THE EFFECT OF DUAL THERAPY OF PEGYLATED INTERFERON AND RIBAVIRIN ON VIRAL LOAD AND LIVER ENZYMES OF HEPATITIS C PATIENTS

Thesis for Fulfillment of Master Degree

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بسم الله الرحمن الرحيم

"رب أوزعنى أن أشكر نعمتك التى أنعمت
على وعلى والدى وأن أعمل صالحا ترضاه
وأدخلنى برحمتك فى عبادك الصالحين "

صدق الله العظيم

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Dedication

To my wife **NADA**, for her support and effort allover my career.

To my **Father**, **Mother** and **my family**, for their praying and help allover my life.

Before them all for my son **KAREEM**, who is shinning my life.

Abstract

Chronic hepatitis C infection is recognized as an important health problem worldwide. Approximately 2–3% of the world population is infected with hepatitis C virus (HCV). Egypt has the highest seroprevalence for Hepatitis C, up to 20% in some areas.

Hepatitis C is caused by a small, single-stranded RNA virus. The virus replicates in the liver at a high rate, resulting in average serum HCV RNA levels of 1 to 2 million genome equivalents per milliliter.

Although some patients with acute HCV infection have an immune response sufficient to clear the virus, chronic infection develops in 55 to 85% of patients. Once established, chronic infection rarely resolves spontaneously. The hepatocellular injury seen in chronic HCV disease appears to be due not to a direct cytopathic effect of the virus but rather to an immunologically mediated injury, with natural killer cells and CD8+ T cells playing a central role.

The currently recommended therapy for chronic hepatitis C is a combination of formulations of interferon alfa and ribavirin.

Key Words:

HCV, Diagnosis of HCV, Treatment and prognosis

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LIST OF ABBREVIATIONS

1	**ARF**	Alternative Reading Frame
2	**ARFP**	Alternate Reading Frame Protein
3	**CPHL**	Central Public Health Laboratories
4	**EOT**	End of Treatment
5	**EVR**	Early Virological Response
6	**Hrp**	Horseradish Peroxidase
7	**IFNAR**	IFN-α Receptor
8	**IFNs**	Interferons
9	**IRES**	Internal Ribosome Entry Site
10	**ISDR**	Interferon Sensitivity Determining Region
11	**ISGF3**	Interferon Stimulate Transcription Factor-3
12	**JAK**	The Janus Kinase
13	**JEV**	Japanese Encephalitis Virus
14	**LFTs**	Liver Function Tests
15	**MPGN**	Membranoproliferative Glomerulonephritis
16	**NANBH**	Non-A Non-B Hepatitis
17	**NAT**	Nucleic Acid Testing
18	**NCR**	Noncoding Region
19	**NS**	Non-Structural
20	**OD**	Optical Density
21	**PKR**	Protein Kinase R
22	**QRT**	Quantitative Real Time
23	**RT-PCR**	Reverse Transcriptase PCR
24	**RT-PCR**	Real Time PCR
25	**s/co**	Sample / Cut Off
26	**SR-BI**	Scavenger Receptor Class B1
27	**STAT**	Signal Transducer and Activator of Transcription
28	**SVR**	Sustained Virological Response
29	**TLR 3**	Toll Like Receptor 3
30	**TMA**	Transcription-Mediated Amplification
31	**TMB**	Tetramethylbenzidine

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INTRODUCTION

Chronic hepatitis C infection is recognized as an important health problem. Approximately 2–3% of the world population is infected with hepatitis C virus (HCV). HCV is one of the leading causes of liver failure and cancer, and the single most common indication for liver transplantation ***(Rosen and Martin, 2000).***

Egypt has the highest seroprevalence for Hepatitis C, up to 20% in some areas. There is a hypothesis that the high prevalence is linked to a now-discontinued mass-treatment campaign for schistosomiasis, which is endemic in Egypt (***Frank et al., 2000)***. Regardless of how the epidemic started, a high rate of HCV transmission continues in Egypt, both iatrogenically, within the community and household ***(Thomas and Seeff, 2005).***

Hepatitis C is often clinically silent. Symptoms of jaundice develop in only one third of patients with acute infection, and most patients with chronic infection have few if any clinical manifestations, at least until cirrhosis is present. The natural history of hepatitis C is variable; cirrhosis eventuates in 20 to 30% of patients with chronic infection, generally after 2 to 3 decades ***(Thomas et al., 2005).*** Once cirrhosis evolves, hepatocellular carcinoma develops in 1 to 4% of these patients per year ***(Fattovich et al., 2004).***

Hepatitis C is primarily a blood-borne or parenterally transmitted infection. Vehicles and routes of parenteral transmission include contaminated blood and blood products, needle sharing, contaminated instruments (e.g., in hemodialysis, reuse of contaminated medical devices, tattooing devices, acupuncture needles, razors and manicure devices), occupational and nosocomial exposures such as needle stick injuries ***(Henderson, 2003).***

Hepatitis C is caused by a small, single-stranded RNA virus ***(Lauer and Walker, 2001 and Lindenbach and Rice, 2005).*** The virus replicates in the liver at a high rate, resulting in average serum HCV RNA levels of 1 to 2 million genome equivalents per milliliter ***(Neumann et al., 1998).*** The six genotypes of HCV vary in nucleotide sequence by 30 to 50% ***(Simmonds et al., 2005).***In the Middle East, almost all anti-HCV-positive individuals identified on blood donor screening are infected with genotype 4 ***(Simmonds et al., 1993).***

Although some patients with acute HCV infection have an immune response sufficient to clear the virus, chronic infection (defined as detectable HCV RNA for more than 6 months) develops in 55 to 85% of patients *(Hoofnagle et al., 2002).*

The currently recommended therapy for chronic hepatitis C is a combination of formulations of interferon alpha and ribavirin *(Strader et al., 2004).* Interferon alpha is a cytokine that has an important function in the innate antiviral immune response *(Feld and Hoofnagle, 2005).* Interferon alpha also induces the expression of genes involved in the immune response, resulting in activation of natural killer cells, maturation of dendritic cells, proliferation of memory T cells, and prevention of T-cell apoptosis *(Tilg et al., 1997).*

Ribavirin is an oral nucleoside analogue with broad activity against viral pathogens *(Feld and Hoofnagle, 2005).* Its mechanism of action against HCV is not completely clear. Ribavirin appears to have minimal direct activity against HCV replication *(Lau et al., 2002)*, but it may lead to rapid and lethal mutation of virions or depletion of intracellular guanosine triphosphate, which is necessary for viral RNA synthesis *(Crotty et al., 2000 and Maag et al., 2001).* Ribavirin also has immune modulatory effects *(Lau et al., 2002)*.

Interferon alpha was approved as a therapy for hepatitis C in 1991. However, the overall rate of sustained virologic response, defined as the absence of HCV RNA in serum at least 6 months after the discontinuation of therapy, was low (generally <20%) with interferon alpha monotherapy *(Myers et al., 2002).* The subsequent addition of the oral antiviral agent ribavirin to interferon led to a marked improvement in rates of sustained virologic response (40 to 45%) *(McHutchison et al., 1998 and Poynard et al., 1998).* Ribavirin alone lowered serum enzyme levels but had little effect on HCV RNA levels *(Brok et al., 2006).*

The most recent important advance in the treatment of hepatitis C was the development of a long-acting interferon, pegylated interferon (peg interferon), produced by the covalent attachment of polyethylene glycol to the interferon molecule. With its increased half-life, peg interferon can be given as a weekly dose *(Glue et al., 2005).* Two peg interferon formulations are currently approved for the treatment of hepatitis C: alfa-2a and alfa-2b. In two large trials of these agents, the rates of sustained virologic response to a 48-week course of peg interferon and ribavirin were 54 and 56%, as compared with 44 and 47%

with standard interferon and ribavirin and only 29% with peg interferon alone *(Manns et al., 2001 and Fried et al., 2002)*.

Patients who achieve a SVR have a greater than 95% chance of still being virus-free 5 years later *(Swain et al., 2007)*. This end point is associated with regression of fibrosis, decreased incidence of hepatocellular carcinoma, and overall reduced morbidity and mortality *(Veldt et al., 2007)*.

Aim of the study

The aim of this study was to assess the effect of dual treatment with pegylated interferon and ribavirin on the viral load and liver enzymes in patients with chronic HCV, in order to determine the efficacy of this combination therapy on hepatitis C patients in Egypt.