukocyte antigen G expression: as a significant prognostic indicator for patients with colorectal cancer. Mod Pathol 20: 375-383.
36. Barrier BF, Kendall BS, Sharpe-Timms KL, Kost ER (2006) Characterization of human leukocyte antigen-G (HLA-G) expression in endometrial adenocarcinoma. Gynecol Oncol 103: 25-30.
37. Wiendl H, Mitsdoerffer M, Hofmeister V, Wischhusen J, Bornemann A, et al. (2002) A functional role of HLA-G expression in human gliomas: an alternative strategy of immune escape. J Immunol 168: 4772-4780.
38. Cantarini MC, Trevisani F, Morselli-Labate AM, Rapaccini G, Farinati F, et al. (2006) Effect of the etiology of viral cirrhosis on the survival of patients with hepatocellular carcinoma. Am J Gastroenterol 101: 91-98.
39. Anzola M (2004) Hepatocellular carcinoma: role of hepatitis B and hepatitis C viruses proteins in hepatocarcinogenesis. J Viral Hepat 11: 383-393.
40. Wands JR (2004) Prevention of hepatocellular carcinoma. N Engl J Med 351: 1567-1570.
41. Tanabe G, Nuruki K, Baba Y, Imamura Y, Miyazono N, et al. (1999) A comparison of hepatocellular carcinoma associated with HBV or HCV infection. Hepatogastroenterology 46: 2442-2446.
42. Ji J, Wang H, Li Y, Zheng L, Yin Y, et al. (2016) Diagnostic Evaluation of Des-Gamma-Carboxy Prothrombin versus a-Fetoprotein for Hepatitis B Virus-Related Hepatocellular Carcinoma in China: A Large-Scale, Multicentre Study. PLoS ONE 11: e0153227.
43. Zhang SY, Lin BD, Li BR (2015) Evaluation of the diagnostic value of alpha-l-fucosidase, alpha-fetoprotein and thymidine kinase 1 with ROC and logistic regression for hepatocellular carcinoma. FEBS Open Bio 5: 240-244.
44. Song P, Feng X, Inagaki Y, Song T, Zhang K, et al. (2014) Clinical utility of simultaneous measurement of alpha-fetoprotein and des-?-carboxy prothrombin for diagnosis of patients with hepatocellular carcinoma in China: A multi-center case-controlled study of 1,153 subjects. Biosci Trends 8: 266-273.
45. Farinati F, Marino D, De Giorgio M, Baldan A, Cantarini M, et al. (2006) Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? Am J Gastroenterol 101: 524-532.
46. Giuseppe Curigliano, Carmen Criscitiello, Lucia Gelao, Aron Goldhirsch (2013) Molecular Pathways: Human Leukocyte Antigen G (HLA-G). Clin Cancer Res 19: 5564-5571.
47. Davidson B, Elstrand MB, McMaster MT, Berner A, Kurman RJ, et al. (2005) HLA-G expression in effusions is a possible marker of tumor susceptibility to chemotherapy in ovarian carcinoma. Gynecol Oncol 96: 42-47.