

## KINETIC STUDY OF GRANZYME B IN PATIENTS WITH CHRONIC HEPATITIS TYPE B

Hussein S. Alshamary<sup>1</sup>, Raid J. M. AL-Timimi<sup>2</sup>, Jalal A. Ali<sup>3</sup> and Qasim S. Al-Mayah<sup>4</sup>

<sup>1</sup>Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Iraq

<sup>2</sup>Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Iraq

<sup>3</sup>Consultant Gastro & Hepatology, College of Medicine, Al-Nahrain University

<sup>4</sup>Medical Research Unit, College of Medicine, Al-Nahrain University

Corresponding author's email : sciencefond2015@gmail.com

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**ABSTRACT :** Chronic hepatitis B ( CHB) virus infection is affecting more than 350 million people worldwide ,and can predispose for serious complications like liver cirrhosis and hepatocarcinoma. The development of CHB from active stage is the direct result of debilitated immune system which mainly depends on cytotoxic T- lymphocyte ( CTL ) and natural killer ( NK ) cells for controlling viral replication. These cells eliminate their targets through the secretion of two proteins : perforin and granzymes (Gzms). Thus, this study aimed to evaluate the activity and concentration of GzmB in patients with CHB. A total of 60 patients (30 newly diagnosed and 30 treated) were recruited for this case-control study. Other 30 age- and gender-matched apparently healthy subjected (negative for HBsAg) were involved as controls. Granzyme B kinetics and concentration were measured using ready commercial kits. Treated patients had significantly higher values of Km ( $8.57 \pm 0.796 \times 10^{-5} \text{ M}$ ), than either untreated patients ( $7.17 \pm 0.69 \times 10^{-5} \text{ M}$ ) or controls ( $7.24 \pm 0.82 \times 10^{-5} \text{ M}$ ), while treated patients showed significantly lower values for Kcat and Kcat/Km ( $3.77 \pm 0.39 \text{ s}^{-1}$  and  $4.55 \pm 0.89 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  respectively) than either untreated patients ( $4.37 \pm 0.31 \text{ s}^{-1}$  and  $6.18 \pm 0.98 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  respectively) or controls ( $4.34 \pm 0.37 \text{ s}^{-1}$  and  $6.187 \pm 1.167 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  respectively). Mean GzmB concentration in control was  $104.4 \pm 26.37 \text{ Pg/mL}$  which significantly higher than that of untreated patients ( $44.3 \pm 8.73 \text{ Pg/mL}$  which did not differ significantly from treated patients ( $42.33 \pm 9.09 \text{ Pg/mL}$ ). These data indicate the reduction in GzmB concentration in CHB which may reflect a defect in the production of this enzyme that predispose un individual to the chronic infection.

**Key words :** Granzyme B, hepatitis B, kinetics.

### INTRODUCTION

Hepatitis B virus (HBV) infection is a major health problem that causes inflammation of the liver. It affects over 350 million people worldwide and over 1 million die each year from this infection. The virus causes either temporary (acute) hepatitis or chronic hepatitis which goes for a long period of time that may develop in the liver fibrosis, cirrhosis and carcinoma [1].

In response to the infection, innate and adaptive immunity have involved in different tasks to control infections. Through recognition of viral nucleic acids, viral proteins or tissue-damage, innate immunity is triggered during the early phases of viral infections [2]. The activation of innate immunity is also necessary for the efficient recruitment of the adaptive immune system, which acts through maturation and expansion of B-cell to produce antibodies specific to Hepatitis B antigen and T-cell clones that specifically recognize and kill infected

hepatocytes [3]. Genetic defects in any arm of immune system could increase the susceptibility of an individual to HBV infection [4].

Cytotoxic T lymphocytes and NK cells eliminate virus-infected cells principally by releasing the contents of cytotoxic granules into the immune synapse formed with their target cell. The granule mediators of target cell are serine proteases, known as granzymes, which induce programmed cell death, after they are delivered into the target cell cytoplasm by the pore-forming protein perforin. Granzyme B is the most important one that induces target cell apoptosis in a mitochondria-dependent manner [5,6].

Granzymes should work at maximum speed with lower Km and should be free of the inhibitors in order for successful initiated apoptosis [2]. In hepatitis B patients, 5% do not respond to treatment in which infected cells do not undergo apoptosis. Some of the possibilities for what is going on with those patients might be either defects in