INTRODUCTION

Cryoglobulinemia is a pathologic condition in which the blood contains immunoglobulins that have the property of reversible precipitation from human serum cooled to 4°C. In 1947, ***Lerner and Watson*** found that these proteins were γ-globulins and introduced the term cryoglobulins (cold precipitable serum globulins). Cryoglobulinemic disease was placed within the vast family of systemic vasculitis and characterized by purpura, weakness, arthralgias, and, in some patients, organ involvement ***(Meltzer et al., 1966).***

The majority (95%) of cryoglobulins are immune complexes (IC) that contain rheumatoid factor (RF). Such cryoglobulins are known as "mixed" cryoglobulins to differentiate them from the cryoglobulins with monoclonal bands that do not contain RF or antigen-antibody complexes. The antigens present in the mixed complexes may include RNA and proteins (eg, hepatitis C virus proteins). A small fraction of cryoglobulins (5%) comprise pure monoclonal gammopathies, which have poor solubility at low temperatures because of their unique amino acid sequences **(*Shihabi, 2006).***

Three types of cryoglobulins have been identified. In type I, the cryoprecipitable immunoglobulin is a single monoclonal Ig. Types II and III cryoglobulinemias are both mixed types (MC), composed of at least two immunoglobulins. In both of them, a polyclonal immunoglobulin G (IgG) is bound to another Ig which

is an antiglobulin and acts as a rheumatoid factor (RF). The main difference between two types of MCs is that in type II, the RF usually of the IgM class is monoclonal, whereas in type III, it is polyclonal ***(Brouet et al., 1974).***

Hepatitis C virus (HCV) chronic infection is recognized as the major cause of mixed cryoglobulinemia (MC) mainly types II and III and in rare cases type I. Its persistence represents a continuous stimulus for host immune system with production of circulating immune complexes (ICs), one-third of them with cryoprecipitate property. Complement factors play a crucial role in the cold-insoluble ICs-mediated vasculitis, involving primarily small blood vessels in different tissues including skin, kidney, peripheral, and central nervous system ***(Lauletta et al., 2012; Ferri et al., 2004).***

It was suggested that cryoglobulin formation is linked to the attempt of the host to clear the significant quantities of virions generated daily by the chronic infection. Thus, management of these patients should be focused on either the reduction of virus production, the elimination of immune complexes, the function of B cells, or the suppression of the inflammatory response of the host to the cryoglobulin immune complex ***(Schamberg et al., 2007).***

Mixed cryoglobulinemia and glomerulonephritis are the most important extrahepatic manifestations of chronic hepatitis C virus (HCV) infection ***(Garini et al., 2005).*** The most significant

accompanying kidney lesion is type I membranoproliferative glomerulonephritis, usually occurring in the context of type II mixed Cryoglobulinemia ***(Fabrizi et al., 2013).***

Central nervous system (CNS) involvement is more rarely reported. Neurological complications in HCV-infected patients occur predominantly in the peripheral nervous system. Peripheral neuropathy in HCV infection is primarily associated with mixed cryoglobulinaemia ***(Cacoub et al., 2005).***

Cutaneous manifestations are ranging from palpable purpura of lower limbs to chronic cutaneous ulcers more frequent in the supramalleolar regions. Skin reactions include Raynaud's phenomenon, livedoreticularis, urticaria, and edema ***(Lauletta et al., 2012).***

AIM OF THE WORK

The aim of this work is to study frequency of occurrence of mixed cryoglobulinemia (MC) manifestations among hepatitis C virus children attending the Pediatric Hematology Oncology Unit, Ain Shams University, to assess its relation to the level of hepatitis C viremia and to compare its frequency among hepatitis C virus beta thalassemia and childhood cancer survivors patients.

Chapter 1

HEPATITIS C VIRUS

The hepatitis C virus is an enveloped single-stranded RNA virus and a member of the hepacivirus genus of the Flaviviridae family. There are eleven major genotypes, indicated numerically from one to six with subgenotypes ***(Khattab et al., 2010).***

Epidemiology

Genotype 1 is the predominant genotype (46%) and constitutes 70% of infections in the US. It also predominates in Europe and Japan. Type 2 is less frequently encountered (22%). Type 3 is predominant in south-east Asia. Genotype 4 is endemic in the Middle East, Egypt and central Africa. The most predominant genotype in South Africa is genotype 5, which constitutes 40% of chronic infections. Genotypes 6-11 are found in Asia ***(Abuelhassan et al., 2012; Gower et al., 2014).***

In 2012, the World Health Organization estimates that there are 150 million infected people worldwide. This constitutes 3% of the world population ***(Petrova et al., 2007).***

The prevalence in Egypt was estimated to be (20%). The extent of the problem in South Africa is not known, but the prevalence is estimated to be between 0.1-1.7%. The hepatitis C virus is a leading cause of chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC). It is also the leading

indication for liver transplants in the US (30%), Europe (up to 50%) and Japan ***(Tampaki et al., 2014).***

Egypt has the highest prevalence of HCV among the world (responsible for 90% of infections), with a predominance of subtype 4a (55%) ***(Omar et al., 2011; Bose et al., 2014).***

Mode of HCV transmission

In the developed world, HCV infection is predominantly associated with sharing contaminated equipment between injecting drug users, while in developing countries, infected blood, nosocomial transmission and unsafe cultural practices have been implicated ***(Dev et al., 2004).***

1) Blood Transfusions

HCV is most efficiently transmitted through transfusion of infected blood and transplantation of infected organs ***(WHO, 2004)***. Blood transfusion remains a major risk of HCV transmission among Egyptian children and more than 75% of children receiving blood transfusion, due to hematological disorders, had HCV seropositivity ***(Khalifa et al., 2002; El-Raziky et al., 2004)***. In Egypt, 8.8% of blood donors' sera in 13 governorates were positive for anti-HCV ***(Tanaka et al., 2004).***

2) Nosocomial HCV Transmission

The three possible modes of HCV nosocomial transmission are patient to patient; Patient to healthcare worker (HCW); and HCW to patient (Viral Hepatitis Prevention Board, 2005). Patient to patient transmission accounts for a substantial

disease burden in areas with inadequate infection control practices. This transmission can occur in various settings as: hemodialysis (HD), digestive endoscopy, dental clinics, surgery and organ transplantation ***(WHO, 2004).***

Currently, the major route of transmission appears to be health-related procedures with inadequately sterilized instruments. Procedures performed by non-medical professionals and traditional healers have been identified as important risk factors for HCV transmission in Egypt. Intrafamilial and sexual transmissions also play a role in the high prevalence of HCV-4 ***(Khattab et al., 2011).***

Some of the well-known mechanisms of HCV transmission include sharing injection material and products, as well as breaches in barrier precautions and in material disinfection. In Egypt, the HCV seroprevalence increased from 45% to 80% among haemodialysis patients within 3 years after the beginning of haemodialysis ***(El-Sherbiny et al., 2004).*** As regards patients-to- HCW transmission, the estimated risk of an individual surgeon acquiring HCV through occupational exposure is low (<1%) even in an area with an extremely high prevalence of HCV ***(Thorburn et al., 2003).***

The risk of transmission of HCV from infected HCW to patients is very small, although a number of outbreaks have been reported ***(Goldmann , 2002).***

3) Sexual Transmission of HCV

In Egypt, it was estimated that the probability of wife-to-husband HCV transmission from infected wives, with and without viremia, was 34% and 10% respectively, compared to 3% and 0% of husband-to-wife transmission for HCV infected husbands with and without viremia respectively ***(Magder et al., 2005).***

4) Mother-to-Infant Transmission

In Egypt, the percent of positive HCV-RNA infants born to HCV infected mothers was 4% and the main risk factor was the level of maternal HCV viremia ***(Abd El Shaheed et al., 2004).*** The risk of HCV transmission through breastfeeding is very low, but may increase when mothers have high viremia or nipple crakes ***(Swan et al., 2004).***

Approximately 10% of HCV- infected adults have no identified risk factor for infection; this frequency is probably higher among pediatric patients ***(Bacon et al., 2007).***

Incubation period:

The incubation period for hepatitis C is 2 weeks to 6 months. Following initial infection, approximately 80% of people do not exhibit any symptoms. Those who are acutely symptomatic may exhibit fever, fatigue, decreased appetite, nausea, vomiting, abdominal pain, dark urine, grey-coloured faeces, joint pain and jaundice (yellowing of skin and the whites of the eyes) ***(Pawlotsky , 2005).***

Communicability occurs during the acute clinical stage of HCV infection and indefinitely in the chronic carrier stage. All HCV positive individuals should be considered potentially infectious although the risk is minimal in the non-viraemic (PCR negative) individual ***(Shivkumar et al., 2012).***

The Pathophysiology of HCV

It is thought that extrahepatic tissues serve as a reservoir for the hepatitis virus, but especially tropism for the lymphoid tissues ***(Flint et al., 2000)***.

HCV replicates within extrahepatic tissues with expression of viral proteins, leading to extrahepatic manifestations. An important feature of HCV is that the virus avoids immune elimination. The consequences are chronic infection, accumulation of immune complexes and autoimmune phenomena. HCV shows lymphotropism in addition to the hepatotropism, which is responsible for many EHM ***(Agnello et al., 2004; Khattab et al., 2011).***

The cellular components leak from the persistent destruction of the infected cells. About 20% of hepatitis C patients are ANA positive ***(Younus et al., 2004).***

1. Autoimmunity

HCV infection and proliferation within lymphocytes leads to functional alteration of lymphocyte and production of excessive auto antibodies and cryoglobulins due to the

molecular mimicry between HCV and auto antigens ***(Saadoun et al., 2008; Jacobson et al., 2010)***.

2. HCV infection of cells

HCV binds several cell surface receptors. Cell tropism required for HCV genome replication are not well characterized ***(Jacobson et al., 2010)***.

Clinical picture

Hepatic

A major fraction of anti-HCV positive children develops chronic liver disease. They may progress to cirrhosis or hepatocellular carcinoma after many years ***(Oza et al., 2012).***

The clinical symptoms of chronic HCV infections are generally nonspecific. Malaise and fatigue are most commonly reported, followed by nausea, abdominal pain, myalgia, and arthritis ***(Sabrya et al., 2005).***

About 75% of people have no symptoms when they first acquire hepatitis C infection. The remaining 25% may complain of fatigue, loss of appetite, muscle aches, or fever. Yellowing discoloration of the skin or eyes (jaundice) is rare at this early stage of infection ***(McCombs et al., 2014).***

Over time, people with chronic infection may begin to experience the effects of the persistent inflammation of the liver caused by the immune reaction to the virus. Patients may become easily fatigued or complain of nonspecific symptoms.

As cirrhosis develops, symptoms increase and may include weakness and loss of appetite ***(Tucker et al., 2013).***

In patients with advanced cirrhosis, the liver begins to fail. Confusion and even coma (encephalopathy) may result from the inability of the liver to process certain toxic substances. Increased pressure in the blood vessels of the liver (portal hypertension) may cause fluid to build up in the abdominal cavity (ascites) and result in engorged veins in the swallowing tube (esophageal varices) that tear easily and can bleed suddenly and massively ***(Jezequel et al., 2015).***

Extrahepatic

Infection with hepatitis C virus (HCV) can lead to several extrahepatic manifestations (EHM). Hematologic diseases such as cryoglobulinemia and lymphoma, autoimmune disorders such as thyroiditis, renal diseases, dermatologic conditions such as lichen planus and porphyria cutanea tarda, Lymphoproliferative disorders ***(El-Serag et al., 2002).***

The most common risk factors associated with extrahepatic manifestations of HCV infection are older age, female sex, and extensive liver fibrosis. Several reports from different parts of the world suggest that hepatitis C virus affects not only the liver, but other tissues, organs and systems as well ***(Petrova et al., 2007).***

Up to 75% of patients will develop an EHM during their illness. In some patients, EHM can be the dominant feature, while hepatic disease is mild ***(Guerra et al., 2012).***

EHM classified into four groups according to degree of association recorded to HCV infection ***(Bose et al., 2014).***

Classification of extrahepatic manifestations of hepatitis C virus

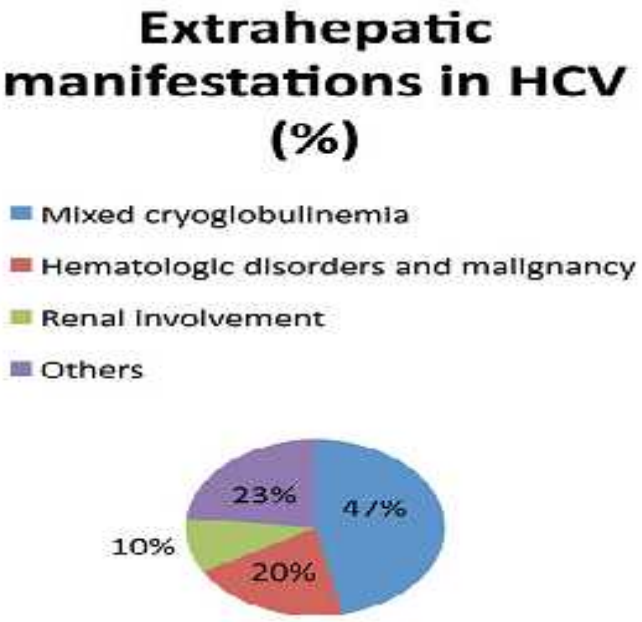

Figure (1): Extrahepatic manifestations of chronic viral hepatitis ***(Tarihi et al., 2012).***

Table (1): Extrahepatic manifestations Classification

Group A	Group B	Group C	Group D
Strong association	**Significant association**	**Similar pathological nature**	**Anecdotal**
-Mixed cryoglobulinemia -B-cell non-Hodgkin's Lymphoma	- Monoclonal gammopathies - Porphyria cutanea tarda - Lichen planus - Diabetes mellitus	-Autoimmune thyroiditis - Thyroid cancer - Sicca syndrome -Idiopathic lung fibrosis -Non cryoglobulinemic nephropathies -Erectile dysfunctions -Carotid atherosclerosis - Psychopathological disorders	- Psoriasis -Peripheral/central neuropathies -Rheumatoid arthritis - Polyarteritis nodosa - Behcet's syndrome - Dermatomyositis - Fibromyalgia - Chronic pruritus -Kaposi's pseudosarcoma - Vitiligo - Cardiomyopathies -Mooren corneal ulcer -Necrolyticacral erythema

(Hanouneh et al., 2008)

A. Renal Disorders

The prevalence of HCV infection in nephropathies is 10 to 20% in the United States, whereas it is 60% in Japan. When HCV-related glomerulonephritis develops, it typically occurs many years, often decades, after initial infection with HCV ***(Tsui et al., 2006)***.

Among Egyptian multitransfused HCV infected children and patients on maintenance hemodialysis, the frequency of

glomerulonephritis has been found to be 44.5% and 46.0%, respectively ***(Omran et al., 2013)*.**

Mechanisms of HCV-Associated Renal Disease:

Three potential mechanisms have been suggested for HCV induced renal disease:

1. Direct viral tissue damage resulting directly from HCV RNA and HCV related proteins.
2. Systemic immune response mediated by cryoglobulins, HCV antibody immune complexes, or amyloid deposition. It is believed to involve deposition of anti-HCV immunoglobulin and an IgM subtype rheumatoid factor ***(Iannuzzella et al., 2010).***
3. Insulin resistance linking HCV and renal disease through elevated levels of fasting serum insulin and insulin resistance. ***(Tsui et al., 2007; Hanouneh et al., 2008)***. Viral proteins themselves or host cell adaptive mechanisms could interfere with the insulin signalling pathway in hepatocytes, or the chronic inflammatory response in the liver could induce inflammatory response indirectly through cytokines that could induce inflammatory response systemically ***(Yaghobi et al., 2012).***

Clinical Syndromes of HCV-Related Renal Disease:

The most common HCV-related nephropathy is membranoproliferative glomerulonephritis, also commonly referred to as mesangiocapillary glomerulonephritis. It occurs in

the context of cryoglobulinemia or mononuclear cell-related membranoproliferative glomerulonephritis) ***(Dalrymple et al., 2007)***.

Less commonly, other types of HCV-related renal disease (mainly glomerular diseases) can develop, including IgA nephropathy, post infectious glomerulonephritis, membranous nephropathy, thrombotic microangiopathies, focal and segmental glomerulosclerosis, and fibrillary glomerulopathy ***(Tsui et al., 2007)***.

Clinical picture

Presentation ranges from hypertension (80%) associated with moderate renal insufficiency. 20%–35% of patients may present as a nephrotic syndrome and up to a quarter of patients will be nephritic. However, 10% of patients will present with rapidly deteriorating renal function ***(Khattab et al., 2010)***.

Treatment:

Treating the underlying HCV infection is important in preventing or limiting renal damage ***(Kamar et al., 2006)***.

Dual therapy with interferon and ribavirin has been shown to reduce viral load, as well as reduce proteinuria but has mixed success in improving glomerular filtration and creatinine levels. Data also support prolonged therapy for at least 48–52 weeks, irrespective of early reductions in HCV loads at 12 weeks ***(Sabry et al., 2002)***.