

Molecular Physical Properties of Cryoglobulin in Patients with Hepatitis B Virus in Al-Najaf Province

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Summary

Cryoglobulins (CGs) are a cold precipitating abnormal immunoglobulins. The incidence of hepatitis B was discovered to be greater in age groups (46-55 years) relative to other age groups. CGs were detected in 50 (33.3%) out of 150 patients with Hepatitis B. Three types of CGs precipitation nature were identified. Gelatinous (76%), flocculate (20%), and crystallineous (4%). No relation was found between patient's age and CGs molecular physical properties.

The cryocrit test of 50 serum samples revealed that CGs are characterized by their reversible precipitation at 4°C between 12 hours to five days and solubility time at 37°C ranged between half an hour and three hours.

Keywords: Hepatitis B virus, Cryoglobulins (CGs), Najaf province.

Introduction

Hepatitis B virus is a public health problem worldwide. It causes acute and chronic liver disease. Chronic hepatitis B can progress to cirrhosis, liver insufficiency or liver cancer¹. The world health organization has prepared a plan in 2016 to eliminate the public health threat of HBV infection globally by 2030².

Fulminant hepatic failure may occur due to acute liver injury caused by HBV infection and acute exacerbations of chronic HBV infection. The mortality rate in this case exceeds 75%³. The most effective method to prevent HBV infection is vaccination⁴.

CGs are immunoglobulins (Igs) characterized by precipitation ability at low temperature (below 37°C) and re-dissolving after warming⁵. CGs can cause damage to many organs such as liver, kidney, peripheral nerve, skin, and others⁶. CGs have different composition, which influences the clinical course and the type of underlying disease. Cryoglobulinemia is classified into two categories: type I, that is exclusively found in clonal hematologic diseases, type II and III, which is known as

mixed cryoglobulinemia and is found in hepatitis C virus infection and systemic diseases such as connective tissue disorder and B-cell lineage hematologic malignancies⁷.

The best description of GCs pathogenicity is the one of Hepatitis C virus (HCV) associated cryoglobulinemia. Clonal B-cell expansion is induced due to HCV infection, B-cell secretes monoclonal IgM that binds anti-HCVIgG and form immune complexes. Endothelial cells bind to these immune complexes via C1q to C1q receptors receptor. This combination promotes the recruitment of inflammatory cells. Consequently, Vasculitis is produced⁷. The extrahepatic manifestation of cryoglobulinemia associated with HBV are rarely reported⁸.

The clinical presentation of cryoglobulinemia is broad and differs between types of diseases, it includes: purpura, arthralgia, glomerulonephritis, skin ulcers, and peripheral neuropathy. Life-threatening manifestations can develop in a small proportion of patients⁷.

The aim of this work was to detect the presence of CGs in the serum of patients with Hepatitis B and study their molecular physical properties.

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Methods

Blood sample processing

Between July 2018 and March 2019, blood samples were collected from 150 patients (15-65 years) were clinically diagnosed with viral hepatitis type B attending public health laboratories in Al-Najaf province. 10ml of blood samples was drawn from each patient and immediately incubated at 37°C to allow the blood to clot. The blood was centrifuged at 1500xg for 5 minutes at 37°C. 2ml of serum samples were used for the detection of GCs.

ELISA detection of hepatitis B surface antigen (HBsAg).

This is a sandwich-type direct immunoenzymatic technique^{9,10} in which anti-HBs covered antibodies on microtiter plat reservoirs act as the peroxidase-marked antibody capture and goat antibodies act as conjugate antibodies. The sample to be analyzed is incubated in one of the antibody coated reservoirs during the test operation. If the sample includes HBsAg, the antigen will attach on the palate to the antibody. To eliminate any unbound material after washing. Goat anti-HBs conjugate is added to the well and permitted to respond with antigen-antibody complex created during the first incubation. An enzyme substratum containing chromogen is added after a second incubation and

subsequent washing. If the sample is positive for HBsAg, the substratum will create a blue color. After blocking with sulphuric acid, the blue color shifts to yellow. The intensity of the color is proportional to the quantity of HBsAg in the test sample¹¹.

Cryoglobulin assay

The experiment was conducted according to Irish Committee of Cryoglobulinemia guidelines¹². 2ml of serum was loaded into Sahli tube. The Sahli tubes were incubated at 4°C for 7 days. The tubes were checked for the appearance of precipitate each 12 hours for the first day. Then the tubes were checked daily.

After the appearance of GCs, the tubes was transported into a water bath at 37°C to solubilize the GCs. The tubes were checked every hour for the disappearance of GCs precipitate.

Results

Age of patients

CGs were detected in the serum of 50 patients out of 150 patients infected with HBV. There was no relations between patient's age and the CGs properties, as both the precipitation and solubility time varied within each age group. HBV patients were divided into seven age groups (Fig1).

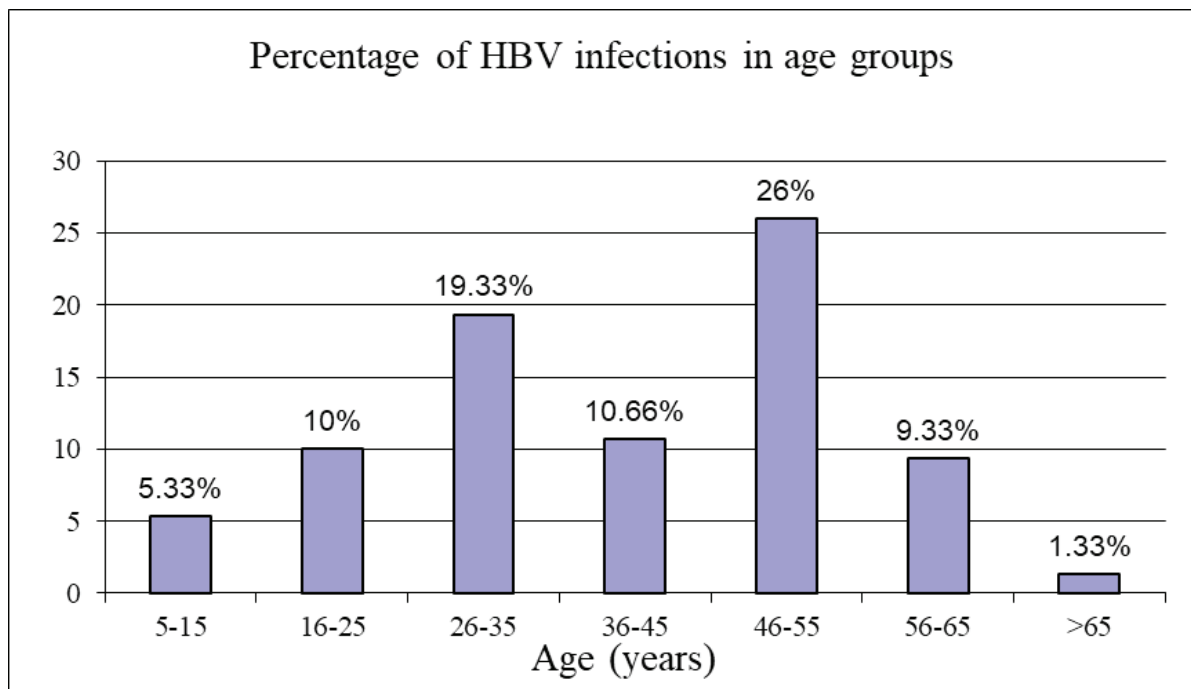


Figure (1). The percentage of HBV infections according to patient's age.

The highest incident of HBV infection was found in age group of 46-55 years, followed by age groups 26-35, 36-45, 16-25, 56-65, 5-15, and over 65 years. No relation was found between the GCs properties and patient's age.

Cryoglobulin molecular-physical properties

Nature of CGs precipitates

CGs are a temperature sensitive proteins, they form different types of precipitates depending on the type of CG, physical nature, and precipitation conditions (13). In this study, three precipitate types were noticed. 38 out of 50 were gelatinous, 10 were flocculent, and 2 were crystalline (Fig 2).

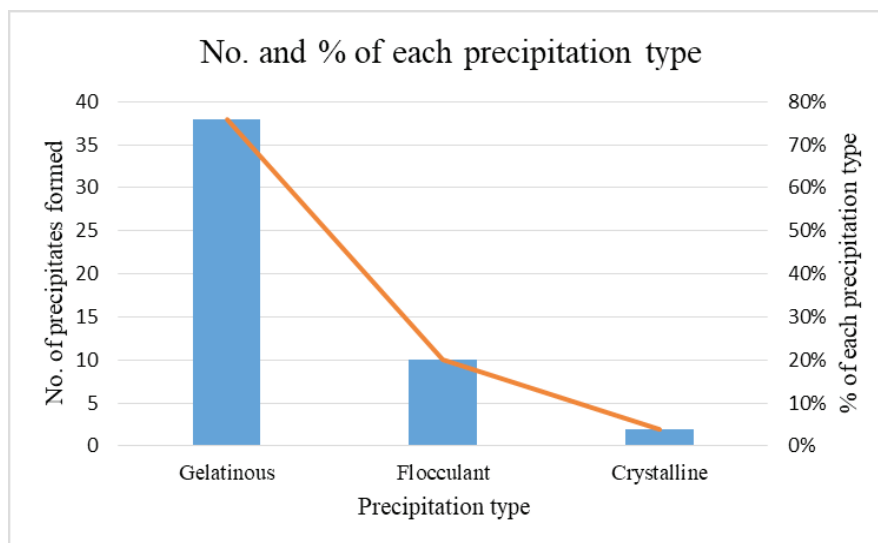


Figure (2). The nature of CGs precipitation. Three types of CGs precipitation was noticed: gelatinous 76%, flocculent 20%, and crystalline 4%. The blue bars refers to the number of each precipitation type and the brown line refers to the percentage of each type.

Cryoglobulin precipitation time

CGs differ in their precipitation time, type I CG may precipitate within 24 hours of incubation at 4°C. While type II and III (mixed) may precipitate within several days⁶. In this study, the first CGs precipitate started to appear after 12 hours of incubation at 4°C in one of the serum samples, while the longest time of GCs precipitation was five days. Most of the cryoglobulins (36%) in the serum of patients with Hepatitis B appeared after one day of incubation (Fig 3).

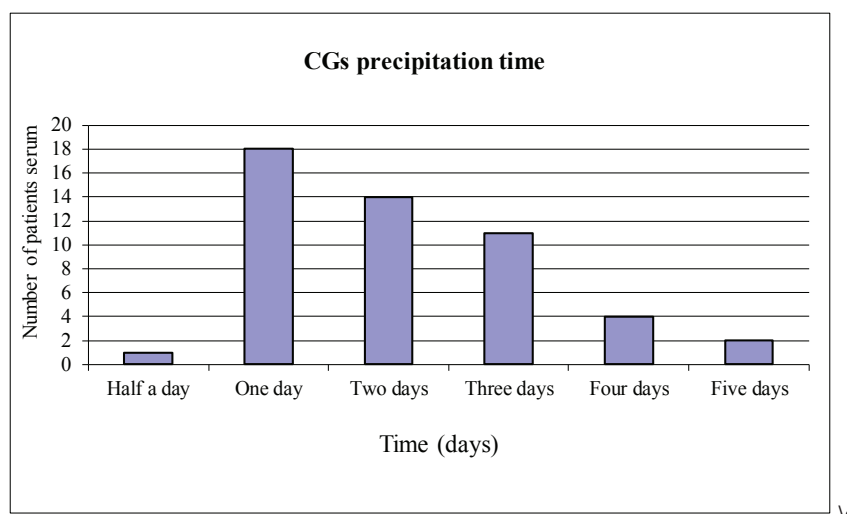


Figure (3). CGs precipitation time at 4°C.

Most of CGs (18 samples, 36%) in HBV patient's serum have precipitated in one day of incubation at 4°C, followed by 28% (14 samples) in two days, 22% (11 samples) in three days, 8% (4 samples) in four days, and 4% (2 samples) in five days. 2% (only one patient's serum) showed a CG precipitation in 12 hours.

Cryoglobulines solubility time

In this study, CGs solubility time was between half an hour and three hours at 37°C. Most of the cryoglobulins (19 samples, 38%) became soluble after one hour and half (Fig 4).

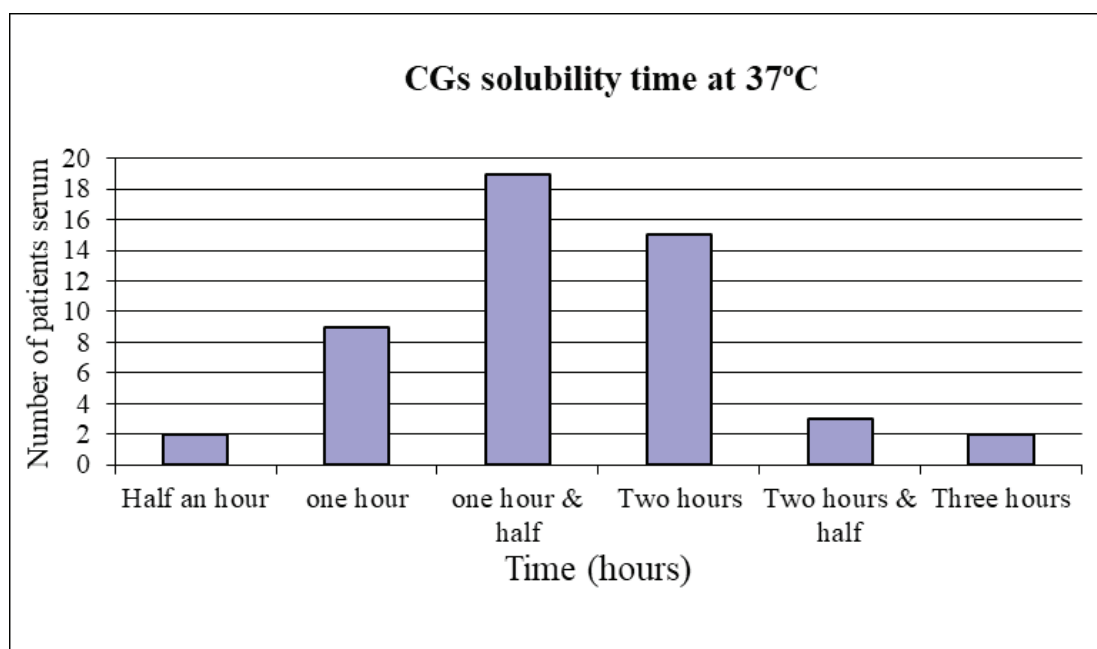


Figure (4). Time of solubility cryoglobulin in 37°C

Most of CGs (19 samples, 38%) in HBV patient's serum was solubilized in one hour and half. followed by (15 samples, 30%) in two hours, (9 samples, 18%) in one hour, (3 samples, 6%) in two hours and half, (2 samples, 4%) in three hours. And another (2 samples, 4%) in half an hour.

Discussion

Chronic Hepatitis B induce a massive monoclonal expansion of B-cell and mixed cryoglobulinemia. About 20% of patients with chronic HBV infection develop mixed cryoglobulinemia¹⁴. In contrast, another report has demonstrated that HBV-associated cryoglobulinaemia is rarely reported¹⁵. Our results showed that 33.33% of HBV patients have developed a cryoglobulinemia. This percentage is much higher than the other reported ones. It seems that the prevalence of CGs in patients with Hepatitis B varies in different populations. Another reason for the low percentage of cryoglobulinemia

in HBV patients reported by Li et al is the improper management of blood samples. Specimens for CGs detection should be transported and centrifuged at 37°C¹⁶.

Gelatinous precipitate is the dominant type of CGs precipitation, crystalline precipitation is rarely seen. The extra-hepatic clinical manifestation varies widely depending on the type and precipitation nature of cryoglobulins produced¹³. Type I CGs (mostly IgM) are found in the serum of lymphoproliferative disease patients¹⁷. Type I CGs cryoprecipitate can appear within 24 hours after incubation, while mixed cryoglobulins (type II and III) cryoprecipitate appears after several days⁶. In this study, 36% of cryoglobulins precipitate appeared after one day of incubation, and 76% of the CGs precipitate have a gelatinous nature. Most likely they are type I cryoglobulins. This finding demonstrates that lymphoproliferative disease is most likely to be the extra-hepatic manifestation in patients with HBV.

We found that 38% of GCs precipitate can solubilize within one hour and half. All the CGs precipitated in this study were soluble at 37°C within the range of half an hour to three hours.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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References

- 1- Zayet, S., Osman, M., Besghaier, H., Ben Moussa, M., Belhadj, A., Bellaaj, R. Prevalence of hepatitis B markers and vaccination status of healthcare personnel: Experience of the Tunis Military Hospital. *Rev Epidemiol Sante Publique*. 2019
- 2- Liu, J. & Liu, M. Progress and challenges in achieving the WHO goal on 'Elimination of Hepatitis B by 2030' in China. *Zhonghua Liu Xing Bing Xue Za Zhi*, 2019;40, 605-609.
- 3- Ichai, P. & Samuel, D.. Management of Fulminant Hepatitis B. *Curr Infect Dis Rep*, 2019;21, 25.
- 4- Liu, F., Guo, Z., Dong, C.. Influences of obesity on the immunogenicity of Hepatitis B vaccine. *Hum Vaccin Immunother*. 2017;13, 1014-1017.
- 5- Sargur, R., White, P., Egner, W. Cryoglobulin evaluation: best practice? *Annals of clinical biochemistry*. 2010;47, 8-16.
- 6- Motyckova, G. & Murali, M. Laboratory testing for cryoglobulins. *American journal of hematology*. 2011;86, 500-502.
- 7- Muchtar, E., Magen, H., Gertz, M. A.. How I treat cryoglobulinemia. *Blood*. 2017;129, 289-298.
- 8- Boglione, L., D'Avolio, A., Cariti, G., Di Perri, G. Telbivudine in the treatment of hepatitis B-associated cryoglobulinemia. *J Clin Virol*. 2013;56, 167-169.
- 9- Fields, H.A.; Davis, C.L.; Bradley, D.W.; and Marynard, J.E. Experimental conditions affecting the sensitivity of enzyme linked immunosorbent assay (ELISA) for detection of hepatitis B surface antigen (HBsAg). *Bull. W.H.O.* 1983;61: 135-142.
- 10- Rose, N.R.; Hamilton, R.G. ; and Detrick, B. *Manual of Clinical Laboratory Immunology* .6th Ed. ASM. Press.USA. 2002.
- 11- Giles, R.E.; Burgees, C.; Gimore, M.; Perry, K.R.; and Parry, J.V. An evaluation of the bioelisa HBsAg colour assay. *Medical Device Agency Evaluation Report*, MDA. 1999;99 (68).
- 12- Lynch PLM. Audit of Cryoglobulin Determination in Northern Ireland . *Clinical Biochemistry Department , Altnagelvin Area Hospital , Londonderry . Full implementation of all new guidelines : 2006*;1-13.
- 13- Vallas, V., Farrugia, W., Raison, R. L., Edmundson, A. B., Ramsland, P. A. Dissimilar aggregation processes govern precipitation and gelation of human IgM cryoglobulins. *J Mol Recognit*. 2007;20, 90-96.
- 14- Visentini, M., Pascolini, S., Mitrevski, M., Marrapodi, R., Del Padre, M., Todi, L., Camponeschi, A., Axiotis, E., Carlesimo, M., De Santis, A., Fiorilli, M., Casato, M. Hepatitis B virus causes mixed cryoglobulinaemia by driving clonal expansion of innate B-cells producing a VH1-69-encoded antibody. *Clin Exp Rheumatol*. 2016;34, S28-32.
- 15- Li, S. J., Xu, S. T., Chen, H. P., Zhang, M. C., Xu, F., Cheng, S. Q., Liu, Z. H. Clinical and morphologic spectrum of renal involvement in patients with HBV-associated cryoglobulinaemia. *Nephrology (Carlton)*, 2017;22, 449-455.
- 16- Chan, A. O., Lau, J. S., Chan, C., Shek, C. Cryoglobulinaemia: clinical and laboratory perspectives. *Hong Kong Medical Journal*, 2008;14, 55.
- 17- Sargur, R., White, P., Egner, W.. Cryoglobulin evaluation: best practice. *Ann Clin Biochem*, 2010;47, 8-16.