Studying the Value of Golgi Protein 73 as a Serum Marker in Hepatocellular Carcinoma Versus Alfa Feto Protein

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Abstract:- Hepatocellular carcinoma (HCC) is the fifth most common cancer. Alpha fetoprotein (AFP) still represents the currently used test for HCC even though its sensitivity of (39 to 65%) is not very satisfactory and there is a high rate of false-negative and false positive results**.** Golgi protein 73 (GP73) is 73 kDa transmembrane glycoprotein that normally resides within the cis-Golgi complex. GP73 was observed in several malignances such as prostate cancer and renal cell cancer. However, knowledge concerning GP73 function and the mechanisms of regulation in normal and neoplastic tissues is still under research. Aim of the study was to determined value of serum Gp73 in diagnosis of HCC in high risk patient (cirrhotic patient). This study was conducted on 80 patients; 50 patients with HCC as **Group A**, 30 cirrhotic patients without HCC as **Group B** and 15 healthy persons as **Group C**. We determined the level of AFP and Gp73 for all cases together with full clinical assessment, liver biochemical profile, viral markers, conventional US, triphasic abdominal CT scan. Serum AFP was elevated in HCC patients (Median= 207.74 ng/ml) when compared with both controls (7.14 ng/ml) and cirrhotics (94.91 ng/ml) groups. Gp73 level was also significantly elevated in the HCC group (Median=14.52 ng/ml) when compared with both the control (1.24 ng/ml) and chronic liver disease (4.70 ng/ml) groups. Positive correlation was found in this study between serum Gp73and serum AFP in HCC group (r=0.35; p=0.01). The cutoff level of Gp73 for diagnosis of HCC in this study was 6.60 ng/ml, with a sensitivity of 92% and specificity of 87%. The combined use of the two markers (AFP and Gp73) led to an increase in the sensitivity, specificity and diagnostic accuracy of AFP from 74%, 73% and 76%, respectively to 81 %, 81.5 % and 74 %. So, Gp73 in a combination with AFP is highly recommended for accurate diagnosis of HCC.

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1. Introduction:-

Hepatocellular carcinoma (HCC) is a major health problem worldwide. It is the fifth most common cancer and the third leading cause of cancer-related death **(Lau and Lai, 2008)**.

Patients with hepatitis B and C related liver cirrhosis are at high risk of developing HCC. The prognosis of patients with HCC is poor when diagnosed at an advanced stage, but when diagnosed and treated at early stage the 5-year survival rate may reach up to 70-80% **(Masatoshi, 2010)**. Therefore, early detection of HCC is a critical goal to improve the patient outcome.

Histo-pathological examination of tumor biopsy is considered the golden standard for diagnosis of HCC. However, it is considered an invasive technique with high risk of seeding the tumor along the biopsy tract **(Masatoshi, 2010).**

As regards serologic screening, alpha fetoprotein (AFP) still represents the currently used test for HCC even though its sensitivity of (39 to 65%) is not very satisfactory and there is a high rate of false-negative and false positive results **(Wei et al., 2006 and Shariff et al., 2009)**. Hence, there is an urgent need for more reliable noninvasive recent biomarkers with better sensitivity and specificity for early diagnosis of HCC.

Golgi protein 73 (GP73) also named Golgi phosphoprotein 2 (GOLPH2) is 73 kDa transmembrane glycoprotein that normally resides within the cis-Golgi complex. GP73 was first identified in normal hepatocyte and biliary epithelial cells **(Iftikhar et al., 2004 and Marrero et al., 2005)**. Given its intracellular localization as a resident Golgi protein, GP73 was also found to be expressed in extrahepatic tissues as the prostate, gut, breast, thyroid, and within the central nervous system. Despite its steady-state localization within the cis-Golgi complex, a slightly smaller form of GP73 in the serum is generated by N-terminal cleavage of the molecule by the proprotein convertase furin **(Bachert et al., 2007 and Li et al., 2008)**.

Some studies demonstrated significant elevation of serum level of GP73 in diverse viral and non-viral

liver diseases, including hepatitis and cirrhosis **(Fimmel and Wright, 2009)**. Moreover, up-regulated GP73 was observed in several malignances such as prostate cancer and renal cell cancer. However, knowledge concerning GP73 function and the mechanisms of regulation in normal and neoplastic tissues is still under research **(Zhou et al., 2011)**.

2. Patients and methods

This study was conducted at Al-Hussein university hospital during period from january 2015 to january 2016, on 95 patients and healthy persons, who were selected to fulfill the study groups:

Group A (HCC group) included 50 patients with HCC. HCC was diagnosed according to ***Bruix et al., (2001).*** **Group B** (cirrhotic liver disease group) included 30 patients with cirrhotic liver disease without any evidence of hepatic focal lesions as excluded by ultrasonography and AFP estimation. Diagnosis of chronic liver disease was based on ultrasonographic criteria. **Group C** included 15 normal subjects who served as the control group.

All patients and controls signed an informed written consent after explanation of the aim of the study and the procedure.

Exclusion criteria:

1. Patients who refused to participate in the study.
2. Inflammatory or septic condition as spontaneous bacterial peritonitis after positive ascetic sample.
3. Carcinoma elsewhere.
4. Pregnancy.

All patients and controls were subjected to full history taking and full clinical examination, laboratory investigations as complete blood count, liver and kidney function tests, also, we determined the level of AFP and Gp73, imaging studies as abdominal ultrasound or tri-phasics CT-scan when needed.

3. Results:-

Serum AFP was elevated in HCC patients when compared with both controls and cirrhotics. (table 1).

Table (1): AFP serum level within the studied groups.

AFP (ng/ml):	Studied groups			Kruskal Wallis test	P value	
	Gp-I (n=50)	GpII (n=30)	GpIII (n=15)			
Mean±SD Range	207.74±99.81 30.00–470.00	94.91±49.04 30.00–276.00	7.19±4.57 2.00–21.20	45.74	<0.001 HS	P1<0.001 P2<0.001 P3<0.001

P1: comparison between group of HCC patients and group of cirrhotic patients.
P2: comparison between group of HCC patients and control group.
P3: comparison between group of cirrhotic patients and control group.

Serum level of Gp73 was also significantly elevated in the HCC group when compared with both the controls and cirrhotics. (Table 2).

The cutoff level of Gp73 for diagnosis of HCC in this study was 6.60 ng/ml, with a sensitivity of 92% and specificity of 87 %. (Table 3)

Table (2): Serum Golgi protein 73 within the studied groups.

Serum GP73 (ng/ml):	Studied groups			Kruskal Wallis test	P value	
	GpI(n=50)	GpII (n=30)	GpIII (n=15)			
Mean±SD Range	14.52±6.57 0.50–20.00	4.70±4.08 0.30–16.00	1.24±1.20 0.30–3.80	52.21	<0.001 HS	P1<0.001 HS P2<0.001 HS P3=0.002 S

P1: comparison between group of HCC patients and group of cirrhotic patients.
P2: comparison between group of HCC patients and control group.
P3: comparison between group of cirrhotic patients and control group.
Positive correlation was found in this study between serum Gp73 and serum AFP in HCC group (r=0.35; p=0.01).

Table (3): Diagnostic validity of serum GP73 (ng/mL) in diagnosis of HCC.

Optimal cutoff point	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Diagnostic accuracy (95%CI)	DOR (95%CI)
6.60	92% (80–97)	87% (73–94)	88% (76–95)	91% (77–97)	89% (81–95)	4.75 (3.38–19.67)

95% CI= 95% confidence interval; PPV=Positive predictive value; NPV= Negative predictive value
DOR= diagnostic odds ratio

The combined use of the two markers (AFP and Gp73) led to an increase in the sensitivity, specificity and diagnostic accuracy of AFP from 74%, 73% and 76%, respectively to 81 %, 81.5 % and 74 %.

Discussion:-

HCC is the most common primary malignant tumor of the liver and represents approximately 85-90% of primary malignant tumors of the liver ***(El Serag and Rudolph, 2007).*** In Egypt, HCC is now a rather common malignancy; both HCV and HBV infections increased the risk of HCC in Egyptian patients ***(Badawy and Micheal, 1991).***

The feasibility of early detection associated with the availability of several effective therapies has permitted encouraging long term survival after diagnosis and as a result, the interest in all aspects related to diagnosis has sharply increased ***(L1overt and Beaugrand, 2003).***

Currently, Ultrasonography (US) is the technique of choice for screening focal hepatic lesions ***(Bruix et al., 2006)***. Any mass detected on US in a cirrhotic liver is suspicious of HCC, particularly if it is > 1 cm in size. As a screening test, US has a sensitivity of 65%-80% and has a specificity of > 90% ***(Bruix et al., 2001)***.

Hepatic Computed Tomography (CT) combines both high sensitivity for focal lesions and high specificity regarding the nature of the lesion ***(Bruix et al., 2001)***.

Alpha fetoprotein (AFP) is the most commonly used tumor marker in early HCC screening in populations at high risk. Ultimately, no single diagnostic modality yields diagnostic accuracy consistently over 50% to 60% in detecting lesions less than 1 cm, a time when curative surgery is most likely. Therefore, screening with AFP, ultrasonography and judicious use of CT provide the best hope for early diagnosis, thus there is an urgent need for new biomarker for detection early HCC **(*Marreo et al., 2005)*.**

Golgi protein 73 (GP73) is belevied to be a new serum marker for liver disease. Sub sequent study revealed minimal Gp73 expression in normal hepatocytes but marked expression in patients chronic hepatitis and liver cirrhosis ***(Iftikhar et al., 2004)***. a circulating form of Gp73 is found in the serum of patients with HCC. ***(Block et al., 2005)***. These data indicate that serum Gp73 is a promising diagnostic serum marker for liver cancer ***(Marrero et al., 2005)***.

In this study some patients of HCC have medical history of Bihariziasis 27 % only and this agreement with ***(Gomaa et al., 2009).***

Regarding the clinical features of the studied patients; hepatomegaly, splenomegaly, lower limb oedema, ascites, pallor, cachexia and jaundice were the main manifestations in both chronic liver disease and HCC groups, with no significant differences in between.

This finding is in agreement of that of **Jakate et al., (2010)** who found that, risk of hepatocellular carcinoma among persons with chronic HBV infection is further increased if they are male or elderly, have been infected for a long time, have a family history of hepatocellular carcinoma.

In this study, we found that there was a statistically highly significant elevation (**p< 0.001**) in the median serum AFP in HCC group (207.74 ng/ml**)** when compared with the healthy group (7.19 ng/ml), this is in agreement with ***Motawa et al., (2001).***

Also, there was astatically difference when compared median serum AFP in HCC group (207.74 ng/ml**)** with median serum AFP in cirrhotic group (94.91 ng/ml**).**

This result is in agreement with ***Li et al., (2008).*** But, in disagreement with ***Sherman, (2001)*** who reported that AFP may be normal in some patients of HCC, this disagreement could be explained as serum AFP is associated with two main problems; first, the transient high rise in the serum level of AFP during exacerbation of hepatitis on top of chronic liver disease patients (serum level >100 ng/ml) and slight rise in the serum AFP in chronic hepatitis and cirrhosis **(**serum level >200 ng/dl**)** causing diagnostic difficulties *(low specificity).* The second is that among all patients diagnosed with HCC, AFP levels may be normal in up to 40% of patients, particularly during the early stages *(low sensitivity)*.

In this study we found that, there is a very high significant elevation in serum GP73 level in HCC group (14.52 ng/ml) when compared with the cirrhotic group (4.70 ng/ml) and control group (1.24 ng/ml).

These results are in agreement with those of ***Mao et al., (2010), Morota et al.,(2011) and Tian et al., (2011)*** who report that serum Gp73 is significantly elevated in HCC patients more than both patients with cirrhosis and normal persons, so this statically significant difference implies the diagnostic role of Gp73 in detection of HCC in cirrhotic patients.

On using the Receiver Operating Characteristic (ROC) curve to reach the value of the best sensitivity and specificity of Gp73 at cut of value of > 6 ng/ml as 92% and 87% respectively, with diagnostic accuracy of 89%**.**

These findings come in agreement with those of ***Mao et al., (2010)*** who reported that, Gp73 sensitivity and specificity at cut of value 8.5 ng/ml, are 75% and 97% respectively.

Marrero et al., (2005) reported that Gp73

sensitivity is 70 % at cut of value 10 ng/ml.

Our results showed that the combined use of the two markers (AFP & Gp73) leads to an increase in the sensitivity, specificity and diagnostic accuracy to diagnose early HCC (81 %, 81.5 % and 74%). These results agree with those of ***Wang et al (2009) and Mao et al.,(2010)***.

Conclusion and recommendations:

The serum level of Gp73 is significantly higher in patients with cirrhosis and HCC, and it has a higher sensitivity, specificity and diagnostic accuracy for prediction of HCC than AFP. Further research is needed to determine the accuracy of Gp73 as a cure marker.

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