OZONE THERAPY IN PATIENTS WITH VIRAL HEPATITIS “C”
A CLINICAL STUDY

BY

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Aim of the Study

• To evaluate the effectiveness of ozone therapy in hepatitis C genotype 4 infection.
• To evaluate a proposed ozone therapy protocol in HCV genotype 4 treatment.
• This is meant to be a provisional study to be followed by another study.
Why HCV?

- Worldwide medical problem (estimated that more than 300 millions suffering from HCV)
- Major medical problem in EGYPT (postulated that more than 15% i.e. more than 10 millions of the population are suffering from HCV)
- Slowly progressing, detected mainly accidentally, devitalizing and difficult to treat
Why HCV? (cont.)

• In most cases it leads to complications e.g. liver cirrhosis, ascitis, liver carcinoma and ultimately liver cell failure

• Not only a medical problem, but also an economic problem (less work, less production and very high costs of usual treatment)
Hepatitis “C”

- There is a worldwide prevalence of genotypes 1,2 & 3.
- In Africa genotype 4 and 5 are more dominant.
- In Asia genotype 6 is more dominant.

*Genotype differences have shown varying susceptibility to antiviral therapy.*
Liver Cirrhosis is estimated to develop in 20-25% of patients with HCV within 20 years.

- Hepato-cellular carcinoma (5% of patients)
- Hepatic decompensation and liver cell failure with ascites
Hepatitis “C” (cont.)

- The main line of treatment nowadays for hepatitis C include interferon and ribavirin.

- Ribavirin and interferon have significant medical and psychiatric side effects.
Why Ozone?

- Known Anti-viral action
- Safe if used by experts
- Less costs
- Classic method of treatment very expensive with major side effects and minimal cure results
Mode of Action of Ozone
Mode of Action of Ozone

• Inactivation of bacteria, viruses, fungi and protozoa:
  – Disrupts the integrity of bacterial cell envelope through oxidation of the phospholipids and lipoproteins.
  – Damages viral capsid and upsets reproductive cycle by disrupting virus-to-cell contact with peroxidation.
  – Inhibits cell growth at certain stages in fungi (V. Bocci & R. Viebahn)
Mode of Action of Ozone (cont.)

• Enhancement of Circulation:
  – Reduce clumping of red cells and restore its flexibility and oxygen carrying ability.
  – Arterial oxygen partial pressure increases and viscosity decreases leading to better tissue oxygenation
  – Oxidizes plaque in arteries allowing removal of the breakdown products.

(V. Bocci & R. Viebahn)
Mode of Action of Ozone (cont.)

- Stimulation of oxygen metabolism:
  - Increases red blood cell glycolysis rate → stimulation of 2,3-diphosphoglycerate (2,3-DPG) → increase of oxygen released to tissues.
  - Activates Krebs cycle by enhancing oxidative carboxylation of pyruvate → stimulating production of ATP.

(V. Bocci & R. Viebahn)
Mode of Action of Ozone (cont.)

• Stimulation of the production of the enzymes which act as a free radical scavengers and cell wall protectors: Glutathione Peroxidase, Catalase, and Superoxide Dismutase

• Dissolution of malignant tumors: Ozone inhibits tumor metabolism, oxidizes the outer lipid layer of malignant cells and destroys them through cell lysis
Mode of Action of Ozone (cont.)

- An inducer of cytokines (other oxidizing agents in appropriate amounts induce the synthesis of cytokines in monocytes and lymphocytes)
- H$_2$O$_2$ crosses the cell membrane and activates the cytoplasmic gene-regulatory nuclear factor kappa B, ultimately causing the transcription of mRNAs of several cytokines, namely interleukin (IL-1,2,4,6,8,10), tumor necrosis factor (TNF-$\infty$) and interferon (IFN $\beta$ and $\gamma$) (V. Bocci 2002)
Ozone and HCV

- Lipid and protein peroxides, produced in small amounts by ozonation, have demonstrable antiviral properties.
- Ozone tends to stimulate leucocyte function and cytokine production.
- Ozone increases the oxygen saturation (P02) in erythrocytes and enhances their pliability so that capillary circulation is facilitated (Gérard & Sunnen 2001).
**Ozone and HCV (cont.)**

- 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 (Gérard & Sunnen 2001).
Antiviral effect of Ozone

- Denaturation of virions through direct contact with ozone. Ozone disrupts viral envelope proteins, lipoproteins, lipids, and glycoproteins.
- The presence of numerous double bonds in these unsaturated molecules makes them vulnerable to the oxidizing effects of ozone.
Antiviral effect of Ozone (cont.)

- Molecular architecture is disrupted and widespread breakage of the envelope ensues.
- Deprived of an envelope, virions cannot sustain nor replicate themselves.
Antiviral effect of Ozone (cont.)

- Ozone proper, and the peroxide compounds it creates, may directly alter structures on the viral envelope which are necessary for attachment to host cells. Peplomers, the viral glycoproteins protuberances which connect to host cell receptors are likely sites of ozone action.
Antiviral effect of Ozone (cont.)

- Alteration in peplomer integrity impairs attachment to host cellular membranes foiling viral attachment and penetration.
- Ozone induces the release of cytokines by leucocytes.
- Stimulation of the immune mechanisms will lead to significant reduction of circulating virions (Gérard & Sunnen 2001).
PATIENTS
and
METHODS
Patients and Methods

- Sixty patients type 4 HCV
  - 45 males
  - 15 females
- Age range 34 to 65 years
- Randomly selected
Patients and Methods (cont.)

• Investigations
  – C.B.C, Liver function tests, AFP
  – Prothrombin time and concentration
  – Antibodies for Bilharziasis
  – PCR quantitative for HCV
  – Abdominal ultrasonography
Patients and Methods (cont.)

- Ozone Treatment Protocol:
  - MAH + RI three times per week for eight weeks followed by two times per week for sixteen weeks
  - The rationale of start low and go slow was respected
Patients and Methods (cont.)

• MAH 25µ/ml ozone \( \rightarrow 25 \rightarrow 30 \rightarrow 30 \rightarrow 35 \)
\( \rightarrow 35 \rightarrow 40 \rightarrow 40 \rightarrow 45 \rightarrow 45 \rightarrow 50 \rightarrow 50 \)
\( \rightarrow 55 \rightarrow 55 \rightarrow 60 \ldots \ldots \ldots \text{The volume was constant 150 ml} \)

• Rectal Insufflations 20µ/ml ozone \( \times 300 \text{ ml} \)
\( \rightarrow 20\mu/ml \times 300 \text{ ml} \rightarrow 25\mu/ml \times 300 \text{ ml} \rightarrow 25\mu/ml \times 300 \text{ ml} \rightarrow 30\mu/ml \times 300 \text{ ml} \rightarrow 30\mu/ml \times 300 \text{ ml} \rightarrow 35\mu/ml \times 300 \text{ ml} \rightarrow 35\mu/ml \times 350 \text{ ml} \rightarrow 35\mu/ml \times 350 \text{ ml} \rightarrow 40\mu/ml \times 350 \text{ ml} \ldots \ldots \)
Patients and Methods (cont.)

- Investigations were repeated after 8 and 24 weeks of treatment (but in this study we are focusing on PCR quantitative and Liver enzymes)
- General health and daily activity were observed
RESULTS
**PCR Average Number**

- **Start**: 1041354
- **After 2 Months**: 423215
- **After 6 Months**: 293150

**Change Percent**
- **59%**
- **72%**

The diagram shows the average number of PCR results over time, with a significant decrease after 2 and 6 months.
Viral Load Category Change

Start

After 2 Months

After 6 Months

High
Moderate
Low
V. Low
Negative

> 200,000
50,000 : 200,000
2000 : 50,000
0 : 2000
0

18
23
28
1
0

11
6
28
3
12

5
4
27
2
22

Start
2 mnth
6 mnth
After 2 Months

Number -ve: 12
% -ve: 20.00%

After 6 Months

Number -ve: 22
% -ve: 37.67%
After 2 Months

- Number: 55
- %: 91.67%

After 6 Months

- Number: 57
- %: 95.00%
**SGOT-AST**

<table>
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<th>Number</th>
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<tbody>
<tr>
<td>Normal</td>
<td>36</td>
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<tr>
<td>Abnormal</td>
<td>24</td>
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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Start</td>
<td>48</td>
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<tr>
<td>2 Month</td>
<td>12</td>
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SGPT - ALT

<table>
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<tr>
<td>Normal</td>
<td>38</td>
<td>51</td>
</tr>
<tr>
<td>Abnormal</td>
<td>22</td>
<td>9</td>
</tr>
</tbody>
</table>

![Bar chart showing the number of normal and abnormal SGPT-ALT results at the start and after 2 months.](chart.png)
**SGPT - ALT**

![Graph showing SGPT levels over time](image)

- **Baseline** and **2 months** are marked on the x-axis.
- The y-axis represents **SGPT** levels.
- The **Normal cutoff value** is indicated.

The graph demonstrates the changes in SGPT levels from baseline to 2 months.
DISCUSSION
and
CONCLUSION
As a preliminary study Ozone therapy was found to be an effective, safe and less expensive method in Hepatitis "C" patients. But further studies are important.
Pilot Studies

- MAH twice/week for 2 months
- MAH three times/week for 2 months
- RI twice/week for 2 months
- RI three times/week for 2 months

Following rationale of start low and go slow

*Good results but not as good as the mentioned protocol*
Pilot Studies (cont.)

- Combined MAH & RI once /week following 2 months of mentioned protocol was not satisfactory from the general condition point of view.
- but if we shift to twice / week the general condition is better.
Comments

• Why Combined MAH & RI?
  * pilot studies
  * hyper-oxygenation of portal circulation (Knoch et al 1987)

• Why the enzymes are normal in some patients before ozone therapy?
  * Medications affecting the enzymes level
Important Postulated Reasons for Less Effectiveness

- Diet
- Exertion
- Hepatotoxic Drugs
Recommendation for Further Study

- Double Blind Randomized Placebo Controlled Study
- Selection of Patients
  - No complications (Cirrhosis, Ascitis, Liver cell failure, etc..)
  - No associated chronic disease (Diabetes, Bilharziasis, etc..)
Recommendation for Further Study (cont.)

- Long term study for one year
- Follow-up by observation and investigations for another year
- Evaluation should be based on many parameters
  - General condition
  - Liver Function tests (synthesis, excretions, integrity)
Recommendation for Further Study (cont.)

- Quantitative PCR as one only parameter for evaluation can be considered as a guide for evaluation but is not conclusive
  - sharp fluctuations of viral load
  - so far there is no precise and accurate method for quantitative estimation of viral load
  - different methods, different units and wide variation from one laboratory to another must be put in consideration
Thank You