EFFECT OF OZONE THERAPY ON PATIENTS AFFECTED WITH THE HEPATITIS C VIRUS

Presented by

Wesam Abdel Rady Younes Abdel Megeed
B.Sc. Suez Canal University
(Microbiology – 2002)

Thesis

In Partial Fulfillment of the Requirements for the Degree of Master of Science
(Microbiology)

Faculty of Science
Botany Department
Cairo University

2012
ABSTRACT

Candidate Name: Wesam Abdel Rady Younes Abdel Megeed

Title of the thesis: **Effect of Ozone Therapy on Patients Affected with the Hepatitis C Virus**

Degree: Master (specialist.) Microbiology

Hepatitis C virus (HCV) is a worldwide medical problem. It is estimated that more than 300 millions on earth are suffering from HCV. Hepatitis C is a major public health problem in Egypt. It is known that more than 15% i.e. more than 12 millions of the populations in Egypt are suffering from HCV. Ozone is a powerful oxidizing agent,. The aim of this study is to evaluate the role of ozone as a safe line of treatment. This study includes 30 HCV patients. They were administered to the ozone treatment for 8 weeks. It was found that, following ozone therapy the viral load decreased in 63.3% of patients with HCV, ALT (57 ± 28 to 43 ± 14 IU/ml) after therapy, AST (52 ± 29 to 40 ± 18.6 IU/ml) after therapy. Ozone therapy was found to be an effective, safe and less expensive method for treating chronic hepatitis “C” patients.

**Keywords:** Hepatitis, Ozone therapy, HCV.

Supervisors:

- Prof. Dr. Hala Mostafa Yousef Habib
- Prof. Dr. Mohamed Nabil Mawsouf
- Prof. Dr. Hanaa Hasan Alam El-Din

Signature:

Prof. Dr. Maimona Abdel Aziz Kord  
Chairman of Botany Department  
Faculty of Science- Cairo University
APPROVAL SHEET

THESIS TITLE
EFFECT OF OZONE THERAPY ON PATIENTS AFFECTED WITH THE HEPATITIS C VIRUS

NAME OF THE CANDIDATE
WESAM ABDEL RADY YOUNES ABDEL MEGEED

This thesis has been approved for submission by the supervisors

1-PROF.DR. HALA MOUSTAFA YOUSEF HABIB
Professor of Virology Botany Department
Faculty of Science
Cairo University

2-PROF.DR. MOHAMED NABIL MAWSOUF
Professor of pain management and Head of Ozone therapy unit
National Cancer Institute
Cairo University

3-PROF.DR. HANAA MAHMOUD HASSAN ALAM EL-DIN
Professor of Virology and Immunology
Virology and Immunology Unit
National Cancer Institute
Cairo University

Prof. Dr. Maimona Abdel Aziz Kord
Head of Botany Department
Faculty of Science
Cairo University
M. Sc. Courses Studied

By

Mrs. Wesam Abdel Rady Younes Abdel Megeed

Beside the work presented in this thesis, the candidate has attended and passed successfully the following postgraduate courses as a partial fulfillment of the degree of Master of Science during the academic year 2003-2004.

1- Host parasite relationship
2- Hydrobiology
3- Industrial microbiology
4- Soil microbiology
5- Virology
6- Bacteriology
7- Tissue culture
8- Instrumental chemistry (spectroscopy)
9- Radiobiology
10- Biostatistics
11- German (as a foreign language)

Head of Botany Department

Prof. Dr. Maimona Abdel Aziz Kord
AKNOWLEDGEMENT

First of all, I do thank Allah for the gifts he has given to me.

I wish to express my deep gratitude to Prof. Dr. Hala Mostafa Yousef Habib, Prof. of Virology, Faculty of Science, Cairo University, for her supervision, advice, help, encouragement throughout the whole work.

My deep thanks and appreciations to Prof. Dr. Mohamed Nabil Mawsouf, Prof. of Pain Management at the National Cancer Institute, Cairo University, for his supervision, advice, help, encouragement, sincere guidance throughout ozone work and writing of the thesis.

I also wish to thank Prof. Dr. Hanaa Mahmoud Hasan Alam El-Din, Prof of Virology and Immunology Unit, Cancer Biology Department, National Cancer Institute, Cairo University. For her supervision, generous help, encouragement, sincere guidance throughout manuscript preparation and revision of the thesis.

I wish to express my deepest gratitude to Dr. Mohamed Mahmoud Hafez, Assistant Professor of Virology and Immunology Unit, Cancer Biology Department, National Cancer Institute, Cairo University. For his advice, help, encouragement, and sincere guidance throughout the practical work.

Finally, thanks are offered to all the staff members and colleagues of Virology and Immunology Unit, Cancer Biology Department, National Cancer Institute, Cairo University, for their help and cooperation.
DEDICATION

For my parents whom have been the wind beneath my wings until I completed this work

For my teacher Dr. Atef

For my kids Judy & Kady

I would like to express my sense of gratitude and thanks to my loving husband, Aly, for his continuous support and understanding. Without him, the finalization of this work would not have been done.
Abbreviations

2.3 - DPG  2.3- diphosphoglycerate
AIDS  Acquired Immuno Deficiency Syndrome
ATP  Adenosine triphosphate
ACTH  Adrenocortiocophic hormone
ALT  Alanine aminotransferase
AST  Aspartate aminotransrerase
AHT  Autohaemotherapy
BOEX  Body Ozone Exposure
CDC  Centers for Disease Control
CFC  Chlorofluorocarbon
COS  Chronic Oxidative Stress
CRP  C-Reactive Protein
CD8+  Cytotoxic T lymphocytes
CMV  Cytomegalovirus
DNA  Deoxyribohnucliaic Acid
ELISA  Enzyme- Linked- Immunosorbent Assay
EBV  Epstein barr virus
GGT  Gamma-glutamyl transpeptidase
GSH-PX  Glutathione peroxidase
GM-CSF  Granulocyt_ monosyte colony stimulating factor
HSPS  Heat shock proteins
CD4+  Helper T Lymphocytes
HAV  Hepatitis A virus
HBV  Hepatitis B virus
HCV  Hepatitis C virus
HDV  Hepatitis D Virus
HEV  Hepatitis E virus
HGV  Hepatitis G virus
HIV  Human Immunodeficiency Virus
IEM  Immune Electron Microscopy
IFN  Interferon
IL  Interleukin
IV  Intravenous
LDL  Low density lipoproteins
MAH  Major auto- hemotherp
NAC  N- acetyl- cysteine
NAD  Nicotinamide Adenine Dinucleotide
O2  Oxygen
O3  Ozone
PSC  Polar Stratospheric Clouds
PCR  Polymerase Chain Reaction
PVC  Polyvinyl Chloride
PTG  Protein Thiol Groups
ROS  Reactive Oxygen Species
RI  Rectal Insufflations
RA  Rheumatoid Arthritis
RNA  Ribonucleic Acid
SDM  Superoxicle Dismutase
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>SODS</td>
<td>Superoxide Dismutases</td>
</tr>
<tr>
<td>SFC</td>
<td>Synovial Fibroblast Cells</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid-reactive substances (marker of peroxidation)</td>
</tr>
<tr>
<td>TAS</td>
<td>Total Antioxidant Status</td>
</tr>
<tr>
<td>TAH</td>
<td>Transfusion-Associated Hepatitis</td>
</tr>
<tr>
<td>TGFα</td>
<td>Transforming Growth Factor alpha</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Transforming Growth Factor beta</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumor Necrosis Factor Alpha</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet light</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cells</td>
</tr>
</tbody>
</table>
# List of Contents

1- Introduction and aim of the work  
2- Review of literature  
2.1. Viral hepatitis  
  2.1.1. Hepatitis of nonviral etiology  
  2.1.2. Hepatitis of viral etiology  
2.2. Hepatotropic viruses  
  2.2.1 Hepatitis A virus  
  2.2.2 Hepatitis B virus  
  2.2.3 Hepatitis C virus  
  2.2.4 Hepatitis D virus  
  2.2.5 Hepatitis E virus  
  2.2.6 Hepatitis GB virus C  
  2.2.7 Hepatitis G virus  
2.3 Hepatitis C virus  
  2.3.1 History  
  2.3.2 Virion structure  
  2.3.3. Nature of the HCV genome and classification  
  2.3.4. Structure and function of HCV genome  
  2.3.5. Replication of HCV  
  2.3.6. Viral diversity  
  2.3.7. Quasispecies nature of HCV genome  
  2.3.8. Genotype of HCV  
2.4. Clinical feature of HCV infection  
  2.4.1. Acute hepatitis C  
  2.4.2. Chronic hepatitis C  
  2.4.3. Long – term outcome of chronic hepatitis  
2.5. Methods of detection  
  2.5.1. Enzyme – linked Immunosorbent Assays (ELISA)  
  2.5.2. Epidemiology  
  2.5.3. Prevalence of HCV infection in low – risk population  
  2.5.4. Parenteral transmission  
  2.5.5. Non parenteral transmission  
2.6. Interferon  
  2.6.1. Type I interferon  
  2.6.2. Type II interferon  
  2.6.3. Interferon – alpha and beta interferon  

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Introduction and aim of the work</td>
<td>1</td>
</tr>
<tr>
<td>2- Review of literature</td>
<td>3</td>
</tr>
<tr>
<td>2.1. Viral hepatitis</td>
<td>3</td>
</tr>
<tr>
<td>2.1.1. Hepatitis of nonviral etiology</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2. Hepatitis of viral etiology</td>
<td>4</td>
</tr>
<tr>
<td>2.2. Hepatotropic viruses</td>
<td>4</td>
</tr>
<tr>
<td>2.2.1 Hepatitis A virus</td>
<td>4</td>
</tr>
<tr>
<td>2.2.2 Hepatitis B virus</td>
<td>4</td>
</tr>
<tr>
<td>2.2.3 Hepatitis C virus</td>
<td>5</td>
</tr>
<tr>
<td>2.2.4 Hepatitis D virus</td>
<td>5</td>
</tr>
<tr>
<td>2.2.5 Hepatitis E virus</td>
<td>6</td>
</tr>
<tr>
<td>2.2.6 Hepatitis GB virus C</td>
<td>6</td>
</tr>
<tr>
<td>2.2.7 Hepatitis G virus</td>
<td>7</td>
</tr>
<tr>
<td>2.3 Hepatitis C virus</td>
<td>8</td>
</tr>
<tr>
<td>2.3.1 History</td>
<td>8</td>
</tr>
<tr>
<td>2.3.2 Virion structure</td>
<td>9</td>
</tr>
<tr>
<td>2.3.3. Nature of the HCV genome and classification</td>
<td>9</td>
</tr>
<tr>
<td>2.3.4. Structure and function of HCV genome</td>
<td>9</td>
</tr>
<tr>
<td>2.3.5. Replication of HCV</td>
<td>11</td>
</tr>
<tr>
<td>2.3.6. Viral diversity</td>
<td>11</td>
</tr>
<tr>
<td>2.3.7. Quasispecies nature of HCV genome</td>
<td>12</td>
</tr>
<tr>
<td>2.3.8. Genotype of HCV</td>
<td>12</td>
</tr>
<tr>
<td>2.4. Clinical feature of HCV infection</td>
<td>14</td>
</tr>
<tr>
<td>2.4.1. Acute hepatitis C</td>
<td>14</td>
</tr>
<tr>
<td>2.4.2. Chronic hepatitis C</td>
<td>14</td>
</tr>
<tr>
<td>2.4.3. Long – term outcome of chronic hepatitis</td>
<td>15</td>
</tr>
<tr>
<td>2.5. Methods of detection</td>
<td>15</td>
</tr>
<tr>
<td>2.5.1. Enzyme – linked Immunosorbent Assays (ELISA)</td>
<td>15</td>
</tr>
<tr>
<td>2.5.2. Epidemiology</td>
<td>17</td>
</tr>
<tr>
<td>2.5.3. Prevalence of HCV infection in low – risk population</td>
<td>17</td>
</tr>
<tr>
<td>2.5.4. Parenteral transmission</td>
<td>18</td>
</tr>
<tr>
<td>2.5.5. Non parenteral transmission</td>
<td>19</td>
</tr>
<tr>
<td>2.6. Interferon</td>
<td>20</td>
</tr>
<tr>
<td>2.6.1. Type I interferon</td>
<td>20</td>
</tr>
<tr>
<td>2.6.2. Type II interferon</td>
<td>20</td>
</tr>
<tr>
<td>2.6.3. Interferon – alpha and beta interferon</td>
<td>20</td>
</tr>
</tbody>
</table>
2.7. Ozone therapy
   2.7.1. History of ozone
   2.7.2. Chemical and physical properties of ozone
   2.7.3. Physical properties of ozone
   2.7.4. Ozone layer
   2.7.5. Ozone depletion
   2.7.6. Biological effects of ozone depletion
   2.7.7. Generation of medical ozone
2.8. Types of medical ozone generation
2.9. Methods of administration of medical ozone
   2.9.1. Systemic approach of ozone therapy
   2.9.2. Local approach of ozone therapy
2.10. How does medical ozone work?
2.11. Immune activating effect
2.12. Some evidence of the anti inflammatory effect of local ozone therapies
   2.14.1. Contraindications of ozone therapy
   2.14.2. Contra applications of ozone
2.15. Viral infections and ozone therapy.
   2.15.1. Chronic hepatitis B and C
3- Material and methods
   3.1. Routine laboratory investigations
   3.2. Collection of samples
3.3. Quantitative detection of HCV by real time – RCR
   3.3.1. Extraction kit preparation
   3.3.2. Extraction procedure
3.4. RT – RCR amplification for quantitative assay
   3.4.1. Preparation of master mix
   3.4.2. Preparation of RT – RCR assay
3.5. Ozone treatment
   3.5.1. Ozone treatment protocol
4- Results
   4.1. Clinical Characteristics
5- Discussion
6- Conclusion
7- Summary
8-References
**List of tables**

<table>
<thead>
<tr>
<th>No. of table</th>
<th>Title of table</th>
<th>Page no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summary of indications &amp; underlying effects of ozone</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>Clinical data of 30 patients having chronic HCV under study</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>30 patients having chronic HCV both before and after treatment</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>log HCV-PCR, HB, TLC, ALT, AST values before and after ozone therapy</td>
<td>55</td>
</tr>
</tbody>
</table>
# List of figures

<table>
<thead>
<tr>
<th>No. of figures</th>
<th>Title of figures</th>
<th>Page no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ozone generator1.</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>Ozone generator2</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>Major Auto-Hemotherapy</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Longevity</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>Local application of ozone for leg</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
<td>Log PCR before and after ozone therapy</td>
<td>52</td>
</tr>
<tr>
<td>7</td>
<td>Mean HB levels before and after ozone therapy</td>
<td>52</td>
</tr>
<tr>
<td>8</td>
<td>Mean TLC levels before and after ozone therapy</td>
<td>53</td>
</tr>
<tr>
<td>9</td>
<td>Normal and abnormal ALT enzyme levels before and after ozone therapy</td>
<td>53</td>
</tr>
<tr>
<td>10</td>
<td>Normal and abnormal AST enzyme levels before and after ozone therapy</td>
<td>54</td>
</tr>
<tr>
<td>11</td>
<td>Pie charts are showing Normal and abnormal ALT &amp; AST levels before and After Ozone therapy.</td>
<td>54</td>
</tr>
<tr>
<td>12</td>
<td>Percentage of negative PCR following therapy</td>
<td>55</td>
</tr>
</tbody>
</table>
Introduction
And
Aim of the work
1. Introduction and aim of the work

Hepatitis C (HCV) is a global disease with an expanding incidence and prevalence base. From massive public health importance, hepatitis C presents supremely challenging problems in view of its adaptability and its pathogenic capacity. The unique strategies that HCV utilizes to parasitize its host make it a formidable enemy and therapeutic interventions need considerable honing to counter its progress. Ozone, because of its special biological properties, has theoretical and practical attributes to make it a potent HCV inactivator.

Chronic hepatitis C is characterized by the presence of HCV RNA and the elevation of liver enzymes for 6 months or longer. Patients may be asymptomatic, or at times suffer an acute exacerbation with a return of symptoms. Approximately 75% of acutely ill patients continue into a chronic phase evidenced by parameters of viral presence. (sunnen, 2001).

Hepatitis C can only be distinguished from other viral hepatic conditions by serological and virological determinations. Liver enzymes characteristically affected by HCV infection include serum alanine transfeferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGTP), and alkaline phosphatase. (sunnen 2001).

Cirrhosis, a diffuse disruption of liver tissue architecture with regenerative nodules surrounded by fibrosis, is an important sequel to hepatitis C. Within 20 years post HCV infection 20 to 25% of patients will develop cirrhosis. Hepatic decompensation ensues with ascites.

Ozone: Antiviral properties recently, there has surged renewed interest in the potential of ozone for viral inactivation. It has long been established that ozone neutralizes bacteria, viruses, and fungi in aqueous media. This has prompted the creation of water purification processing plants in many major municipalities worldwide.
Some viruses are much more susceptible to ozone's action than others. It has been found that lipid-enveloped viruses are the most sensitive. This group includes, amongst others, HCV, Herpes 1 and 2, Cytomegalus, HIV1 and 2.

In HCV, viral load appears to be a major factor in the invasiveness and virulence of the disease process. Preliminary research has shown that reduction of viral load in Hepatitis C by means of ozone therapy can significantly normalize hepatic enzymes and improve measures of global patient health. Volunteers administered ozone therapy according to the method outlined below achieved a viral load reduction in the order of 5 log, or 99.9%, along with a normalization of liver enzyme levels. (Sunnen 2001)

The present work aims to evaluate the effectiveness of ozone therapy on viral load and other important comparing parameters in chronic hepatitis C virus.
Review of literature
1. **Review of literature**

2.1. **Viral Hepatitis:**

Viral hepatitis is a systemic infection affecting the liver. Five different human hepatitis viruses have been recognized and characterized in detail. The five established agent are hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV). Three additional viruses designated GB virus C hepatitis G, TT virus (TTV), and SEN virus have also been described; however, these blood borne agents have not been established as human hepatitis pathogens. All human hepatitis viruses are RNA viruses except for hepatitis B, which is DNA virus despite difference in their genomes molecular structure and virus.

Hepatitis is an inflammation of the liver. It is usually caused by viral infections or non viral agents (toxic agents or drugs) but may be an autoimmune response. It is characterized by jaundice, abdominal pain, liver enlargement and sometimes fever. It may be mild, or can be acute leading to fulminant hepatitis, others, usually viral infections are chronic, and can lead to cirrhosis and liver cancer. The different types of viral hepatitis are:-

A) Formerly called infectious hepatitis (Francis et al., 1984).
B) Serum hepatitis (Robinson and Marion 1984).
C) Formerly called parentally transmitted non-A non-B hepatitis (Choo et al., 1989).
D) Delta hepatitis (Wang et al., 1986).
E) Enterically transmitted non-A non-B (McCaustland et al., 1991), (Linnen et al., 1996) and GB virus C (Simons et al., 1995), cryptogenic (caused by a virus as yet unidentified).

Non hepatotropic agents, such as yellow fever, Epstein Barr Virus (EBV) and cytomegalovirus (CMV) as well as parasites and bacteria, can cause hepatitis. A famous drug which can do damage of the liver (if taken in excess) is acetaminophen. Other types of hepatitis are autoimmune and alcoholic hepatitis.

2.1.1. **Hepatitis of Nonviral Etiology**

Not all diseases clinically or biochemically resembling acute viral hepatitis can be attributed to known or unknown viral agents. Unusual but instructive examples include Q fever and *Pneumocystis cannii infection*. Q fever, due to infection by rickettsial organism *Coxiella burnetti*, may be associated with hepatitis like illness with hepatomegaly, jaundice and abnormal liver...
Review of literature

chemistries (Yale et al., 1994). Hepatitis due to drugs must always be a consideration in the
evaluation of hepatic dysfunction. The variety of drugs associated with hepatitis is extraordinarily
broad (Gradon et al., 1992).

2.1.2 Hepatitis of Viral Etiology

2.2 Hepatotrophic viruses:

Hepatitis A and E viruses are enterically transmitted (via oral ingestion of fecal material from
infected patients), whereas the hepatitis B, C, and D viruses are transmitted by parenteral rout (via
exposure to infected body fluids). The enterically transmitted hepatitis viruses generally produce a
self-limiting hepatitis followed by complete recovery. The parentally transmitted hepatitis viruses
can persist as chronic infection in the form of chronic hepatitis and eventual development of
cirrhosis and hepatocellular carcinoma.

2.2.1. Hepatitis A virus:

Hepatitis A virus was the first virus to be isolated from patients with enterically transmitted
hepatitis.

The virus is hepatotropic, and, following replication in infected hepatocytes, is excreted in
the bile and feces. The virus is remarkably stable in the environment, and is most commonly
transmitted by the ingestion of facially contaminated foods and materials. HAV is transmitted by
person-to-person contact in a variety of settings in households (24%), in such institutions as day
care centers (18%), with male homosexual activities (11%), and during travel to endemic areas
(4%) (Francis et al., 1984). HAV can also be transmitted parentally.

HAV produces a mild clinical hepatitis lasting several weeks, followed by complete
recovery. Fulminant hepatitis can occur, particularly in older patients (over 50 years) and in patients
with existing chronic liver disease. The fatality rate is 3% reported cases of hepatitis A (lender et
al., 1985). More commonly, the viral infection is effectively cleared by the host immune system
with neutralizing antibodies and virus-specific cytotoxic T lymphocytes. HAV infection is not
generally associated with chronic hepatitis, cirrhosis, or hepatocellular carcinoma.

2.2.2. Hepatitis B virus:

Hepatitis B virus consists of a partially double-stranded DNA genome enclosed by envelope
proteins (HBsAg). The genome is packaged with a core protein (HBcAg) and a DNA polymerase.
HBV is transmitted parentally from infected patients (Robinson and Marion, 1984), where concentrations in the blood may approach a high level of $10^{10}$ per milliliter (Heathcote et al., 1974). Settings where HBV may be transmitted include parenteral exposure to infected blood products, such as during transfusions (Alter et al., 1975), use of contaminated needles in intravenous drug administrations, sexual intercourse (Szmuness et al., 1975) and from mother to infant parentally or in uterus (Beasly et al., 1977). Infants of HBeAg-positive mothers have a 70 % chance of infection; following acute infection these infants have a 90 % chance of developing chronic infection (Gerty et al., 1977).

2.2.3. Hepatitis C virus:
Hepatitis C virus was first isolated from non-A, non-B infectious plasma (Choo et al., 1989). HCV is a member of the family Flaviviridae, which includes yellow fever virus, dengue viruses, and Japanese encephalitis virus (Miller et al., 1990). HCV is a single stranded, linear, positive-sense RNA genome, which is approximately 9.5 Kb in length (Choo et al., 1989). Hepatitis C is the most common cause of nonalcoholic liver disease in the USA. More than 150,000 individuals are acutely infected with HCV annually (Alter et al., 1989). Hepatitis C is transmitted through blood and blood products. Risk factors include blood transfusion and intravenous drug abuse (Alter et al., 1991). Sexual and perinatal transmissions are less important routes in hepatitis C. Chronic HCV infection, however, develops in up to 80% of cases and progress to cirrhosis in 50% of them (Seeff et al., 1992).

2.2.4. Hepatitis D virus:
Hepatitis D virus is an enveloped RNA virus whose envelope proteins are derived from proteins synthesized by hepatitis B virus (Wang et al., 1986). It, therefore, requires the presence of HBV for infection. Acute HDV infection may occur concurrently with acute HBV infection (HDV/HBV co-infection), or may take place in the setting of an established chronic HBV infection. Acute HDV/HBV co-infection usually produces a self-limiting hepatitis, with only 2 to 5 % persisting as chronic HDV infection. Acute HDV super-infection in 70 to 90% of cases and the progression to cirrhosis is more accelerated as compared with chronic HBV infection alone (Smedile et al 1994). Generally, acute HDV super-infection presents as an acute exacerbation of chronic HBV hepatitis. However, 17% of patients with acute HDV super-infection develop fulminate hepatitis (Buti et al., 1987).

HDV has not been associated with hepatocellular carcinoma. Patients with HBsAg-positive cirrhosis and HCC have a prevalence of chronic HDV infection similar to that of patients with
HBsAg-positive cirrhosis without HCC. Chronic HDV infection, however, may accelerate the development of cirrhosis, thereby increasing the risk for hepatocellular carcinoma.

2.2.5. Hepatitis E virus:

Hepatitis E virus is enterically transmitted without serologic evidence for HAV infection was first noted in a retrospective study of an epidemic in Delhi, India, in 1956. More than 29,000 cases were reported after the water supply in the region became contaminated with sewage. Hepatitis E virus was identified as the etiology for enterically transmitted non-A, non-B hepatitis following confirmatory transmission studies in nonhuman primates (McCaustland et al., 1991).

Like HAV, HEV is a non-enveloped RNA virus that produces a transient clinical hepatitis followed by complete recovery. Acute HEV infection in pregnant women, particularly those in the third trimester, can be fulminant and fatal in 20% of cases (Khuroo et al., 1981). There is no evidence for chronic hepatitis or persistent viremia following acute HEV infection. HEV virus particle can be detected in the stool by immune electron microscopy and HEV antigen in infected hepatocytes by immunofluorescent probes.

2.2.6. Hepatitis GB virus C:

Evidence for the existence of additional hepatitis viruses is largely circumstantial but intriguing nonetheless. In addition to the absence of markers of HAV, HBV, HCV, HDV and HEV in 5% of community-acquired hepatitis and 10% of transfusion-associated hepatitis in the United States, marker-negative hepatitis has been widely reported elsewhere. For example, non-A, B, C, D, E hepatitis has been recognized as an important problem in Spain (Buti et al., 1994). Among 341 Spanish patients with acute hepatitis, seen over a 2 years period beginning in 1989, 33% could be identified as hepatitis A, 20% as hepatitis B, 6% as hepatitis D, and 22% as hepatitis C, no cases of hepatitis E were identified. Thus, about 20% of acute hepatitis could not classify serologically. A small proportion of patients with post transfusion hepatitis in China may also be attributable to the presence of additional hepatitis viruses. From 57 patients with acute transfusion-associated non-A, non-B hepatitis, 53 (93 %) were positive for anti-HCV or HCV RNA (Luo et al., 1993). One-third of those with evidence of HCV infection were also positive for HBV DNA, although HBsAg was undetectable. Four (7 %) of patients had no detectable markers, including HCV RNA and HBV DNA suggesting the presence of other agents.