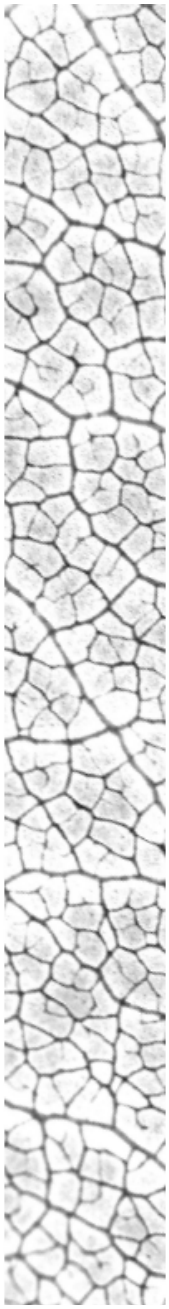

EurAsian Journal of BioSciences
Eurasia J Biosci 13, 633-638 (2019)

Environmental health investigation of the partial purification and study of Guanase in sera of hepatitis B and C patients

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Abstract
Viral hepatitis is one of the most common type of diseases in the world, it is associated with high risk of cirrhosis and liver cancer, the objective of this study is to evaluate the efficacy of enzyme guanase and purifying enzyme in patients with hepatitis B and C virus. The study included 63 hepatitis B and C patients, it also included 28 people who had no infection and a similar age for patients where it was considered a control group, the age group ranged from 25-55 years for each group of patients and control, measure the level of guanase enzyme in both the patients and control group, the study also included purification of the enzyme from hepatitis B and C patients using ammonium sulphate, ion exchange technology and gel filtering technology, using DEAE-Cellulose and Sepharose-6B, samples were collected between April 2018 and July 2018 at Kirkuk general hospital. The results showed that there was significant increase (P 0.05) in the level of guanase activity in hepatitis B patient compared with control group and a highly significant increase (P 0.05) in the level of guanase activity in hepatitis C patient compared with control group. There was no difference in guanase activity in serum of HBV and HCV both in male and female the present study included partial purification of guanase from serum of hepatitis B and C patients using salt precipitation, ion exchange chromatography and gel filtration by using DEAE- Cellulose and sepharose-6B,the purification degree of HBV and HCV by ammonium sulfate precipitation was(4.358) fold, while it was by Ion exchange (8.939) fold and by gel filtration (39.446) fold, while enzyme yield was (64.084%,50.5%, 41.535%) respectively, and specific activity was (226.179,463.914, 8921,99) pg/ml, also this study included the determination of molecular weight of the purified guanase in serum of HBV and HCV by using standard plot of proteins, the results showed that molecular weight was (71.131KD), the study concluded that guanase can be considered as a predictive function in diagnosis and follow-up of viral hepatitis before and after infection.

Keywords: Guanase, hepatitis, molecular weight of enzyme

Rasheed MN, Ali SJ (2019) Environmental health investigation of the partial purification and study of Guanase in sera of hepatitis B and C patients. Eurasia J Biosci 13: 633-638.

INTRODUCTION

Viral hepatitis is a common disease, which may be serious or small and occurs as a result of liver injury or inflammation, viral infection is the main cause of infection and less as a result of bacterial infection and others, when the liver gets infected, its cells are able to regenerate themselves quickly and restore their cells to normal to perform their functions in cases where the disease develops in patients with chronic hepatitis, liver cirrhosis or liver scarring may lead to liver failure or liver cancer, these diseases lead to the deaths of approximately 1-2 million people annually around the world (Essam et al. 2012). Hepatitis B and C viruses are responsible for 78% of liver cancer cases and 57% of cases of liver cirrhosis, where 60% of cirrhosis and 80% of liver cancer are due to Hepatitis B and C virus infection respectively, studies show that hepatitis B virus causes 1.4 million deaths each year, it is estimated that around 248 million people worldwide live with chronic hepatitis B virus infection, and that 110 million people have antibodies to the virus and hepatitis C and 80 million people infected with chronic hepatitis C. (Abubakar et al. 2015, Gower et al. 2014, Schweitzer et al. 2015).

Guanine deaminase (EC 3.5.4.3, Guanase, or GDA), is an enzyme that hydrolyzes guanine to form xanthine that is unsuitable for DNA/RNA buildup (Lewis and Glantz 1974) this enzyme has been found in normal or transformed human organs and sera (Knights et al. 1965). Even though no direct cause and effect relationship has been established between increased guanase activity and post-transfusional hepatitis (PTH),

Received: November 2018
Accepted: April 2019
Printed: May 2019

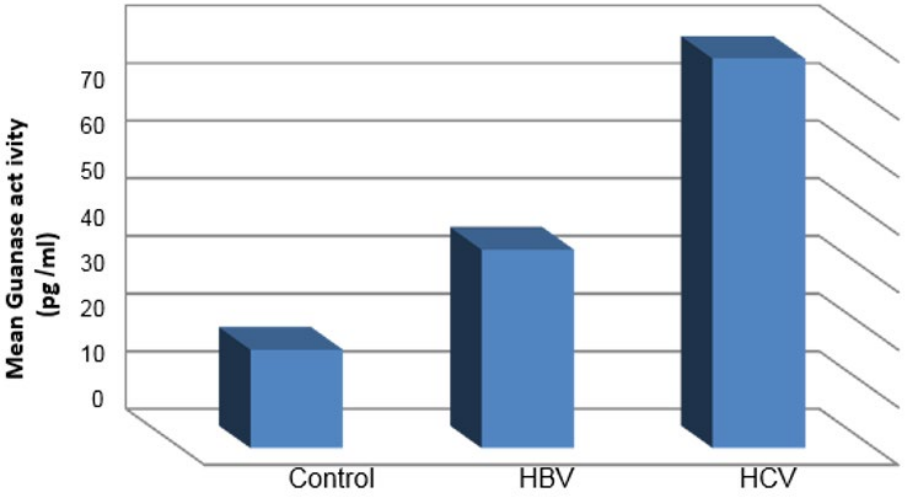

Fig. 1. Serum guanase enzyme level in patients with hepatitis B and C

it is reasonable to assume that the guanase level is responsible, in part, for the development of PTH. There are only a few and scattered reports on the specific physiological or metabolic role of guanase. Nonetheless, there are important observations made on this enzyme as reported in the literature. For example, it is known that high serum guanase activity is a clear biochemical indicator of organ rejection in liver transplant patients (Crary et al. 1989). It has also been shown that patients with multiple sclerosis (MS) have significantly elevated levels of guanase activity in their cerebral spinal fluid (CSF), and that there is a clear correlation between the extent of disability and the level of guanase activity (Chakraborty et al. 2011). In this regard, it is important to consider reports of abnormal levels of guanase activity in various cancer tissues. An increase in guanase activity has been reported in lung (Greengard et al. 1980), gastric (Durak et al. 1994), kidney (Durak et al. 1997, Paletzki 2002, Yuan et al. 1999), and breast cancer tissues (Canbolat et al. 1996) There are several documented reports on detection of abnormally high levels of serum guanase activity in patients with liver diseases like hepatitis, therefore, the elevated enzyme activity has been suggested as a marker of hepatitis and hepatoma, there are also various reports of patients developing hepatitis C when they are transfused with blood containing high levels of serum guanase activity (Ito et al. 1988, Nishikawa et al. 1989, Shiota et al. 1989).

EXPERIMENTALS

Collection of sample: The total number of these samples was (63) samples, serum samples collected from hepatitis B and C patients for both sexes. Type B was (30) samples while type C was (33) samples. Blood was drawn from the vein using a 5 ml plastic syringe with one use. The blood was placed in clean and free anticoagulant tubes, and left to coagulant at room temperature. The blood serum was then separated by centrifuge at a velocity 4000 G for 15 minutes to ensure adequate serum red blood cell extraction. The effectiveness of the enzyme was measured directly and the study was done outside the body (in vitro).

Diagnosis of samples: Guanase activity in serum was measured by using Elisa kit supplied from sun long biotech-China).

2-3-Sepration and purification of Guanase from serum of hepatitis B and C patients: Guanase was purified from the serum of hepatitis B and C patients using the following steps: 1-Addatoin ammonium sulphate (75%) 2-Ion exchange (using DEAE- Cellulose) 3- Gel Filtration Chromatography (using Sepharose-6B).

RESULTS AND DISCUSSION

The current study showed high serum guanase (pg/ml) activity in patients with hepatitis B compared to control group (34.46±2.17 and 17.1±5.65 respectively; P≥0.05). There was also a significant increase in the enzyme in patients with hepatitis C compared with the control group (67. 6±84.2 and 17.1±5.65 respectively; P≤ 0.05), as shown in **Fig. 1**.

GU is abundantly expressed in the liver, brain and kidney. It is quite scarce in the skeletal muscles, myocardium, pancreas and other organs in which aspartate aminotransferase (AST) and ALT are relatively abundant (Knights 1984, Matsunaga et al. 2003). Therefore, it has been reported that a rise in blood GU activity may be a sign specific to liver disease, and that measurement of his enzyme may serve as an excellent means of screening for liver disease (Ito et al. 1984, Mandel et al. 1970, Takaoka and Ito 1979). The results of this study are consistent with the results Matsunaga et al. (Ito et al. 1981) which showed high efficacy of serum guanase in patients with hepatitis B and C. This study agrees with the results Ito et al. (1982) who reported that serum guanase was significantly

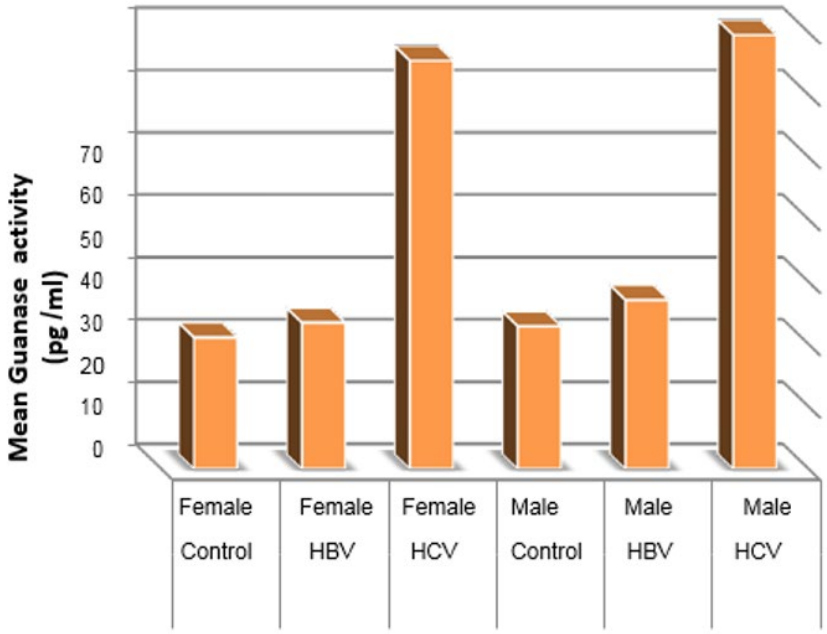

Fig. 2. Serum guanase enzyme level in patients with hepatitis B and C with the gender difference

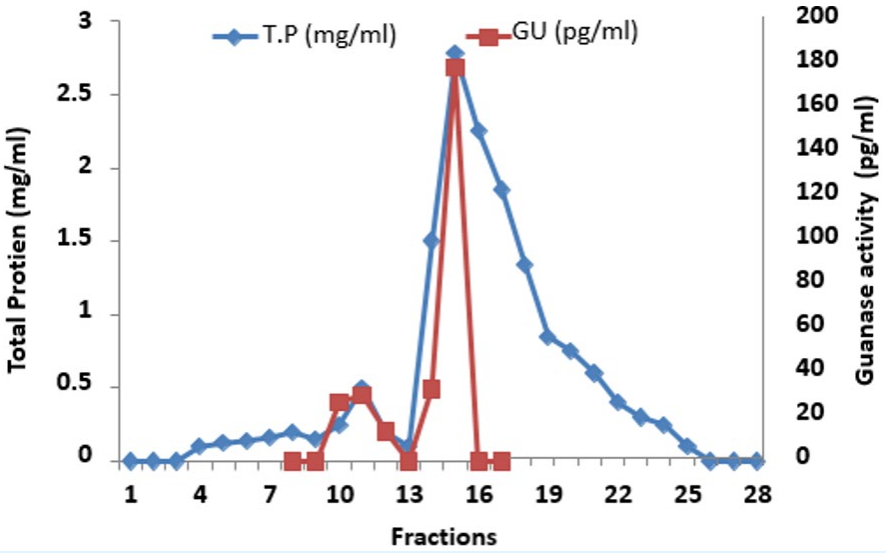

Fig. 3. Purification of guanase with exchange(using DEAE- Cellulose) (Elution curve)

higher in patients with HBV and HCV than controls. As agreed with the results Shiota et al. (1989) which showed high efficacy of serum guanase in patients with hepatitis B. As the results showed no difference in guanase activity in serum of HBV and HCV both in male and female as shown in **Fig. 2**.

Partially Purification of Guanase from Serum of Hepatitis B and C Patients

The basic principle is to equalize the charges on the surface of the protein (enzyme) and the degradation of the water layer surrounding the protein and reduce the degree of the watering, solubility of the protein and sedimentation (Nelson and Cox 2017). Therefor separation and purification process of guanase was made from serum of hepatitis B and C patients by steps where in the first stage of purification the enzyme was precipitated using ammonium sulphate salts (NH4)2SO4 to concentrated enzyme, the purification degree by ammonium sulfate precipitation was (4.358) fold and yield of enzyme 64.084% ,specific activity 226.179 pg/ml as shown in the **Table 1**, the stage of purification were complete by using Ion exchange (using DEAE-Cellulose) and gel filtration (using Sepharose-6B) which showed a single peak with the degree purification was (8.939, 39.446) fold respectively and yield of enzyme (50.5%, 41.535%) respectively ,specific activity (463.914,8921.99) pg/ml respectively, as shown in the **Table 1**.

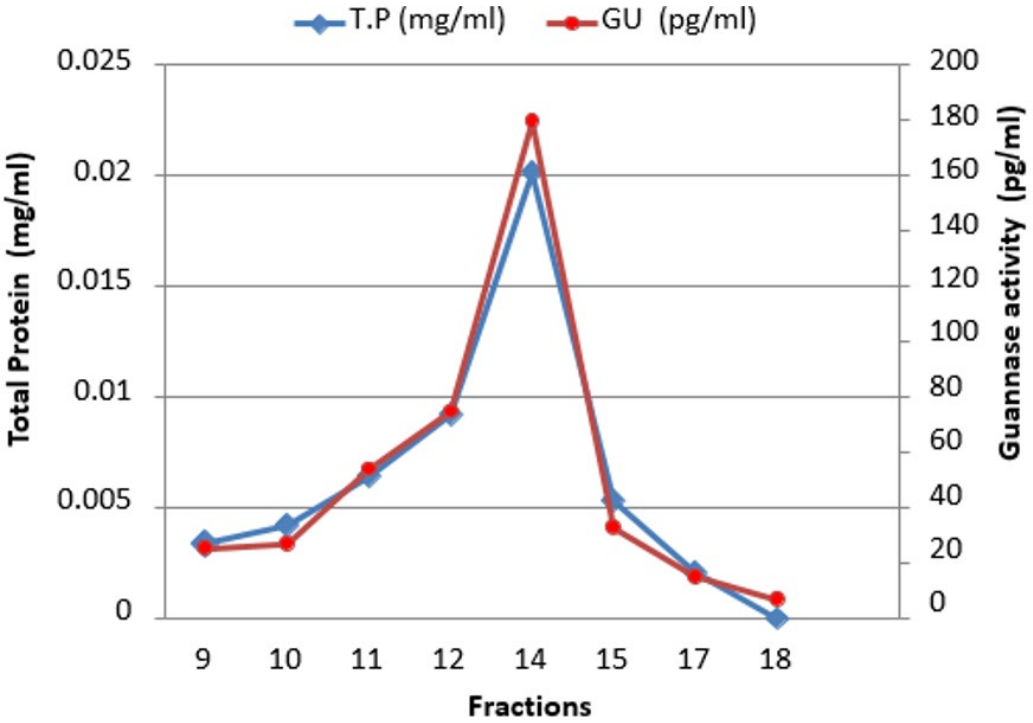

Fig. 4. Purification of guanase with gel filtration on Sepharose-6B (Elution curve)

Table 1. Steps of purification

Purification steps	Volume (ml)	Guanase activity (pg/ml)	Total protein (mg/ml)	Specific activity (pg/mg)	Total activity	Yields %	Purification fold
Crude extract	24	26.985	0.520	51.894	647.64	100	1
Ammonium sulfate	5	83.008	0.367	226.179	415.04	64.084	4.358
Ion exchange	3	109.02	0.235	463.914	327.06	50.500	8.939
Gel Filtration	1.5	179.33	0.0201	8922	269	41.535	39.446

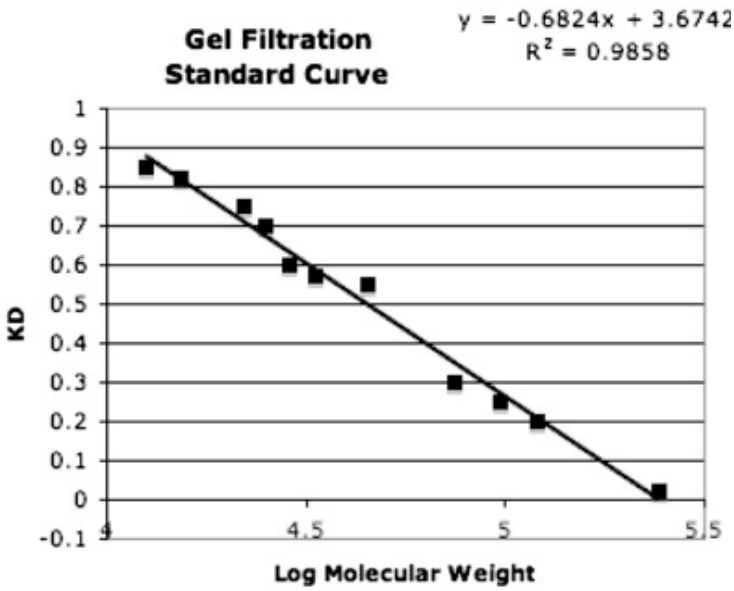

Fig. 5. The standard curve for finding the molecular weight of the enzyme

Determination of Molecular Weight of Guanase

The molecular weight of the enzyme was calculated by gel filtration method using Sepharose-6B gel, where the linear relationship was plotted between the ratio of recovery volume per standard protein and recovery volume of blue dextran, where it reached its molecular weight (71.131KD) as shown in **Fig. 5**.

Discrepant results have been obtained on the molecular weight of purified guanase. Fogle and Bieber (1975) reported that guanase of rabbit liver consists of molecules of 100,000 and 207,000 daltons. Bergstrom and Bieber (1979) reported that the molecular weight of guanase of rabbit liver was 110,000, and consisted of subunits of 52,000. They obtained several subunits and multiple forms of the native enzyme. Rossi *et al.* (1978) reported that guanase of porcine brain has a molecular weight of 110,000 and is composed of two identical subunits of 50,000 daltons. Similarly, Miyamoto *et al.* (1982) found that the molecular weight of guanase from rat brain was 105,000, and consisted of two identical subunits of 52,000 daltons. Gupta and Glantz (1985) reported that the molecular weight of human liver guanase was 120,000 and consisted of two identical subunits of 59,000 daltons. Kuzmits *et al.* (1979) found two isozymes of guanase of human liver with different pH optima and iso-electric points, but with the same molecular weight of 100,000. Nishikawa *et al.* (1985) reported a monomer and polymer of human liver guanase with molecular weights of 64,000 and 110,000, respectively. We observed a single peak of guanase with a molecular weight of 71,131 on gel filtration. The discrepant results obtained by various groups were probably due to differences in purification procedure and sources of enzyme (Kilitci et al. 2018, Niyazmand et al. 2018, Rabbani et al. 2014).

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