LABORATORY EVIDENCE OF AUTOIMMUNITY IN HCV INFECTED PATIENTS AFTER INTERFERON TREATMENT

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ABSTRACT

Objective: To determine the significance of interferon alpha therapy in emergence of autoantibodies in HCV infected patients in a local population

Design: Quasi experimental study

Place and duration of study: Department of Immunology, Armed Forces Institute of Pathology (AFIP) Rawalpindi from Mar 2007 to Oct 2007.

Methods: A total of 106 HCV infected individuals (not on any antiviral therapy), were screened for laboratory evidence of autoimmunity (ANA, SMA, AMA, Anti LKM, Anti GPC, Anti Thyroid microsomal & RA factor). HCV infected patients without any laboratory evidence of autoimmunity were included in the study and this population was divided in to a test group (36) who were treated with interferon alpha and a control group (32) who did not receive anti viral treatment during this period. All were retested for the same autoantibodies after a period of 3 months. All autoantibodies were tested by indirect immunofluorescence except RA factor which was tested by agglutination method.

Results: Out of 106 patients scarred, 32 showed autoimmunity that were excluded from study. Six denied to participate in study. After 3 months, 61% of the patients showed autoimmunity in study group and frequencies of ASMA, anti-TPO (*p* 0.036), ANA (p 0.14) and RA were 36%, 25%, 25% and 33% respectively. Control group showed autoimmunity in 37.5% of the patients with frequency of 28%, 15.6%, 6.3% and 3% for ASMA, RA, TPO and ANA respectively. AMA anti-LKM antibodies were not found in both groups.

Conclusions: ANA and anti thyroid antibodies emerge in increased frequency in HCV infected patients after treatment with IFN-α.

Key words: Autoantibodies, Autoimmunity, HCV, IFN- α.

INTRODUCTION

Hepatitis C virus (HCV) infects an estimated 170 million individuals world wide[1] and true prevalence of HCV infection in Pakistani population is not known, however, it varies from 2 to 3.5% in various risk groups. According to world health organisation (WHO) assessment it is 1.5-2.0%[2-5]. Since the discovery of the hepatitis C virus (HCV) in 1989, an increasing number of studies report an association of chronic HCV infection and interferon therapy with autoimmune phenomena. In various studies the prevalence of autoantibodies in HCV-infected patients varies from 25% to 66%[6,7]. The most commonly detected autoantibodies are anti-smooth muscle antibodies (SMA), followed by anti-nuclear antibodies (ANA), anti thyroid peroxidase (TPO) and anti-liver/kidney microsomal antibodies (LKM). Besides the detection of autoantibodies, the natural course of HCV infection is also accompanied by an increased prevalence of autoimmune diseases[6,7].

Various studies investigated whether the appearance of autoantibodies in chronic HCV infection is influenced by interferon alpha (IFN-α) treatment and it was observed that frequency of different autoantibodies is increased in HCV infected patients during treatment with IFN-α[8,9]. One of the most common phenomena after IFN-α treatment is the appearance of autoantibodies against the thyroid. A significant percentage of patients with anti-thyroid antibodies develop signs of thyroid dysfunction, predominantly hypothyroidism. Several studies observed that IFN- α treatment increased the risk of thyroid dysfunction whereas in most cases, thyroid dysfunction is reversed after discontinuation of IFN-α

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treatment[10,11]. It is important to note that IFN- α treatment itself is associated with autoimmune manifestations. There is ample evidence that IFN-α plays a role in another set of autoimmune diseases, including SLE, autoimmune gastritis and diabetes[12,13]. We therefore aimed to determine the significance of interferon alpha therapy in emergence of autoantibodies in HCV infected patients in a local population.

PATIENTS AND METHODS

A quasi experimental study was designed and carried out at the Department of immunology, Armed Forces Institute of Pathology (AFIP) Rawalpindi Pakistan from Mar 2007 to Oct 2007. The study was approved by the AFIP review board and the CPSP (College of Physicians and Surgeons Pakistan). Patients who were found to be positive for one or more than one autoantibodies and those not willing to participate in the study were excluded. HCV infected patients without any laboratory evidence of autoimmunity were included and this population was divided in to a test group who were treated with interferon alpha and a control group who did not receive anti viral treatment during this period. Both groups were retested for autoantibodies after 3 months. Three ml clotted blood sample was collected each time. ANA, ASMA, AMA (anti mitochondrial antibody), anti-LKM, anti GPC (anti gastric parietal cell antibody) and antithyroid microsomal antibodies were tested by indirect immunofluorescence (IIF) on tissue sections of rat liver, kidney and stomach and human thyroid using a serum dilution of 1:10. Rheumatoid factor was determined by the agglutination method. Data was analysed in SPSS (Statistical Package for Social Sciences) version 10.0. Descriptive statistics were used to describe the data. Chi-square test was applied to compare frequencies of different antibodies between the two groups. A p value < 0.05 was considered significant.

RESULTS

There were 36 patients in study group and 32 patients in control group. Average age of patients in study group was 36±3.12 years and in control group was 35±2.95 years p-value >0.05. In study group there were 29 (80.6%) males while in control group they were 28 (87.5%) p-value>0.05. Overall 22 (61%) patients in study group and 12 (37.5%) patients in

Table: comparison of different antibodies between study and control groups.

Features	Study Group (n=36)		Control Group (n=32)			
	Frequency	%)	Frequency	(%)	Relative risk	*p*-value
Total patients of autoimmunity	22/36	61	12/32	37.5	1.19	0.052NS
ANA	9/36	25	1/32	3	1.93	0.014*
ASMA	13/36	36	9/32	28	1.18	0.49
TPO	9/36	25	2/32	6.3	1.73	0.036*
RA	12/36	33	5/32	15.6	1.5	0.09 NS
GPC	2/36	5.6	0/32	0	1.94	0.276 NS
Patients with one autoantibody	4/36	11	8/32	25	0.018*	
Patients with> one autoantibody	18/36	50	4/32	12.5		

* = Significant
NS = insignificant

control group (0.052) (p=0.052, RR =1.19) showed the evidence of autoimmunity. Frequency of autoimmunity in males was 62% (18) in study group and 36% in control group (p=0.046). As number of females was very less in both groups so comparison was not done.

Frequency of each of ANA and anti TPO antibodies in study group after IFN-α treatment was significantly higher than control group. Frequencies of other autoantibodies like ASMA, anti-GPC and RA factor in study patients were statistically insignificant. Anti LKM and AMA were not found in both groups (Table).

DISCUSSION

In this study, we investigated about the role of interferon alpha in development of laboratory evidence of autoimmunity in HCV infected patients during treatment. We observed that overall frequency of autoantibodies in our study group (who were given IFN-α) was statistically insignificant when compared with control group but the frequency of only ANA and anti TPO antibodies was found to be significant. The frequency of ANA was observed in 25% of patients who received interferon alpha treatment (study group) and were initially negative for ANA. Preziati et al also described significant increase in ANA after IFN-α treatment. He observed prevalence of ANA in 14% of HCV infected patients before interferon alpha therapy which increased upto 36% after treatment (p-value 0.04).

Regarding the effect of interferon alpha treatment in HCV infected patients our second statistically important finding was frequency of anti thyroid antibody (anti TPO) in study group. This autoantibody was noted in 25% of patients of study group while in 655 patients in control group. Majority of the studies have described a statistically significant effect of IFN-α treatment on development of thyroid autoantibodies in HCV infected patients in which different frequencies of anti thyroid microsomal antibodies ranging from 7% to 40% have been reported[14-16,19]. Mandac et al[10] emphasizes the clinical significance of thyroiditis in patients receiving interferon therapy and suggest the classification as autoimmune type and non-autoimmune type. The presence of antithyroid antibodies does not absolutely contraindicate the use of IFN–α[17].

IFN-α treatment for chronic hepatitis C may appear to be associated with occurrence of autoimmune gastritis, particularly in female patients. In this study autoantibodies related to autoimmune gastritis were observed only in 6% of the patients after IFN treatment. Fabbri et al. observed the frequency of anti GPC antibodies upto 13% in his patients after interferon alpha treatment[9].

The effect of IFN-α on the immune system is the enhancement of cell cytotoxicity which is sustained by suppression of T helper (Th) 2 and an increase in Th1 immune response. Although these effects seem to be transient, it has been suggested that the generalized Th1 activation induced by IFN-α may be important for the occurrence of thyroid autoimmunity. In thyroid autoimmunity both Th1 and Th2 responses are found with different intensity in relationship with the expression of the disease process. The type-1 response seems to be dominant in hypothyroid patients whereas type-2 immune response has been found in patients with thyroid autoimmunity and normal thyroid function[18,19].

AMA and anti LKM antibodies have been rarely found in patients with hepatitis C and after IFN-α therapy, which was also observed in our patients.

As in the majority of adult studies, no one has reported any connection between autoantibody appearance and treatment outcome. However, a study carried out by Wasmuth et al. in adults observed a better response in autoantibody-negative patients treated with IFN-α plus ribavirin[8].

CONCLUSION

The emergence of ANA and anti thyroid antibodies is correlated with IFN- α treatment. Since serum thyroid antibodies and ANA

significantly develop in HCV infected patients during treatment with IFN-α so it must be emphasized that close monitoring of thyroid function and other autoimmune phenomena, is mandatory. Serial estimation of autoantibodies during treatment with IFN-α would be helpful for early diagnosis and monitoring of any immunological alteration.

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