A Double Blind Study Comparing Virucare and Interferon as Treatment for Hepatitis C Virus and its Complications

Maher El-Aaser[1]; Nabil Omer[2]; Hosam A. Hamid[3]; Monir Hamad[4] and Samy Omer[5].

[1]Faculty of Medicine–Cairo University, Chairman of Hepatology & Oncology Centre–Maadi; [2]Faculty of Medicine Monifia University, Chairman of Hepatology Centre–Giza; [3]Medical Genetics Center, Ain Shams University; [4]Hepatology Consultant of Rashid Hospital- Dubai, UAE; [5]Hepatology Consultant of Maadi Millitary Hospital.

ABSTRACT

Objective: To present the results of a comparative double blind study between Virucare and Interferon to evaluate their efficacy, safety and tolerability in treating Hepatitis C Virus and its complications.

Key Words:

Hepatitis C, polymerase chain reaction, interferon.

Corresponding Author:

Maher El-Aaser
E-mail: melaaser@yahoo.com

INTRODUCTION

Hepatitis C Virus (HCV) is a significant cause of morbidity and mortality. This infectious disease has spread to more than 200 million people worldwide with steady increases in the number of cases each year[1]. Despite recent progress, current therapies (First generation–nonspecific) remain inadequate for the majority of patients as it is a mutant virus due to the lack of editing control during its replication (With 6 different Genotypes in at least 25% of the HCV nucleotides sequence, in addition to many subtypes).[2]

The study of HCV molecular virology is providing an increasing number of new anti-HCV targets (second generation therapies–specific for anti-protease and anti-polymerase drugs) that are still striving to achieve their cure rate, side effects and cost for treating each of the different HCV genotypes. Virucare is a newly invented drug of plant origin that has achieved and measured its milestones without the need for differentiation between the genotypes to result in a successful treatment reducing HCV viremia and its complications.[3]

Virucare treats HCV and its complications by acting through many mechanisms as: anti-protease, anti-polymerase, anti-oxidant, anti-fibrosis, anti-carcinogenesis, and anti-steatosis; as well as helping hepatic cell regeneration, increases liver vitality and functionality, regulates bile

and acts as a liver decongestive by lowering portal hypertension. Clinical studies including more than 20,000 patients with statistical analysis had abstracts published in many medical journals showing the mechanism of actions of each active ingredient of Virucare in treating HCV and its complications. Those studies revealed a high cure rate efficacy that was well tolerated without any side effects hindering the patient's general health throughout the duration of treatment.[4,5]

Virucare has passed all phases of pharmaceutical researches successfully from 1994 up to 2002 in the National Organization of Drug Control and Research (NODCR), Ministry of Health and Population for Egypt. Virucare has also fulfilled all certificates of composition, analysis of the active ingredients, and methodology of identification tests for active ingredients by Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC); as well as certificates of disintegration specification of its tablet form, stability and toxicity analysis.

First registered in the Ministry of Health– UAE in 2004 under No. 796/04, Virucare was subsequently registered as an Invention Patent under No. 33673 in 2006 in Academy of Scientific Research and Technology – Patent Office, Ministry of State for Scientific Research in Egypt.

PATIENTS AND METHODS

The study was performed from January 2007 until October 2007 on patients of different sex (Males and females), age, nationality, and HCV genotypes. These studies were carried out in multi-hepatology centers throughout Egypt, United Arab Emirates, Kuwait, Yemen, and Kingdom of Saudi Arabai.

The study compared 200 patients consisting of 100 patients (84 male and 16 female) for Group A (Virucare and Eprex), their age ranged from 15 years to 65 years, and another 100 patients (87 male and 13 female) for Group B (Peginterferon and Ribavirin) with the same age range as group A. Both patient groups were given treatment for 10 months in the form of weekly subcutaneous injections and daily oral capsules.

There were single weekly injections as follows:

- Group A received Eprex 4000u (An erythropoietin hormone as bone marrow stimulant to form red blood cells in patients).
- Group B received Peginterferon alpha-2a (40 kilo-dalton) 180 microgram as the anti-HCV agent.

The oral tablets were as follows:

- Group A received a daily dose of Virucare in 3 tablets (600 mg per tablet) every 12 hours given 15 minutes before the meal as the anti-HCV agent.
- Group B received a daily dose of Ribavirin in 3 tablets (200mg per tablet) every 12 hours after meals as adjuvant treatment.

The genotypes that were found in the two groups are as follows:

Genotyope	Type 1	Type 2&3	Type 4	Total
Group A	18	15	67	100
Group B	16	20	64	100
Total	34	35	131	200

All patients were examined prior to the administering of any treatment and periodically checked to monitor their progress. All data was collected unbiased and blindly as Group A patients or Group B patients; and the results were analyzed and certified by the Medical International Statistics Institute (M.I.S.), which is the largest statistical consultant and analysis office for research studies in Egypt.

The following is a list of the tests that were used to gauge the effects of the two treatments:

1. Full clinical Examination to assess the general condition of each patient was performed monthly to monitor the progress throughout the treatment. There was a scoring system created (From 1 to 10) to measure the severity of symptoms that are caused by HCV (This scoring system is established and used as a clinical practice protocol in Cairo University Hospital for assessment of HCV severity). There were four categories to analyze these symptoms: Bad (1-3), Impaired (4-6), Moderate (6-8), and Good (8-10). In the patients who scored a "6" or "10", additional laboratory and ultrasonography data was used to assess their overall condition. Each score was assessed by the following criteria:

a. Bleeding gums.
b. Oedema of lower limbs.
c. Nausea and vomiting.
d. Fatigue.
e. Gastritis.
f. Irritable bowel.
g. Urine color.
h. Pruritus.
i. Skin pigmentations.
j. CNS disorientation.

2. Laboratory examinations were performed at the start of the study and then every month or every second month, depending on the examination, throughout the duration of the treatment. However, the genotype was only assessed once at the beginning of the study for each patient. Monthly Examinations

a. CBC, renal function tests, creatinine and urea levels.
b. Liver function tests: total bilirubin, ALT, AST and prothrombin time and concentration.
c. AFP (Alpha feto-protien).

Bi-monthly Examinations.
a. HCV – RNA PCR showing the HCV viremia present in the blood (IU/ml).

3. Ultrasonography of the abdomen was performed at the start and every month of the treatment to assess the size and texture of the liver and spleen; as well as monitor the portal vein diameter and any other abnormality that may be detected.

4. Liver biopsy and histopathological examination was only performed at the start and the end of the treatment.

a. HCV chronic active hepatitis (CAH) activity score was rated as follows: minimal (0-2/18), mild (3-8/18), moderate (9-14/18), and severe (15-18/18).
b. Liver fibrosis score was rated as follows: minimal (0-1/6), mild (2-3/6), moderate (3-4/6), and severe (5-6/6).

RESULTS:

I. General Condition and Mean Score:
At the completion of the study it was

observed that there was a 100% improvement of Group A-Virucare patient's general condition; where as 98% of Group B-Interferon patient's general condition degenerated with 23% resulting in a "bad" general condition. Due to the natural origin of active ingredients and zero side effects from the usage of Virucare, the Group A patient's general health was not prohibited from making a recovery while HCV was treated.

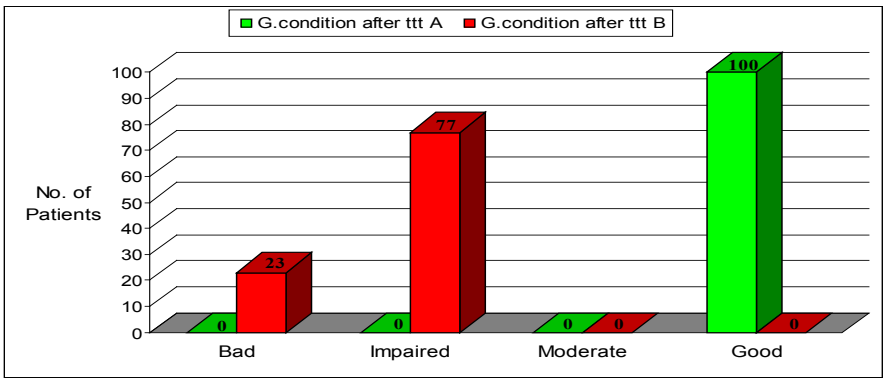


Fig.1: Comparison between group A and B as regards general condition after treatment.

I. The general condition mean score (1 to 10) was determined for both groups, before and after the treatment. After treatment the scores were:

i. Group A–Virucare patient's had a mean score of 8.59, and

ii. Group B–Interferon patient's had a mean score of 4.16.

This significant difference between the two scores is a representation of the intrinsic benefits that Virucare treatment offers patients in addition to curing their HCV, when compared to the present Interferon treatment and its adjoining side effects.

II. Renal Function Tests:

There was a favorable result seen for Group A–Virucare patient's serum creatinine and serum urea levels, which surpassed the results that was recorded for the patient's of Group B–Interferon:

i.The serum creatinine mean for Group A–Virucare was significantly reduced (before ttt 1.14±0.18; after ttt 0.93±0.14) , while the mean for Group B–Interferon was increased (Before ttt 1.16±0.14; after ttt 1.18±0.19).

ii. The serum urea mean for Group A-Virucare was reduced (Before ttt 33.43 ± 10.82; after ttt 25.26±6.96), where as the mean for Group B–Interferon has increased (Before ttt 32.44±7.27; after ttt 38.18±8.51).

N.B.

- We randomly select the patients and the serum creatinine was within normal range in all patients before the study and throughout the study period (N: 0.2–1.4 mg/dl).
- Estimation of serum creatinine help us for assessment of drug toxicity.

III. Liver Function Tests:

The results below show the significant

effects that Virucare and Interferon have on the liver's function and resulting condition. Taking notice to the mean results after the two treatments you will see a close comparison in the favorable results.

However, the Virucare treatment had more effective results than Interferon as seen in the improvement of the mean level of total bilirubin, the mean serum level of ALT and AST, and the mean value of prothrombin concentration after completing the treatment course.

i. Total Bilirubin:
Comparison between mean values of total bilirubin level before and after treatment with virucare (Group A): (Before ttt 1.72±0.56; after ttt 0.18±0.13) ($p<0.001$ [HS]), where as the mean for Group B–Interferon was (Before ttt 1.6±0.49; after ttt 0.84±0.11) ($p<0.001$ [HS]).

ii. ALT:
Comparison between mean values of ALT level before and after treatment with virucare (Group A): (before ttt 89.54±59.26; after ttt 25.37±5.56) ($p<0.001$ [HS]), where as the mean for Group B–Interferon was (Before ttt 92.48±53.74; after ttt 27.06±7.76) ($p<0.001$ [HS]).

iii. AST:
Comparison between mean values of AST level before and after treatment with virucare (Group A): (Before ttt 104.45±64.77; after ttt 32.56±12.49) ($p<0.001$ [HS]), where as the mean for Group B – Interferon was (Before ttt 114.4±62.66; after ttt 39.08±14.06) ($p<0.001$ [HS]).

iv. Prothrombin Time and Concentration:
Comparison between mean values of prothrombin time and concentration before and after treatment with virucare (Group A): (before ttt 57.09±10.33; after ttt 73.81±7.74) ($p<0.001$ [HS]), where as the mean for Group B–Interferon was (Before ttt 56.18±9.42; after ttt 71.64±9.02) ($p<0.001$ [HS]).

N.B.: We assessed prothrombin concentration percentage (Normal value is 70-110%).

IV. AFP: (Normal range up to 10.9ng/ml):
It was noticed that Virucare is more effective in improving the mean value of serum AFP rather than Interferon, as there was a highly significant difference between the mean levels of AFP after the treatment.

Comparison between mean values of AFP level before and after treatment with virucare (Group A): (Before ttt 18.86±13.13; after ttt 7.69±3.95) ($p<0.001$ [HS]), where as the mean for Group B – Interferon was (Before ttt 16.39±9.36; after ttt 11.38±7.46) ($p<0.001$ [HS]).

N.B.: Estimation of AFP is not diagnostic but it is prognostic follow up assessment for HCV activity and also for liver fibrosis and malignancy etc…

V. Platelets:
The results from this test clearly show an advantage received from the effects of Virucare beyond Interferon to improve the mean value of the platelet count.

Comparison between mean values of platelets count before and after treatment with virucare (Group A): (Before ttt 97.34±43.77; after ttt 164.16±48.5) ($p<0.001$ [HS]), where as the mean for Group B–Interferon was (Before ttt 144.65±35.54; after ttt 76.57±19.22) ($p<0.001$ [HS]).

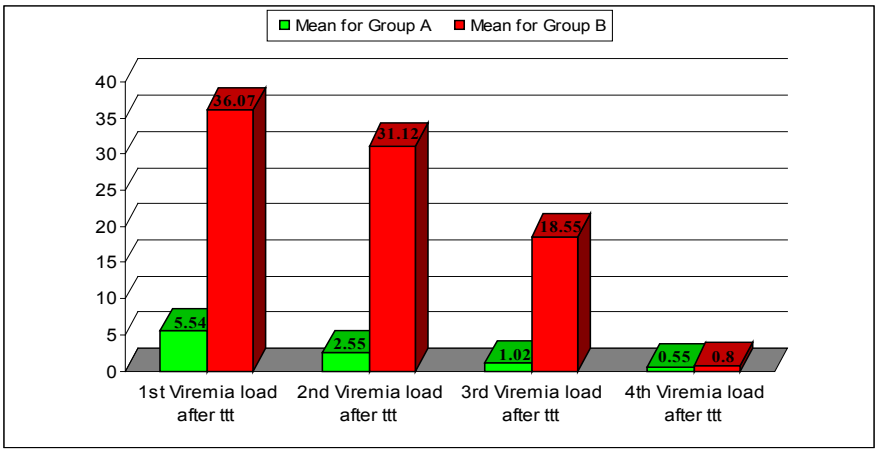
N.B.: The mechanism of increase platelet count: decrease portal vein pressure which lead to splenic decongestion so decrease hypersplenism and this will improve bone marrow activity as liver detoxification, function is improved.

VI. HCV-RNA PCR:

Comparing the results between the mean values of viremia load after treatment, it is obvious that Virucare is more effective than Interferon. In addition, it is also necessary to notice the quick reduction of viremia load experienced by Virucare patients, which is highly significant, and not experienced by the Interferon patients. The mean viremia load before the treatment was 18.22 and 15.09 (100,000 IU/ml) for Group A and Group B, respectively.

Comparison between mean values of Viremia Load after treatment with Virucare and Interferon (10,000 IU/ml)

	1st Viremia load after ttt	2nd Viremia load after ttt	3rd Viremia load after ttt	4th Viremia load after ttt
Mean for Group A	5.54	2.55	1.02	0.55
Mean for Group B	36.07	31.12	18.55	0.8



P<0.001 (Highly Significant).

Fig. 2 : Comparison between mean values of Viremia Load after treatment with Virucare and Interferon (10,000 IU/ml)

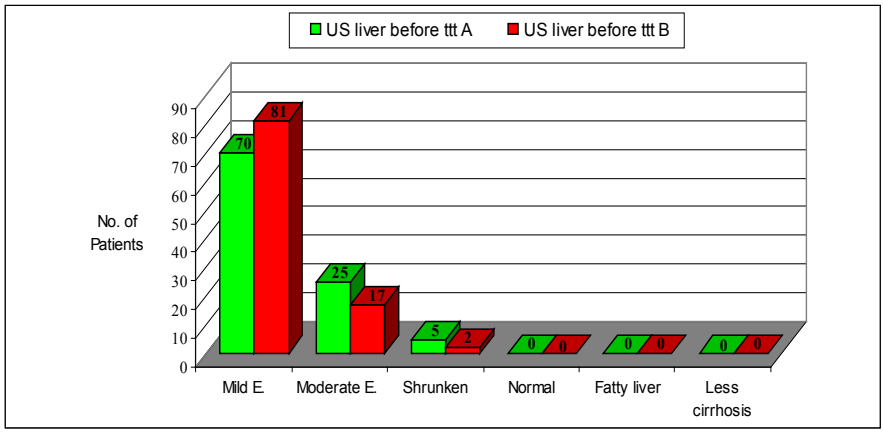
Comparison between mean values of Viremia Load after treatment with Virucare and Interferon (10,000 IU/ml).

VII. Ultrasonography (US):

Both groups of patients experienced similar positive results for the liver US

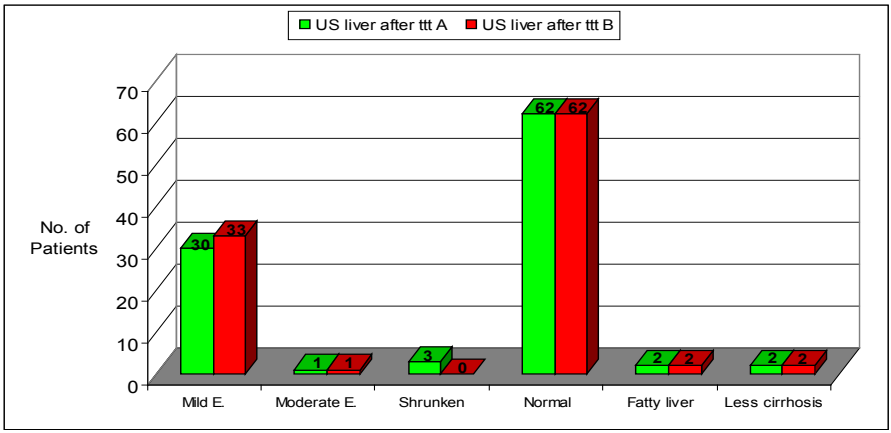
examination, spleen US examination, and portal vein US examination.

i. Liver US examinations (Size and texture) for both Virucare and Interferon found that a majority of livers (62%) achieved normal size at the close of the treatment course.



P<0.001 (Highly Significant)

Fig. 3 : Liver US examinations (size and texture) for both Virucare and Interferon (before treatment).

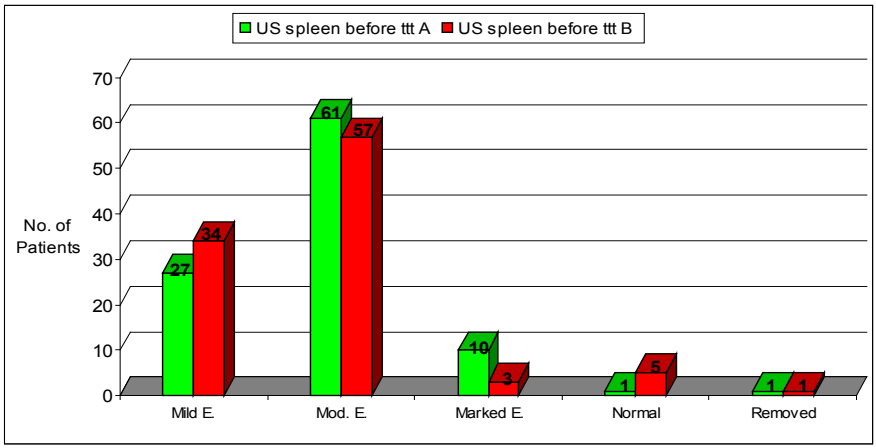


P<0.001 (Highly Significant)

Fig. 4 : Liver US examinations (size and texture) for both Virucare and Interferon (after treatment).

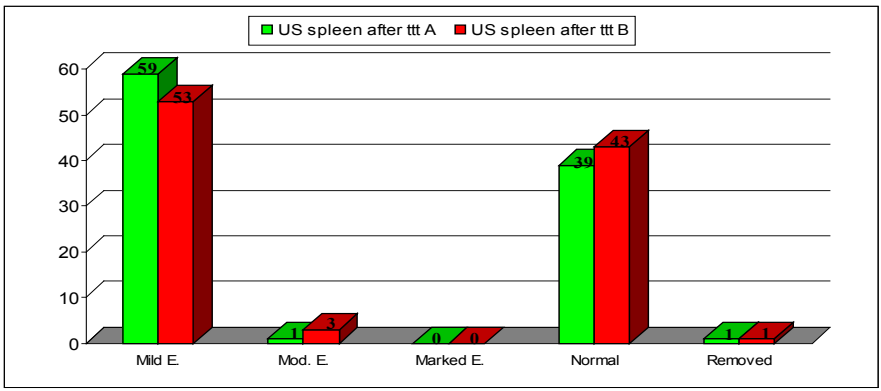
N.B.: The assessment of size of the liver is a clinical as well as US assessment (Mild: 2 cm below costal margin, moderate: 4 cm below cost margin, while huge enlargement is>5cm below costal margin.

ii. Spleen US examinations were similar in their outcome as 39% and 43%, for Virucare and Interferon respectively, had achieved normal spleen sizes for their patients.



P<0.001 (Highly Significant)

Fig. 5 : Spleen US examination for both Virucare and Interferon (before treatment).



P<0.001 (Highly Significant)

Fig. 6 : Spleen US examination for both Virucare and Interferon (after treatment).

N.B.: Removed: means splenectomy.

iii. Portal Vein US examination (Size; normally 8–12mm) achieved similar positive results for both the Virucare and Interferon.

Comparison between mean values of U/S portal vein diameter before and after treatment with virucare (Group A): (Before ttt 13.55±1.41; after ttt 10.19±1.77) (p<0.001 [HS]), where as the mean for Group B–Interferon was (Before ttt 13.3±1.33; after ttt 10.21±1.78) (p<0.001 [HS]).

VIII. Liver Biopsy and Histopathological Examination:

i. Chronic Active Hepatitis (CAH) patients had a greater improvement as a result of the Virucare treatment than the Interferon treatment; accounting for 80% of the patient's. Comparing the changes in the number of "Moderate" level CAH patients between Group A–Virucare and Group B–Interferon

you can see that there was a substantial improvement in Group A from 93 patients to 20 patients (A 78% reduction) versus Group B from 90 patients to 43 patients (A 46% reduction), which is highly significant. In addition, there

Comparison between CAH activity before and after treatment with Virucare (Group A).

	CAH activity before ttt A	CAH activity after ttt A
Arrested	0	4
Minimal	1	15
Mild	6	61
Moderate	93	20

P<0.001 (Highly significant)

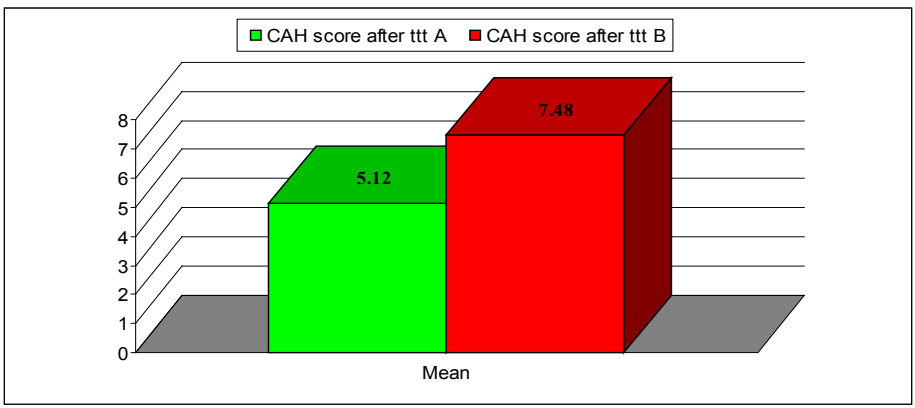
Comparison between CAH activity before and after treatment with Interferon (Group B).

	CAH activity before ttt B	CAH activity after ttt B
Arrested	0	0
Minimal	1	9
Mild	9	48
Moderate	90	43

P<0.001 (Highly significant)

were 4 patients whose results achieved the "Arrested" level of CAH activity from Group A, but there were zero patients from Group B to experience the same progress.

ii. CAH Mean Score was rated from 0–18 as follows: Minimal (0-2/18), Mild (3-8/18), Moderate (9-14/18), and Severe (15-18/18). At the completion of the study, Group A (Score 5.12) re-

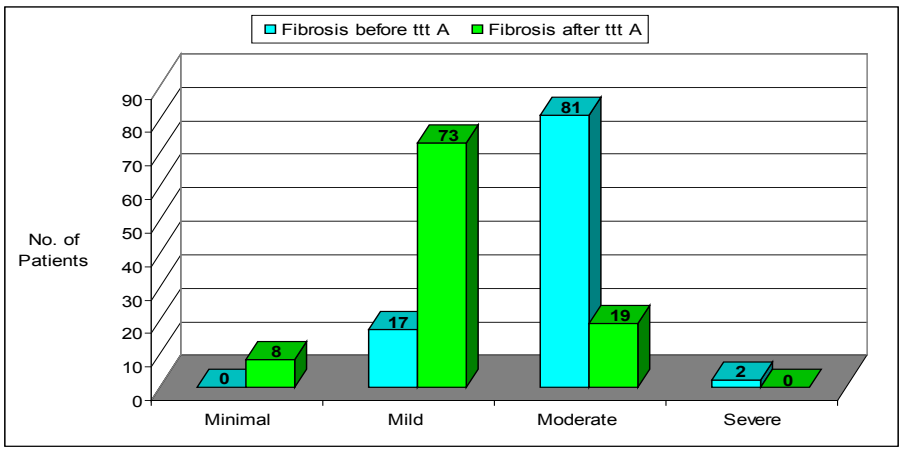


P < 0.001 (Highly Significant)
Fig. 7 : CAH score for both Virucare and Interferon (after treatment).

sulted in a lower CAH mean score that Group B (Score 7.48) showing the effectiveness and greater response to the Virucare treatment than to the Interferon treatment.

iii. Fibrosis:

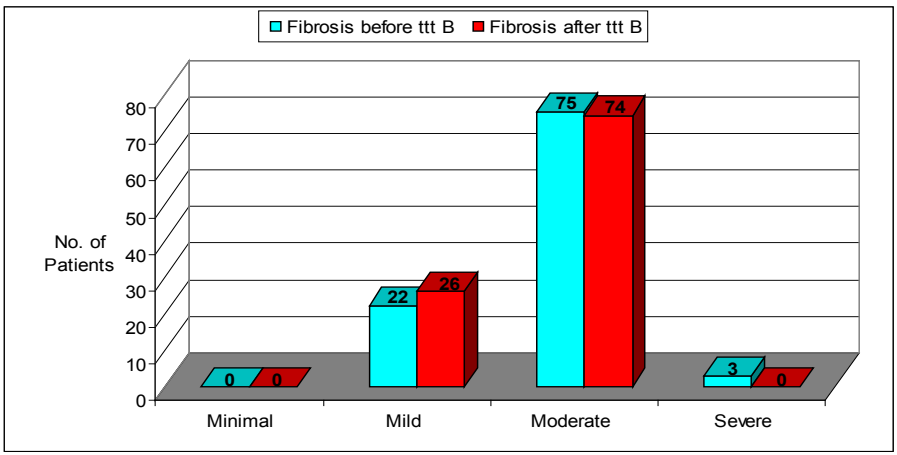
There is a highly significant difference of improvement between Group A-Virucare patient's fibrosis condition, before and after the treatment, which



P<0.001 (Highly Significant)

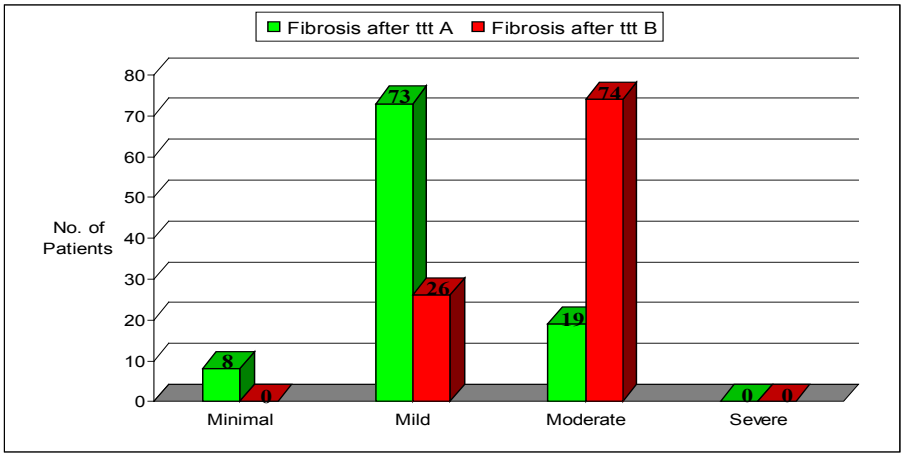
Fig. 8 : Assessement of liver fibrosis before and after treatment with Virucare.

However, Group B–Interferon patient's fibrosis remained almost constant from the start to the finish of the treatment.



P < 0.001 (Highly Significant)

Fig. 9 : Assessement of liver fibrosis before and after treatment with Interferon.



P<0.001 (Highly Significant)

Fig. 10 : Comparison between Virucare and Interferon as regard liver fibrosis (after treatment)..

was not shared by Group B–Interferon patients. The liver biopsy demonstrates that Virucare has an excellent impact on improving fibrosis of the liver as shown in the data chart below.

iv. Fibrosis Mean is based out of a possible score of 6 and was rated as follows: Minimal (0-1/6), Mild (2-3/6), Moderate (3-4/6), and Severe (5-6/6). There was a highly significant improve-

Comparison between fibrosis score of 6 before and after treatment with Virucare (Group A)

	Mean	SD
Fibrosis score before ttt A	3.41	0.73
Fibrosis score after ttt A	2.14	0.49

$p < 0.001$ (Highly significant)

Comparison between fibrosis score of 6 before and after treatment with Interferon (Group B)

	Mean	SD
Fibrosis score before ttt B	3.16	0.73
Fibrosis score after ttt B	2.8	0.61

$p < 0.001$ (Highly significant)

N.B.: CAH mean score and fibrosis score are according to Ishak scoring basis.

ment in Group A–Virucare fibrosis mean value of 37%, while Group B-Interferon fibrosis mean value only improved by 11%.

DISCUSSION

The study of HCV molecular virology is providing an increasing number of new anti-HCV targets. These in turn can and are being translated into the development of new drugs which offer the prospect of more effective antiviral therapies.

HCV is a positive single stranded RNA virus, its 9.6kb genome encodes a single-3000 amino acid polyprotein which is proteolytically processed by cellular and viral proteinases into structural (Components of mature virus) and non-structural (Elements proposed to help replicate new virons) proteins.[6,7]

The structural protein include the core and envelope proteins the former is thought to serve as a nucleocapsid protein. It also appears to interact with several host cells, signaling pathways and has been implicated in steatosis.

Both the core protein and the viral RNA genome are encapsulated in a lipid envelope in which are embedded the two HCV envelope proteins E1 and E2. A characteristic of all viruses which like HCV, replicate via an RNA dependent RNA polymerase is a relatively high rate of spontaneous mutations (Presumably due to the lack of an editing function of the polymerase. The resulting genetic heterogeneity means that the virus present at any given time in an individual is best thought of as a population of related but slightly different and ever changing genomes (Genotypes) which provide the virus with an increased ability to respond to change selective pressures which arise from the host's immune response or the administration of antiviral drugs. The heterogeneity is particularly pronounced in certain regions of the genome such as the hypervariable region (HVR) of envelope E2. Understanding of the function of individual non-structural proteins is better for some than others. For example, the enzymatic activities of the regions NS3 and NS5. NS3 has a protease activity responsible for librating and down stream NS proteins from the polyprotein precursor.[8]

NS5 encodes the catalytic activity for RNA directed RNA polymerase. Several viral proteins also localize to cellular lipid layers and under certain circumferences may be able to gain access to the cell's nucleus which is significant for the viral life cycle.[9]

Anti-HCV therapies are passed through phases and generations. The first one would include currently approved drugs (Interferon and Ribavirin) which are not really specific for HCV and the benefit of ribavirin is only achieved when used in combination with interferon.[10]

The second generation drugs are specifically designed for and directed at HCV-specific targets (NS3 and NS5B). NS3 is a 630 amino acid protein having 2 domains with different genetically enzymatic activities, both thought to be essential for the HCV life cycle which are serine protease and RNA helicase. The protease activity is most efficient when combined with NS4 which serves as a cofactor segments promotes the NS3 activities. RNA helicase activity is required to help facilitate unwinding

of duplex RNA during replication of the viral genome. The crystal structure of each isolated NS3 domain as well as the complete HCV molecule, anti-HCV therapy drugs are designed and optimized to be more specifically NS3 enzymes inhibitors in various stages of development.[11]

NS5B, is another obvious target for drug development as its RNA-dependent (RNA-polymerase) is a virus specific feature as our human cells are not supposed to have such enzymes and therefore the potential for selective target exists.

Moreover, like protease inhibitor drugs there is precedence for a collection of drugs successfully targeting polymerases in HCV.[12] The newly invented virucare drug has many active ingredients which show antiprotease, antihelicase and antipolymerase activities, hence, it has specific anti-HCV actions put it in the category of the second generation of specific anti-HCV therapy with higher cure rate and less side effects than the currently approved first generation non-specific anti-HCV therapy drugs.

CONCLUSION

Virucare is a more effective and a safer line of treatment for HCV infection and its complications than Interferon. Results from this study, show the Virucare treatment was equal to more successful than the Interferon treatment with major differences being the improvement in health due to a lack of side effects and an inexpensive manufacturing costs.

REFERENCES

1. Durand J, Lefevre P, Kaplanski G, Soubeyrand J. Patient-to-patient transmission of hepatitis C virus. Lancet 1995; 3; 345 (8962): 1442-3.

2. Foy E, Li K, Wang C, Sumpter R,Jr, Ikeda M, Lemon SM, et al. Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. Science 2003; 16; 300 (5622): 1145-8.

3. Houghton M, Weiner A, Han J, Kuo G, Choo QL. Molecular biology of the hepatitis C viruses: Implications for diagnosis, development and control of viral disease. Hepatology 1991; 14 (2): 381-8.

4. Meisman TA. In: Peach K, Tracey V, editors. Modern methods in plant analysis. Berlin: Springer-Verlag; 1995: 450-98.

5. Gisolvold O, Rogers CH. The chemistry of plant constituent. Minneapolis: Bargress Publishing Co.; 1983.

6. Bartenschlager R, Lohmann V. Replication of hepatitis C virus. J. Gen. Virol. 2000; 81 (Pt 7): 1631-48.

7. Branch AD. Hepatitis C virus RNA codes for proteins and replicates: Does it also trigger the interferon response? Semin.Liver Dis. 2000; 20 (1): 57-68.

8. You S, Stump DD, Branch AD, Rice CM. A cis-acting replication element in the sequence encoding the NS5B RNA-dependent RNA polymerase is required for hepatitis C virus RNA replication. J. Virol. 2004; 78 (3): 1352-66.

9. Reed KE, Rice CM. Overview of hepatitis C virus genome structure, polyprotein processing and protein properties. Curr. Top. Microbiol. Immunol. 2000; 242: 55-84.

10. Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, et al. Peginterferon alfa-2a in patients with chronic hepa-

titis C. N. Engl. J. Med. 2000; 7; 343 (23): 1666-72.

11. Yao N, Reichert P, Taremi SS, Prosise WW, Weber PC. Molecular views of viral polyprotein processing revealed by the crystal structure of the hepatitis C virus bifunctional protease-helicase. Structure 1999; 15; 7 (11): 1353-63.

12. Lesburg CA, Cable MB, Ferrari E, Hong Z, Mannarino AF, Weber PC. Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site. Nat. Struct. Biol. 1999; 6 (10): 937-43.