ORIGINAL ARTICLE

INSINUATION OF PROPHETIC VARIABLES AND THEIR ROLE IN DEVELOPMENT OF AUTOIMMUNE THYROID DYSFUNCTION IN HCV PATIENTS RECEIVING INTERFERON THERAPY

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Background: Chronic liver disease has remained a major threat to human health. Hepatitis C patients administered combined interferon with ribavirin therapy, and lacking vitamin-D, are susceptible to extra-hepatic manifestations, particularly autoimmune thyroid disorders (AITDs). The present study was designed to assess the effects of interferon and vitamin D in attaining sustained virological response in autoimmune thyroid disorders. **Methods:** Seventy-five patients of HCV of age 25–70 years were enrolled and screened at Ganga Ram Hospital Lahore, and 50 age and sex matched healthy individuals served as control. Their sera were separated and estimated for thyroid profile, lipid peroxidation and Vitamin D. Real time HCV PCR was performed with the serum of patients before and after the therapy. **Results:** Variables of oxidative and inflammatory like malondialdehyde (MDA), interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) differed significantly. The levels of MDA (3.68±1.14 ηmoles/ml), IL-6 (6.80±0.79 ρg/ml) and TNF-α (31.95±4.35 ρg/ml) were recorded in HCV patients. The thyroid profile of HCV patients showed highly significant differences among the studied groups [FT4 (10.11±1.93), FT3 (19.18±2.06), and TSH (19.68±2.09) ρmol/ml]. Higher levels of thyroid antibodies were recorded in HCV patients subjected to interferon therapy. The levels of vitamin D (9.36±1.22 ηg/ml) in HCV patients differed significantly compared to controls (13.22±0.81 ηg/ml). **Conclusion:** Hepatitis C patients receiving interferon therapy and deficient in vitamin D dys-regulate autoimmune response rendering them to develop thyroid disorders.

Keywords: INF-alpha, HCV, Vitamin D, Malondialdehyde, Interleukin-6, TNF-alpha, Thyroid stimulating hormone receptor, Thyroid peroxidase, Thyroglobulin

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INTRODUCTION

Chronic liver disease mainly occurs due to hepatitis C virus (HCV) infection resulting in fibrosis, cirrhosis and eventually hepatocellular carcinoma, having higher incidence in developing countries due to unawareness of needle exchange program and lack of medical facilities.[1] About 3% of population is infected with HCV infection worldwide and most of them are ignorant of their disease.[2] Despite latest therapeutic approaches, pegylated interferon (IFN) alpha in combination with ribavirin has been still used as a standard therapy against HCV infection but causes several side effects like hematological problems, neuropsychiatric issues and of particular concern thyroid disorders.[3] Thus, the dose of IFN-α should be reduced or discontinued by clinicians depending on the seriousness of any side effect resulting in compromised therapeutic response. Approximately 5-10% HCV patients receiving IFN-α therapy will develop thyroid disorders, of which hypothyroidism (Hashimoto's thyroiditis) was the most frequent complication but few cases of hyperthyroidism (Graves disease) were also reported.[2,4]

It has been revealed that HCV can independently mediate thyroid dysfunction by directly attack on thyrocytes, inhibiting thyroid hormogenesis and enhances immune response. IFN and ribavirin therapy has the ability to amplify the immune response resulting in early sustained virological response (SVR) in HCV patients suffering from hypothyroidism and autoimmune thyroid disorders (AITDs).[5,6] 1, 25 OH-Vitamin D_3, an important mediator of proper function of both innate and acquired immunity thus has an ability to enhance the effects of anti-viral therapy and inhibit fibrosis progression by making T-cells active to HCV so to achieve SVR.[7] Vitamin D is an antioxidant and anti-inflammatory thus it can suppress oxidative insult and inflammatory mediators, important in prevention and inhibit progression of fibrosis.[8]

The aims and objectives of the present study were to assess the significance of interferon and vitamin D in attaining sustained virological response by inducing the development of thyroid disorders.

MATERIAL AND METHODS

All of the patients included in the study were screened at Ganga Ram hospital Lahore. Seventy-five (75) patients with hepatitis C of age group 25–70 years were obtained for the study. Informed consent was taken from them and then they were included in this study. Fifty age and sex-matched controls were included. All of the performed experimental protocols were under the guidelines of Research Ethical Committee of The

Institute of molecular biology and biotechnology, The University of Lahore. Hepatitis C diagnosis was based on the criteria: 1) Increased serum levels of amino-transferases for at least six months; 2) Absence of anti-thyroid antibodies before the start of interferon therapy; 3) Finally, the patients were selected who complete the interferon therapy with presence of anti-thyroid antibodies, detected using third-generation ELIZA. Those who had the history for any drugs (or even alcohol and cigarette), pre-diagnosis medications (e.g. anti-parkinsonian/anti-psychotic), were not included in the study. Serum from these subjects were separated and was centrifuged at 3000rpm for about 10 minutes and it was then stored at -80 ^{o}C until appropriate biochemical analysis was done.

Lipid peroxidation was determined by the method of Ohkawa *et al*[9], which employs the use of calorimeter. About 200µl of the sample was obtained in the test tube and subsequently 200µl of 8.1% SDS, afterwards acetic acid (20%) and TBA (0.8%) having volume 1.5ml were finally added in the tube and were allowed to heat for 60min. It was then cooled down, and about 4ml of n-butanol was then added and was allowed to centrifuge for 10mins at 3000rpm. At last, supernatant was separated in a cuvette and absorbance was measured with the help of spectrophotometer at 532nm against the blank. Thyroid profile including T3 (Triiodothyronine), T4 (Thyroxine), TSH (Thyroid stimulating hormone), TSHr (Thyroid stimulating hormone receptor) and TPO (Thyroid peroxidase) and Tg (Thyroglobulin) IgG were determined by human ELIZA kit (BioVendor). Vitamin-D was estimated by the ELISA kit method of ALPCO, USA. (Heaney RP2010). Out of 75 HCV patients specimens were collected (Before and after the completion of therapy) were estimated by using assay Abbott Real Time HCV and final results were then matched with another assay by NCCLS Document EP9-A2.[10]

RESULTS

Variables of oxidative and inflammatory like malondialdehyde (MDA), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) differed significantly (p<0.05). The higher levels of MDA (3.68±0.46 ηmole/ml), IL-6 (6.80±0.33 ρg/ml) and TNF-α (31.95±0.26 ρg/ml) were recorded in HCV patients as compared to normal healthy controls (1.43±0.32 ηmole/ml, 5.64±0.26 ρg/ml, 29.90±0.18 ρg/ml respectively). The level of vitamin-D (9.36±0.48 ηg/ml) in HCV patients differed significantly (p=0.0042) compared to control (13.22±0.52 ηg/ml).

The thyroid profile of HCV patients showed a highly significant difference among the studied groups. The lower levels of FT4 (10.11±1.32 ρmol/ml) and higher levels of FT3 (19.18±1.26 ρmol/ml) and TSH (19.68±1.05 ρmol/ml) were recorded in HCV subjects receiving interferon therapy parallel to healthy controls FT4 (22.73±1.52), FT3 (11.91±1.08) and TSH (11.98±0.92) ρmol/ml respectively. Thyroid auto-antibodies present highly significant differences among the study groups. The higher levels of auto-antibodies were recorded in HCV patients subjected to interferon therapy. The levels of TgAb (48.33±2.43 ρmol/ml), TPOAb (10.16±0.52 IU/ml), and TSHrAb (3.35±0.16 IU/ml) were observed in HCV patients while these variables remained 26.55±1.42, 5.19±0.36, and 1.19±0.12 in controls respectively.

The lower levels of antioxidants such as SOD (0.659±0.049 U/ml), GSH (5.26±0.24 µmol/L) and CAT (2.44±0.21 U/L) were recorded in HCV subjects receiving interferon therapy parallel to healthy controls SOD (1.06±0.062 U/ml), GSH (7.265±0.41 µmol/L) and CAT (4.58±0.31 U/L) respectively. The higher levels of isoprostanes (31.26±0.80 ρg/ml), 8-OHdG (1.19±0.066 ρg/ml), MMP-9 (95.35±4.28 ηg/ml) and 4-HNE (1.756±0.12 g/mole) were also recorded in HCV subjects receiving interferon therapy parallel to healthy controls isoprostanes (1.40±0.09 ρg/ml), 8-OHdG (0.10±0.05 ρg/ml), MMP-9 (31.28±3.88 ηg/ml) and 4-HNE (0.645±0.091 g/mole) respectively. (Table-1)

Table-1: Expression of prophetic variables of medical importance involved and their interplay in the development of thyroid dysfunction

Variables	Controls (n=50)	Patients (n=75)	*p*
MDA (moles/ml)	1.43±0.32	3.68±0.46	0.014
IL-6 (ρg/ml)	5.64±0.26	6.80±0.33	0.018
TNF-α (ρg/ml)	29.90±0.18	31.95±0.26	0.011
Vitamin D (ηg/ml)	13.22±0.52	9.36±0.48	0.0042
FT4 (ρmol/ml)	22.73±1.52	10.11±1.32	0.002
FT3 (ρmol/ml)	11.91±1.08	19.18±1.26	0.024
TSH (ρmol/ml)	11.98±0.92	19.68±1.05	0.021
TgAb (ρmol/ml)	26.55±1.42	48.33±2.43	0.0011
TPOAb (IU/ml)	5.19±0.36	10.16±0.52	0.012
TSHrAb (IU/ml)	1.19±0.12	3.35±0.16	0.021
SOD (U/ml)	1.06±0.062	0.659±0.049	0.032
GSH (µmol/L)	7.265±0.041	5.26±0.24	0.029
CAT (U/L)	4.58±0.31	2.44±0.21	0.021
Isoprostanes (ρg/ml)	1.40±0.09	31.26±0.80	0.000
8-OHdG (ρg/ml)	0.10±0.05	1.19±0.066	0.001
MMP-9 (ηg/ml)	31.28±3.88	95.35±4.28	0.000
4-HNE (g/mole)	0.645±0.091	1.756±0.12	0.023

DISCUSSION

A correlation between thyroid and IFNα was distinguished as early as in 1985 with patients who have been received IFNα therapy for breast cancer. The clinical demonstration of IFN-induced thyroid autoimmunity can be divided into various types including thyroiditis, GD and profound subclinical hypothyroidism.[11] The genome of HCV may be react with integral part of thyroid dysfunction. The HCV virus is able to convince endogenous interferon to a greater potency; in this result the formation of thyroid

auto-antibodies is increased and activate autoimmune thyroid disease in vulnerable individuals. It is possible that synergy between exogenous and endogenous IFNa may emphasize the effect on the thyroid thus causing additional hypothyroidism.[12] The present study showed that the chronic hepatitis C (CHC) patients receiving anti-viral therapy, including interferon (IFN) have abnormal thyroid function tests, higher levels of thyroid auto-antibodies and malondialdehyde (MDA) while lower levels of vitamin D result in thyroid disorders. It has been revealed that hepatitis C virus (HCV) induces IFN α and β synthesis which mediate maturation, proliferation and prevention of apoptosis of T-lymphocytes by "bystander mechanism" in which HCV glycoprotein E2 combines with thyrocytes CD81 receptors stimulate tissue inflammatory reaction thus, triggering organ specific auto-immunity (particular concern thyroid auto-immunity) leading to auto-immune thyroid disorders (AITDs).[13] The thyroid disturbances may be due to immunological processes and not directly by HCV infection[14] but in contrast to it HCV can directly attack thyrocytes and mediate their apoptosis.[15] The occurrence of thyroid disorders may initiate an up-regulated immune response resulting in increased viral eradication thus, sustained virological response (SVR) can be achieved more efficiently relatively to CHC patients wsithout thyroid problems resulting in reduced cirrhosis and its associated complications.[16] With clinical and biochemical evidences within the group of patients they were observed with increased oxidative stress and decreased levels of antioxidants i.e., SOD, CAT, GSH and end-product of lipid peroxidation MDA remained elevated. Moreover, pro-inflammatory cytokines as mentioned earlier in case of TNF-alpha was increased which resultantly increased the levels of ILs in the rats receiving interferon therapy for their thyroid dysfunction. Significant correlations can be observed in such rats for the variables TNF-alpha and Interleukins.

Recently, it has been reported that the occurrence of thyroid problems in HCV patients might be independent of IFN therapy.[17] The exogenous IFN administration can potentiate the effects of HCV on thyroid gland by the amplification of cytotoxic stimulation of T-helper 1cells and interaction with major histocompatibility complex class 1 antigen (MHC1), aberrantly expressed on thyrocytes resulting in their apoptosis and destructive thyroiditis.[18] The most prevalent finding was hypothyroidism (Hashimoto's thyroiditis) as TSH levels were higher, similar results were proposed by Hamza *et al*.[19] It has been estimated that IFN-α mediated AITDs can arise as early as 4th week after initiation of IFN-α therapy.[20] After IFN withdrawal, thyroid auto-antibodies persist indefinitely or there may be partial reversal but thyroid functions recover completely.[21,22]

IFN-alpha binds to interferon receptors, which are transmembrane glycoproteins that contain cytoplasmic domains which activate different signaling pathways, including the JAK-STAT pathway, the Crk-pathway, the IRS signaling pathway, and the MAP kinase pathway. More than 25 interferon-induced proteins have been identified. In the past several decades IFNa has emerged as a major therapeutic modality for several malignant and nonmalignant diseases. By far the most common indication for IFNa treatment is hepatitis C virus (HCV) infection. In two pivotal randomized trials, approximately 50% of patients with chronic hepatitis C, who were treated with peginterferon alpha–2a plus ribavirin, achieved a sustained virologic response.

It has been suggested that CHC patients having Vitamin D deficiency which may be due to decreased expression of cytochrome P27A1 show poor response to anti-viral treatment and mediate fibrosis progression by making T-cells inactive to HCV.[23,24] The present study showed an inverse correlation between Vitamin D, MDA and pro-inflammatory cytokines including interleukin-6 and TNF-α (Vit.D Vs MDA, r= -0.724**, Vitamin D Vs IL-6, r= -0.422* and Vitamin D Vs TNF-α, r= -0.782**), similar findings were reported by Arooj M, *et al*.[25] Thus, vitamin D supplementation can reverse pro-inflammatory cytokines and MDA levels to their normal ranges as Vitamin D may enhance glutathione production by increasing glutamate and p53 levels, and inhibits iron mediated liposomal lipid peroxidation.[8,26] Vitamin D also hampers production of T-helper 1 cells while may enhance T-helper 2 response thus it can enhance anti-inflammatory response. A recent study reported poor response to anti-viral therapy as SVR cannot be achieved [23,27], contradictory to the present study. This variation in response to Vitamin D supplementation may depend on HCV genotype as genotype 1 has a good prognosis while genotype 4 has a poor prognosis.[24,28] It has been suggested that IFNa stimulate the JAK-STAT pathway upon react to its receptors leading to stimulate of a various interferon-stimulated genes (ISGs) like Adhesion molecule genes and also activate macrophages and neutrophils. IFNa therapy also increase the secretion of cytokines specifically IL6[29] like present study.

Thyroid disease was defined as any value of these markers (TSH, FT3 and FT4) which was greater or less than normal value the reference range (RR) for TSH was 0.35–5.5 uIU/ml, FT4 0.61–1.12 ng/dl and FT3 was 2.52–3.90 pg/ml.[30] In the present study there is an abrupt decrease in the level of FT4 in subjects as compared to control whereas levels of other parameters of thyroid function test such as FT3 and TSH are increased in the subjects that depicts that there is high production of T3 and TSH and less production of T4. Workup should also include checking TSH receptor

antibody (TRAb), and TPO-Ab, and Tg-Ab levels. If the etiology of hyperthyroidism cannot be revealed by these tests a thyroid I-123 uptake and scan may be performed, as well. If serum TSH is high, fT4 and fT3 levels should be measured to confirm the diagnosis of primary hypothyroidism.[31]

CONCLUSION

The chronic hepatitis C patients have a susceptibility to develop hypothyroidism and AITDs while receiving interferon therapy. This results in sustained virological response and worsens thyroid related problems leading to compromised IFN therapy. Vitamin D is also important in achieving early SVR and limiting fibrosis progression by regulating the immune, inflammatory and oxidative responses. It should be mandatory to evaluate the thyroid function tests, thyroid auto-immunity status and vitamin D levels prior to administration of the therapy. Vitamin D can be used as a co-treatment with antiviral therapy.

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