*Research Article*

# Study of serum glucoregulatory enzymes as biomarker in patients with hepatocellular carcinoma

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**Abstract**
**Introduction:** Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide (Llove , 2005). It is a major health problem worldwide, HCC develops most often in cirrhotic liver and this condition is the strongest predisposing factor (Llovet et al., 2002& El-Serag et al., 2007). Many trials are carried out all over the world to implement specific early markers for detection and prediction of HCC, hoping to set a more precise strategy for liver cancer prevention. cancer cells possess an abnormal pattern of energy metabolism when compared to normal cells (Brown et al.,1993). During the progression of tumours, the genetic and epigenetic alterations accumulate and the rapidly growing tumours need to overcome hypoxia and nutrient deprivation owing to the inadequate blood supply, leading to changes in the energy demands of cancer cells (Wang et al, 1995 Guillemin , 1997). Glucose is the primary energy source and high rate of glycolysis is one of the earliest discovered hallmarks of cancer, it provides the tumour with metabolic and survival advantages (Beckner et al., 1990, Greiner et al., 1994). Aim Of The Work: "The present study aims to investigate serum levels of glucokinase,glucose-6-phosphate dehydrogenase and glucose-6-phosphatase to demonstrate their role in the pathogenesis of HCC and evaluating their possible application in serving as recent serum markers in diagnosis and for easy monitoring of HCC". **Patients and Methods:** This study is across-sectional prospective case control hospital based study was conducted in Internal Medicine department and outpatient clinic Minia University Hospital From March 2016 to October 2016. A total 100 subjects were recruited from inpatient and outpatient clinic and divided in to 3 groups: Group I:- It included 45 patients HCV induced cirrhosis with HCC. Diagnosis of liver cirrhosis is based on abdominal ultrasound & laboratory data (Dufour et al., 2000). HCC was diagnosed by abdominal ultrasound as screening and confirmed by triphasic abdominal C.T, The Diagnosis of HCC was based on characteristic criteria of the national guidelines for HCC, where, imaging data show either one of the following three cases: (1) one or more liver masses 2 cm in diameter via both CT and MRI, (2) one imaging data with early enhancement and a high level of AFP 400 ng/ ml, and (3) one imaging data with early arterial phase contrast enhancement plus early venous phase contrast washout regardless of AFP level (Attwa, 2015). Diagnosis of HCV infection was based on positivity of anti-HCV and detection of viremia by PCR. All patients were seronegative for HBsAg, and none consumed alcohol. Group II: It included 30 patients who had chronic HCV induced cirrhosis without HCC, diagnosis of chronic hepatitis (CHC) was based on detection of HCV antibodies by ELISA and confirmed by detection HCV RNA by PCR ≥6 months (Pham et al., 2010) whenever available . diagnosis of LC based on clinical , laboratory and ultrasonographic data ( Iacobellis). **Group III:-** It Included 30 healthy persons with normal physical examination , laboratory findings and abdominal ultrasonography. They were negative for HCV antibodies. **Results: Our study revealed that;** patients with HCC had higher blood urea and α-fetoprotein than healthy control. CHC related liver cirrhosis complicated with HCC patients had higher level of serum ALT and AST compared to healthy control. HCC patients had higher percentage of portal vein thrombosis and α-fetoprotein & INR than cirrhotic patients without HCC. There is a significance increase in serum G6PD level among HCC patients more than cirrhotic patients and healthy control. There is increase in the serum level of glucose 6 phosphatase than both cirrhotic patients and healthy group.

There is decrease in glucokinase serum level in both HCC and cirrhotic patients than healthy control.

**Kay words: AASLD:** American Association for the Study of Liver Diseases, **AcCoA:** Acetyl-CoA, **ADP:** Adenosine diphosphate

## Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide [(1)] that typically occurs on top of cirrhosis and chronic hepatitis virus infections. HCC develops most often in cirrhotic liver and this condition is the strongest predisposing factor [(2)].

Many trials are carried out all over the world to implement specific early markers for detection and prediction of this disease, hoping to set a more precise strategy for liver cancer prevention. cancer cells possess an abnormal pattern of energy metabolism when compared to normal cells[(3)]. During the progression of tumours, the genetic and epigenetic alterations accumulate and the rapidly growing tumours need to overcome hypoxia and nutrient deprivation owing to the inadequate blood supply, leading to changes in the energy demands of cancer cells[(4)]. Glucose is the primary energy source and high rate of glycolysis is one of the earliest discovered hallmarks of cancer, it provides the tumour with metabolic and survival advantages[(5)]

HCC receives blood from the hepatic artery and hypoxia forces the cancer cells to make a metabolic switch to ensure the energy sources[(6)]**.** The crucial role of glucose utilization to HCC growth was confirmed by HCC cell lines that undergone acute cell death upon glucose starvation. Further study demonstrated an increased glycolysis and a decreased glycogenesis in liver cancer**.** Several key genetic alterations associated with tumor development were recently shown to affect glycolysis directly, such as p53 mutation and the activation of hypoxia inducible factor (HIF)[(6)].

Elevated glucose catabolism is important for the production of energy and required anabolic precursors in rapidly growing tumour. It was established that abnormalities of glucose metabolizing enzymes in the transformation of normal livers is referred to high glucose utilization in hepatoma cells.[(7)]

Tumour cells often metabolize glucose to lactate even in the "presence" of oxygen which is called aerobic glycolysis. This is unusual and stands in contrast to normal cells that only metabolize glucose to lactate in the "absence" of oxygen [(6)] .
Since a definite correlation exists between tumour progression and the activities of glycolytic enzymes. [(9)]

## Aim of the Work

"The present study aims to investigate serum levels of glucokinase,glucose-6-phosphate dehydrogenase and glucose-6-phosphatase to demonstrate their role in the pathogenesis of HCC and evaluating their possible application in serving as recent serum markers in diagnosis and for easy monitoring of HCC ".

## Patients and Methods

This study is across-sectional prospective case control hospital based study was conducted in Internal Medicine department and outpatient clinic Minia University Hospital From March 2016 to October 2016. A total 100 subjects were recruited from inpatient and outpatient clinic and divided in to 3 groups:
Group I:- It included 45 patients HCV induced cirrhosis with HCC (35 men and 10 women), their ages ranged from 45 to 74 years old. Diagnosis of liver cirrhosis is based on abdominal ultrasound & laboratory data (Dufour et al., 2000). HCC was diagnosed by abdominal ultrasound as screening and confirmed by triphasic abdominal C.T, The Diagnosis of HCC was based on characteristic criteria of the national guidelines for HCC, where, imaging data show either one of the following three cases: (1) one or more liver masses 2 cm in diameter via both CT and MRI, (2) one imaging data with early enhancement and a high level of AFP 400 ng/ ml, and (3) one imaging data with early arterial phase contrast enhancement plus

early venous phase contrast washout regardless of AFP level (Attwa, 2015). Diagnosis of HCV infection was based on positivity of anti-HCV and detection of viremia by PCR. All patients were seronegative for HBsAg, and none consumed alcohol.

**Group II:** It included 30 patients who had chronic HCV induced cirrhosis without HCC, They were 12 men and 18 women, their ages ranged from 45 to 69 years old. Diagnosis of chronic hepatitis (CHC) was based on detection of HCV antibodies by ELISA and confirmed by detection HCV RNA by PCR ≥6 months (Pham et al., 2010) whenever available. diagnosis of LC based on clinical, laboratory and ultra-sonographic data ( Iacobellis).

**Group III:-** It Included 30 healthy persons with normal physical examination , They were 10 men and 20 women, their ages ranged from 45 to 66 years old. laboratory findings and abdominal ultrasonography. They were negative for HCV antibodies.

## Results

**Table (1) variables in age ,gender ,ascites between the study group**

| | Group I HCC (n=45) | Group II LC (n=30) | Group III Control (n=30) | P value | | |
|---|---|---|---|---|---|---|
| **Age** | | | | **0.005*** | | |
| Range | (45-74) | (48-69) | (45-66) | **I vs II** | **I vs III** | **II vs III** |
| Mean ± SD | 59.6±7.1 | 59.4±5.9 | 54.7±7.2 | 0.994 | **0.007*** | **0.021*** |
| **Sex** | | | | **<0.001*** | | |
| Male | 35(77.8%) | 12(40%) | 10(33.3%) | **I vs II** | **I vs III** | **II vs III** |
| Female | 10(22.2%) | 18(60%) | 20(66.7%) | **0.001*** | **<0.001*** | 0.592 |
| **Ascites** | | | | **<0.001*** | | |
| No | 13(28.9%) | 11(36.7%) | 30(100%) | **I vs II** | **I vs III** | **II vs III** |
| Yes | 32(71.1%) | 19(63.3%) | 0(0%) | 0.479 | **<0.001*** | **<0.001*** |

**Table (2): Correlation between AFP and G-6-PD, Glucose-6-Phosphatase and Glukokinase**

| Variables | AFP | |
|---|---|---|
| | R | P-value |
| **G-6-PD** | -0.313 | 0.036* |
| **GLUCOSE-6-PHOSPHATASE** | -0.341 | 0.022* |
| **GLUKOKINASE** | 0.216 | 0.154 |

## Discussion

Hepatitis C virus (HCV) infection is a major worldwide epidemic disease. It is estimated that more than 170 million individuals are infected with HCV (plat et al., 2016). The vast majority of HCV infections persist, with up to 80% of all cases leading to chronic hepatitis associated with liver fibrosis, cirrhosis, and hepato-cellular carcinoma (Quarleri & Oubiña., 2016). In Egypt, HCC contributes to 14.8% of all cancer mortality (Bertuccio et al., 2017). This high incidence is attributed to the high prevalence of Hepatitis C virus (HCV) infection (Razavi et al., 2014).

Cancer cells are characterized by an abnormal pattern of energy metabolism that is manifested by an increase in glucose, fatty acid and amino acid metabolism and a decrease in oxidative phosphorylation (Haussinger et al., 2007) Glucose is the primary energy source, and a high rate of glycolysis, which is one of the earliest discovered hallmarks of cancer cells, provides the tumor with metabolic and survival advantages (Beckner et al., 1990,

Greiner et al., 1994), Anomalies of the glycolytic pathway are well-known biochemical disturbances in hepatomas (Permalatha et al, 1997; Sivanesan et al., 2007).

Early studies established the presence of abnormalities in the glucose-metabolizing enzymes with the transfor-mation of normal liver cells into high glucose-utilizing hepatoma cell lines (Parimala, 1993). The high rate of aerobic glycolysis exhibited by some cancer cells is called the Warburg effect, in recognition of Otto Warburg's discovery some 80 years ago, Warburg championed the notion that aerobic glycolysis is necessary during carcinogenesis (Harris et al., 2004. Xu RH et al., 2005 & Zhivotovsky et al, 2009).

A definite correlation was reported between tumor progression and the activities of the glycolytic enzymes (Vander Heiden et al., 2017)

## Conclusion & Recommendation

This study indicated activation of a group of glucoregulatory enzymes and suppression of another group in the direction of getting advantage for carcinogenesis. Our data presented significantly higher serum levels of G-6-PD and hexokinase enzymes in cirrhotic patients with HCC compared to cirrhotic patients without HCC. On the opposite side our results indicated significantly lower serum levels of glucose-6-phosphatase enzyme in patients having HCC than those without HCC. Whether these findings may offer a diagnostic privilege to predict HCC, this may need larger sample size than ours.

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