ORIGINAL ARTICLE

EFFECT OF VITAMIN "E" SUPPLEMENTS IN THERAPY OF CHRONIC HEPATITIS C: A HISTOLOGICAL STUDY

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ABSTRACT

Background: Chronic Hepatitis C is a major healthcare problem worldwide. Current therapies are alpha interferon used either alone or in combination with ribavirin. Antioxidant approaches have not been investigated sufficiently. The aim of this study was to evaluate the efficacy of vitamin E in combination with interferon and ribavirin in reducing hepatic inflammation and fibrosis**.**

Material & Methods: This experimental study was conducted at Khyber Teaching Hospital Peshawar, Pakistan, from October 2002 to March 2003. Fourteen male patients suffering from chronic hepatitis C were divided into two groups; In group A interferon and ribavirin combination therapy was given and in group B vitamin E was added. Pre and post treatment percutaneous liver biopsy was taken and examined under light microscopy. Both groups were graded according to Knodell Histology Activity Index numerical scoring system. The slides were scored for grades of necrosis and stages for fibrosis.

Results: It was observed that in vitamin E treated patients the progression of fibrosis was prevented or even reversed. In group A, post-therapeutic mean of Knodell score decreased from 11.00±3.74 to 6.60±1.50 ($p<0.02$). In group B post-therapeutic mean of Knodell score decreased from 10.5± 4.24 to 4.37± 2.06 ($p<0.005$). Comparing the post-therapeutic Knodell scores in A and B, it was 6.60 ±1.50 and 4.37 ±2.06 respectively ($p<0.05$).

Conclusion: Addition of Vitamin E to combination therapy with interferon and ribavirin prevents the progression of fibrosis in chronic hepatitis C patients.

Key words: Hepatitis C, Vitamin E, Antioxidant, Liver fibrosis.

INTRODUCTION

Chronic infection with Hepatitis C virus (HCV) is a major healthcare problem and a leading cause of chronic liver disease worldwide. After acute infection with HCV, 50–85% of patients develop chronic infection which can lead to hepatic decompensation, cirrhosis and hepatocellular carcinoma.[1] Since the identification of the virus in the late 1980s,[2] significant progress has been made in understanding the epidemiology of HCV,[3] but substantial uncertainty persists regarding risks of progression to advanced liver disease.[4]

Chronic hepatitis C has a prevalence of at least 1% world wide.[5] In Pakistan, up to 10% adults have hepatitis C virus infection. [6] Generally, patients develop chronic hepatitis in 10 years from the day of initial infection, cirrhosis in 20 years and hepatocellular carcinoma in 30 years.[7] Factors such as age, gender, use of alcohol and age at infection, influence the progression to cirrhosis but cannot accurately predict the risk of developing cirrhosis in patients with chronic hepatitis C.[8]

The main causes of liver fibrosis in industrialized countries include chronic HCV infection, alcohol abuse, and nonalcoholic steatohepatitis. Patients with cirrhosis can remain free of major complications for several years (compensated cirrhosis). Decompensated cirrhosis is associated with short survival, and liver transplantation is often the only effective therapy.[9] Hepatic fibrosis is the result of the wound healing response of liver to repeated injury.[10] Following hepatic injury, hepatic stellate cells transdifferentiate to become extracellular matrix (ECM) producing myofibroblasts, promoting hepatic fibrogenesis.[11] If hepatic injury persists, eventually the liver regeneration fails and hepatocytes are substituted with abundant ECM, including fibrillar collagen. As fibrotic liver disease advances, disease progression from collagen bands to bridging fibrosis to frank cirrhosis occurs.[12]

The pathogenesis of HCV-induced liver fibrosis is poorly understood due to lack of a rodent model of persistent HCV infection.[13] HCV escapes immune response and infects hepatocytes, causing oxidative stress and inducing the recruitment of inflammatory cells. Both factors lead to Hepatic stellate cells (HSC) activation and collagen deposition. Moreover, several HCV proteins directly stimulate the inflammatory and fibrogenic actions of HSCs.[14]

Liver biopsy is considered the gold-standard method for assessment of liver fibrosis.[15] Histologic examination is useful in identifying the underlying cause of liver disease and assessing the necro-inflammatory grade and the stage of fibrosis.[16] Clinically relevant progression of chronic hepatitis C would be better estimated by the fibrosis stage than by the grade of histological activity alone.[17]

Since the demonstration in 1990s, that even advanced liver fibrosis is reversible, researchers have been stimulated to identify antifibrotic therapies.[18] In contrast to the traditional view that cirrhosis is an irreversible disease, recent evidence indicates that even advanced fibrosis is reversible.[19] In experimentally induced fibrosis, cessation of liver injury results in fibrosis regression.[20] In humans, spontaneous resolution of liver fibrosis can occur after successful treatment of the underlying disease.[20, 21] It may take years for significant regression to be achieved. Importantly, nearly half of the patients with cirrhosis exhibit reversal to a significant degree.[22]

Antifibrotic therapies for liver disease take many forms, from antioxidants to anti-inflammatory to cytokine antagonists and growth factors. A variety of compounds have been identified that have the potential to prevent or even reverse fibrosis. Most of the studies so far conducted, regarding the treatment of patients suffering from chronic hepatitis C, are based only on the role of interferon alone or in combination with ribavirin without a particular antioxidant. Results of these studies are assessed by the biochemical analysis or the grades and stages of microscopic observations with no morphological details.

The present study was undertaken to study the necro-inflammation and fibrosis in liver on light microscopy before and after treatment with interferon (a cytokine antagonist), ribavirin (an oral antiviral) and vitamin E (an anti-oxidant).

MATERIAL AND METHODS

This experimental study was conducted at Khyber Teaching Hospital Peshawar, Pakistan, from October 2002 to March 2003. Adult males, 18 to 60 years of age, suffering from chronic hepatitis C with the following minimum hematologic, biochemical and histological criteria were included in the study:

- Hemoglobin > 13g/dL for males, > 12g/dL for females
- WBC count >3000/mm^3
- Granulocyte count >1500/mm^3
- Platelets count >100,000/mm^3
- Serum Bilirubin and Albumin within normal limits
- Elevated ALT levels prior to entry into the study
- Hepatitis C Antibody (MEIA/4th generation ELISA)
- HCV-RNA by DNA assay or PCR

Exclusion Criteria:

- Patients unwilling for liver biopsy
- Co-infection with HBV
- Wilson's disease
- Autoimmune hepatitis
- Alcoholic liver disease
- Obesity induced liver disease
- Drug related liver disease
- Evidence of advanced liver disease such as history or presence of ascites, bleeding varices, spontaneous encephalopathy

Fourteen male patients suffering from chronic hepatitis C were included in the study. They were grouped as A and B. (Table-1)

Table-1: Experimental design.

Groups	Subgroups	Number of patients	Treatment received
A	A1 (before treatment)	6	Interferon with Ribavirin
	A2 (after treatment)	6	
B	B1 (before treatment)	8	Interferon + Ribavirin with Vitamin–E
	B2 (after treatment)	8	

Group A included six patients treated with Interferon and Ribavirin only. This group was called A1 before treatment and A2 after treatment. Group B included eight patients treated with Interferon, Ribavirin and vitamin E. This group was called B1 before treatment and B2 after treatment.

The patients were started on 3 MIU of Interferon 2b three times a week subcutaneously along with 800-1200 mg of Ribavirin (depending on the weight of the patient) and Vitamin-E 600mg BD orally daily. The duration of treatment was six months.

A pre and post-treatment percutaneous liver biopsy was taken directly through the skin from the liver with a Tru-cut disposable liver biopsy needle of 1.5 cm notch size. The size of biopsy specimen, varied between 1 and 3mm in length and between 1.2 and 2mm in diameter. Both groups were graded and staged according to Knodell Histology Activity Index (HAI) numerical scoring system of liver biopsy specimens.[23]

Biopsy specimens were fixed in neutral buffered formalin (pH o.7) for 24 hours, embedded in paraffin and sectioned at 4-5 micron thickness.[24]

Three slides of each case were prepared in a serial order and stained by the following methods.

- Hematoxylin and eosin for routine microscopy.[24]
- Periodic Acid Schiff for demonstration of carbohydrates. [24]
- Masson's Trichrome for demonstration of collagen fibers.[25]

The measurements were made by means of an occulometer at magnification of 10x and 40x. stromal connective tissue and infiltration with inflammatory cells was labeled as minimum (±), mild (+), moderate (++) and marked (+++).

RESULTS

In group A1, connective tissue component was moderately increased (++) (Fig.1), and remained as such in most of the patients, rather it was markedly increased (+++) in one patient in group A2. (Fig.2).

In group B1, a moderate increase (++) in connective tissue was observed (Fig.3), which was decreased after treatment to a minimum (±) level in group B2. (Fig. 4)

Pre treatment microscopic observations revealed that the lobular architecture of liver was disturbed with nodule formation and fatty change in a few cases both in group A and B, while the architecture was almost normal with few atypical or incomplete nodules after the treatment. This improvement was more marked in vitamin E treated patients of group B.

Necrosis with fatty change was seen in one patient in group A1 while no such alteration could be observed in A2 (Figure-1 & 2). Well marked necrosis and abnormal cells with fatty change were seen in two cases in group B1 while less number of such cells was observed in B2 group (Figure-3 & 4).

Slides of both groups A and B were scored for grades and stages according to which, there was a significant decrease in activity grade in both the groups after the treatment. (Table-2 & 3)

While in the fibrosis, the change was less significant in group A as compared to the change in group B after the treatment. (Table-4)

Table-2: Comparison of grades and stages in Group A1 and A2.

Observations (Mean±SD)	A1	A2	P value
Grades	9.00 ± 2.82	4.00 ± 1.09	P < 0.001
Stages	2.00 ± 1.09	2.66± 1.03	P > 0.10
Knodell Score	11.00 ± 3.74	6.60 ± 1.50	P < 0.02

Table-3: Comparison of mean grades and stages in Group B1 and B2.

Observations (Mean±SD)	B1	B2	P value
Grades	8.50 ± 3.20	3.00 ± 1.13	P < 0.001
Stages	2.00 ± 1.41	1.37 ± 1.06	P > 0.20
Knodell Score	10.5 ± 4.24	4.37 ± 2.06	P < 0.005

Table-4: Comparison of mean grades and stages in Group A2 and B2.

Observations (Mean±SD)	B1	B2	P value
Grades	4.00 ± 1.09	3.00 ± 1.13	P < 0.10
Stages	2.66 ± 1.03	1.37 ± 1.06	P > 0.05
Knodell Score	6.00 ± 1.50	4.37 ± 2.06	P < 0.05

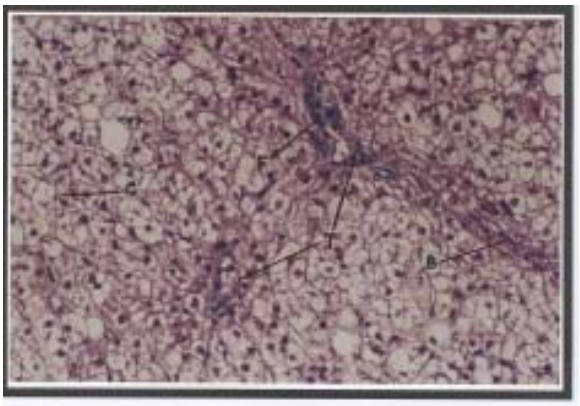

Fig. 1: Photomicrograph from liver of group A2 patient showing an area between two hepatic lobules with a portion of triad illustrating (N) a normal cell with a nucleus and nucleolus, (B) a binucleated nucleus with nucleoli, (I) inflammatory cells and (F) fibrous tissue bridging between triads.

Masson's Trichrome x 580

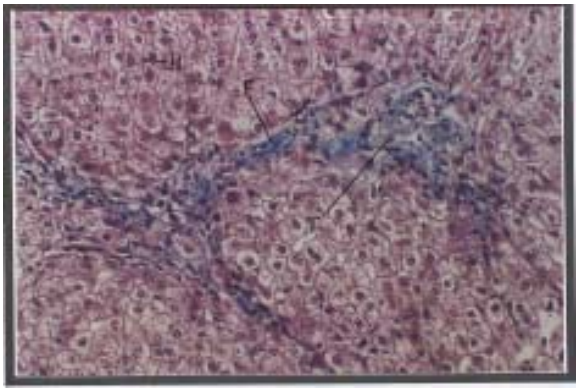

Fig. 2: Photomicrograph of a 5¼m thick section of liver from Group A2 showing (T) hepatic triad with an area between adjacent hepatic lobules, (F) fibrous tissue bridging between the triads and (H) normal hepatic cell.

Masson's Trichrome x 290

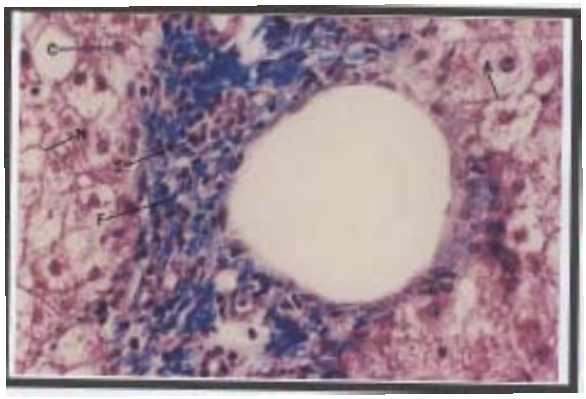

Fig. 3: Photomicrograph illustrating a section of liver from patient of group B1, showing (N) an abnormal cell with fatty change having no nucleus, (F) fibrous tissue, (I) inflammatory cells in portal triad, (A) an abnormal cell with ballooning and vacuolation and (C) nucleus with nucleolus.

Masson's Trichrome x 580

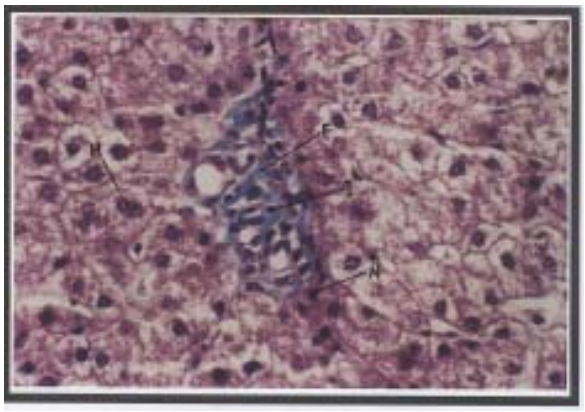

Fig. 4: Photomicrograph of group B2 illustrating an area around a portal triad, showing (I) inflammatory cells, (F) fibrous tissue, (N) necrotic cells around the triad and (H) a normal binucleated hepatic cell.

Masson's Trichrome x 580

DISCUSSION

Chronic Hepatitis C has become the most common cause of liver disease worldwide, and is on the top of the list for discussion and research among the hepatologists. So for, the line of treatment followed includes either interferon alone or in combination with ribavirin, with no second option. Our study mainly focused on the impact of treatment with interferon and ribavirin alone and in combination with vitamin E, the most effective antioxidant. Efficacy of oral vitamin E is also supported by Ertsoz G et al who evaluated it as an antioxidant in combination, moreover it is available at affordable price and carries little side effects.[26] Several reports suggest that vitamin E, either alone or together with other antioxidants, protects tissues from reactive oxygen species damage.[27] This background prompted Lavine[28] and Hasegawa et al.[29] to investigate the possible therapeutic role of vitamin E in two uncontrolled pilot groups of adolescents and adults with liver dysfunction.

The rationale of vitamin E dosing in our study was based on published clinical trials performed on adults, in whom a dose of 300 to 400 mg/d

was considered satisfactory to obtain an antioxidant effect.[30-31]

After treatment in our patients necro-inflammation significantly decreased as compared to fibrosis in both A and B groups; a finding that is in accordance with the observations of Ghany et al.[32]and Patrick et al.[33] In group A patients, fibrosis almost remained unchanged or even increased in some patients, which is in accordance with the observations of Elizabeth,[34] according to which, necro-inflammation may wax and wane but fibrosis generally progresses or remains stationary while it rarely regresses. Contrary to the findings in Group A, we observed that in vitamin E treated patients (Group B), the progression in fibrosis was prevented or even reversed in few cases as revealed by Harrison et al; in which vitamin E and vitamin C treatment improved fibrosis in adult patients with hepatitis. [35]

In both the groups after treatment, there was significant decrease in activity grade as compared to the stage. On the other hand the stage score was altogether arrested or decreased in group B as compared to A, highlighting the role of vitamin E in treating fibrosis. This observation contradicts the statement of Elizabeth[34] about progression or regression of fibrosis.

CONCLUSION

Addition of Vitamin E to combination therapy with interferon and ribavirin prevents the progression of fibrosis in chronic hepatitis C.

Vitamin E addition to the treatment is safe and effective option in patients with liver disease.

Since more effective new therapeutic options are lacking, patients with chronic liver disease should be encouraged to take vitamin E supplements, which are safe and affordable.

For additional confirmation of our results and determination of the optimal daily dosage of vitamin E it may be necessary to carry out large-scale collaborative studies.

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