ORIGINAL ARTICLE

Alteration of Serum Biochemical Markers and Malondialdehyde Levels in Patients with Thyroid Gland Dysfunction Receiving Interferon Therapy

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ABSTRACT

Thyroid dysfunction is the most frequent autoimmune reaction to interferon therapy. Thyroid diseases are frequently associated with liver injuries and abnormal results for biochemical tests, such as increased levels of alanine transaminase (ALT), aspartate transaminase (AST) and other liver-specific enzymes. In the present study, serum levels of AST, ALT, alkaline phosphatase (ALP), total bilirubin and malondialdehyde (MDA) were investigated as markers of liver injuries in patients with thyroid dysfunction. The focus of the present study was to assess the biochemical markers and MDA status in cases of interferon-induced thyroid gland dysfunctions. Interferon (IFN) therapy leads to an increase in liver enzymes such as AST, ALT, ALP and total bilirubin in patients with thyroid dysfunction. MDA, which is an important biochemical parameter and one of the products of lipid peroxidation, was also found to be significantly increased in patients with interferon-induced thyroid dysfunction. The estimation of these circulating biochemical parameters indicated that in cases of interferon-induced thyroid dysfunctions, some liver enzymes and the oxidative status of the cells were imbalanced, which is related to hepatic injury. IFN therapy leads to an increase in liver enzyme levels, particularly AST, ALT, ALP and MDA, in patients with thyroid dysfunction. Therefore, patients treated with IFN therapy should be informed of their risk of developing thyroid dysfunction, and screening of thyroid function should be conducted before and during treatment for HCV.

Keywords: Thyroid, interferon, total bilirubin, lipid peroxidation, malondialdehyde, liver enzymes

INTRODUCTION

The hypothalamic pituitary axis is a frequently studied component of the endocrine system that produces various hormones that act on the pituitary gland to stimulate the release of pituitary hormone. Thyroid hormones are very important for the proper differentiation and development of all cells in the human body[1]. Thyroid gland dysfunction often results in imbalanced production of thyroid hormones[2]. Abnormalities in thyroid function, such as hyperthyroidism (thyrotoxicosis) and hypothyroidism, are among the two most common endocrine disorders. Hyperthyroidism and hypothyroidism reflect the overactivity (excess) and underactivity (deficiency) of thyroid hormone secretion[3], respectively. Hypothyroidism is characterized by biochemical and clinical manifestations of thyroid hormone deficit in its target tissues, consequently leading to a reduction in metabolic rate[4]. During IFN-α therapy, the development of thyroid dysfunction has been reported to occur in 4.7% to 38.8% of patients, with a mean incidence of 12.1%[29].

Hepatitis C virus infection is one of the major worldwide causes of chronic liver inflammation and cirrhosis. To date, the most effective treatment is a combination of ribavirin and interferon (IFN) therapy. However, although treatment with IFN is widely used, it is frequently associated with major adverse effects, such as the development of thyroid disease (TD) during therapy. A vast range of autoimmune TDs, ranging from Graves' disease to thyroiditis and hypothyroidism, have been reported[5,6,7]. Various organ-specific and systemic pathological changes represent the adverse effects of IFN treatment. Many of these manifestations occur as consequences of the deregulations of the immune system that are induced by IFN itself[8]. For the development of TD, the activation of the immune system, which occurs by IFN therapy, is integral. IFN therapy has direct effects on the thyroid hormone by inhibiting thyroid synthesis, release and metabolism[9,10,11]. In some cases of TDs, the aberrant expression of major histocompatibility antigens on thyroid cells is

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modulated by IFNs[12,13,14]. and favors the cytokine microenvironment, which may result in the immune-mediated damage of thyroid tissues (14). The synthesis of thyroid hormones requires the formation of hydrogen peroxide (H_2O_2), which is a highly reactive oxidant[15].

Thyroid peroxidase catalyzes the peroxidation reaction, which immediately utilizes the H_2O_2 and oxidized iodine[16]. In cases of hyperthyroidism, the increased activity of glutathione peroxidase, catalase and superoxide dismuthase (SOD) in the liver has been observed[17]. Malondialdehyde (MDA), which is both carcinogenic and mutagenic, is a natural product of lipid peroxidation and prostaglandin biosynthesis. MDA not only is an indicator of lipid peroxidation but also acts as a potential biomarker for oxidative stress. In IFN-induced thyroid dysfunctions, the lipid peroxidation process is also perturbed and can manifest as abnormalities in the MDA levels (18). Though the liver has a significant correlation with endogenous antioxidant enzymes, it also has dynamic mitochondrial and microsomal systems that produce reactive oxygen species (ROS) during normal metabolic processes (19, 20). In fact, lipid peroxidation has been reported as a major cause of hepatocyte damage, which may lead to an elevated level of MDA, a biomarker of oxidative stress[21].

TDs are frequently associated with liver injuries and abnormal biochemical test results, i.e., the elevation of alanine transaminase (ALT), aspartate transaminase (AST) and other specific enzymes[22]. Liver damage may cause AST and ALT to leak into the bloodstream[23]. In addition to AST and ALT, other enzymes involved in liver dysfunction include alkaline phosphatase (ALP), 5'-nucleotidase, lactate dehydrogenase (LDH) and γ-glutamyltrans peptidase[24]. Liver function tests (LFTs) are abnormal in approximately 50% of hypothyroid cases, indicating that some abnormalities in LFTs are also associated with thyroid dysfunction[25]. In cases of hyperthyroidism, LFTs may reveal distracted elevations of the transaminases, ALP and mild indirect hyperbilirubinemia with some minor changes observed in the liver histology[26].

Although treating hepatitis with IFN therapy is common, this treatment can lead to adverse effects such as changes in liver enzymes, particularly the AST, ALT, ALP and MDA levels, in patients with thyroid dysfunction. If these changes can be confirmed, then the screening of thyroid function to identify patients at risks of developing thyroid dysfunction before interferon therapy can be recognized and included in common practice. In the present study, we investigated the circulating biochemical markers and MDA status in IFN-induced thyroid gland dysfunctions.

MATERIALS AND METHODS

This study was conducted at the University of Lahore (UOL) and Gangaram Hospital, Lahore, Pakistan, from August 2011 to March 2012. Two hundred twenty-four patients were initially screened using the inclusion/exclusion criteria, and 10 patients were excluded due to the presence of cirrhotic changes in the liver. A total of 214 non-cirrhotic patients with chronic hepatitis C were enrolled in the study.

The age range of the patients was 18-75 years of age, and both sexes were included. The subjects were divided into treatment (n=204) and control (n=10) groups. The control group consisted of healthy individuals. The presence of hepatitis C virus (HCV) had previously been confirmed among the patients based on their long-term serum ALT levels, positive HCV antibodies by ELISA, qualitative positive HCV ribonucleic acid (RNA) by polymerase chain reaction and positive histopathological findings in liver biopsies. Other diagnostic markers of HCV were excluded from the exclusion criteria. IFN was administered to treat the HCV infection at various intervals for six months of therapy. The majority of the patients received follows up and was taking IFN four to six months prior to the study.

Inclusion Criteria: Patients suffering from hepatitis C with no previous thyroid dysfunction symptoms, and no administration of ribavirin and/or IFN for treatment were included. In later stages, the patients may show symptoms of thyroid dysfunction.

Exclusion Criteria: Patients with cirrhotic changes observed on a liver biopsy, prior history of treatment with IFN and/or ribavirin, history of pre-existing TD, neoplastic, autoimmune, severe pulmonary or cardiac disease, presently using immunosuppressants or any steroids and diabetic patients were excluded from the study.

Blood samples (3ml) were obtained for thyroid function and liver function tests in IFN-induced HCV patients to investigate the status of thyroid gland dysfunction. The levels of free thyroxine (FT4), free triiodothyronine (FT3) and TSH were measured using a radioimmunoassay (RIA) method, and the liver enzymes were estimated using a RANDOX kit (London, UK). The total amount of lipid peroxidation products in the serum was estimated in both patients and controls using the thiobarbituric acid method (TBA) test, which measures the MDA levels to determine the oxidative status in the cells.

All data were expressed as the mean ± standard deviation (mean±SD). Statistical analysis was performed by a one-way ANOVA using SPSS (version 16 for Windows, Armonk, NY, USA). Pearson's correlation was used to correlate the

biochemical parameters. A *P* value less than 0.05 was considered to be statistically significant.

RESULTS

The FT4 level was lower (14.74±0.19pmol/L) among hypothyroid patients compared to the controls (23.6±0.21 pmol/L; Table 1). However, the remaining biochemical markers (AST, ALP, TSH, FT4 and MDA) were significantly and markedly higher among the hyperthyroid patients compared to those of the controls. Decreased levels of the circulatory biomarkers were observed in patients with hypothyroidism, with the exception of MDA, for which increased levels were observed. Hence, the MDA levels were significantly elevated in cases of both hypo- and hyperthyroidism compared to those of the controls.

Table 1: Groups showing the mean ± SD of various biochemical markers in the control, hypothyroid and hyperthyroid.

	Control	**Hypothyroid**	**Hyperthyroid**
ALT (IU/L)	34.36 ± 0.83	39.93 ± 5.38	37.46 ± 2.44
AST (IU/L)	31.73 ± 0.72	36.03 ± 1.04*	34.30 ± 1.61
ALP (IU/L)	277.40 ± 5.53	260.16 ± 5.59*	301.85 ± 5.06*
Total bilirubin (mg/dL)	0.53 ± 0.01	0.52 ± 0.01	0.54 ± 0.01
TSH (IU/L)	2.41 ± 2.00	1.70 ± 0.01*	2.58 ± 0.23*
FT3 (pmol/L)	6.47 ± 0.46	3.61 ± 0.25	6.62 ± 0.36
FT4 (pmol/L)	23.6 ± 0.21	14.74 ± 0.19*	42.53 ± 0.43*
MDA (µmol/mL)	2.02 ± 0.13	9.97 ± 0.19*	13.22 ± 0.33*

*p<0.05

Table 2: Correlation Matrix of Biochemical Markers and MDA Status in thyroid gland dysfunction receiving IFN therapy

	ALT	**AST**	**ALP**	**Total bilirubin**	**TSH**	**FT3**	**FT4**	**MDA**
ALT	1.000	0.094	0.039	0.082	0.083	0.058	-0.008	0.020
		0.173	0.573	0.235	0.228	0.395	0.902	0.776
AST		1.000	0.137*	-0.109	0.188**	0.086	-0.208**	0.090
			0.045	0.112	0.006	0.211	0.002	0.191
ALP			1.000	0.124	0.275**	0.048	-0.440**	0.343**
				0.070	0.000	0.489	0.000	0.000
Total bilirubin				1.000	-.0091	-0.034	-0.122	0.066
					0.185	0.623	0.076	0.338
TSH					1.000	0.009	0.057	-0.042
						0.893	0.406	0.536
FT3						1.000	-0.108	0.118
							0.114	0.084
FT4							1.000	-0.353**
								0.000
MDA								1.000

**Correlation is significant at the 0.05 level (two-tailed).* ***Correlation is significant at the 0.01 level (two-tailed).*

DISCUSSION

Treatment of non-cirrhotic chronic hepatitis C patients with IFN therapy produced symptoms of thyroid dysfunction that indicated the conditions of hypothyroidism and hyperthyroidism. Notably, these patients did not have symptoms of thyroid dysfunction before therapy. Moreover, elevated levels of MDA in therapy-induced thyroid dysfunction reflected oxidative stress in those patients. Before treatment, patients tend to show symptoms of hepatitis, but they may develop thyroid disease in later stages of the disease.

In the thyroid glands of patients with HCV, the presence of HCV particles may lead to the induction of IFN-α and IFN-β production as part of the innate immune response[27]. There are two possible reasons for the induction of TD following exposure to IFN. First, TD induction can be attributed to the pharmacokinetics of IFN therapy, during which the drug is expected to clear within four to six weeks. Consequently, most TDs that occur after therapy tend

to do so within that timeframe[28]. Second, after the six-month period during which the immunomodulation effect is finished and the complete removal of the hepatitis C viral particle has occurred and a continuous sustained viral response has been achieved, the influence on thyroid tissues is no longer significant. During IFN-α therapy, the development of thyroid dysfunction has been reported to occur in 4.7% to 38.8% of patients, with a mean incidence of 12.1%[29]. In our study, TFTs have been performed to investigate the FT4, FT3 and TSH levels and thereby rule out the potential coexistence of thyroid dysfunctions in IFN-induced patient with unexplained abnormal results of liver biochemical tests.

Increased lipid peroxidation in red blood cells can also occur due to the inhibition or alteration of the activity of various enzymatic and non-enzymatic components of the oxidative system, such as glutathione (GSH), catalase (CAT) and superoxide dismuthase (SOD). In the present study, we evaluated the extent of peroxidative processes in the liver of IFN-induced patients. The high MDA levels in the subjects showed that the damaged cell membranes of the hepatocytes in therapy induced hypo- and hyperthyroidism (9.97±0.19 and 13.22±0.33 µmol/ml) thiobarbituric acid-reactive substances (TBARS) because an elevated MDA level is an indicator of lipid peroxidation. Increased levels of serum AST, ALT, ALP and total bilirubin are indicative of cellular damage and the loss of functional integrity of cell membranes in the liver. Therefore, in the present study, the levels of the three liver enzymes ALT, AST, ALP and total bilirubin in patients with IFN-induced TDs were estimated and compared with controls and were ultimately found to be elevated in patients after IFN therapy.

A correlation matrix established among the biochemical parameters and oxidative stress in patients with a dysfunctional thyroid state indicated that ALT and TB showed an inverse but non-significant ($P > 0.05$) correlation with FT4. However, there was a significant correlation between FT4 with AST and ALP, indicating that oxidative stress is responsible for not only the release of these enzymes but also the secretion of FT4. Therapy both induced damage to cells and disturbed the metabolic control of biological systems.

A strong, positive and highly significant correlation between the AST level with TSH and ALP was observed, indicating that therapy affects the release of thyroid hormone. ALP showed a positive and highly significant correlation with MDA, indicating that the increase in ALP and MDA levels was dependent upon one another in a diseased state. FT4 also exhibited a highly significant inverse correlation with MDA, showing that any thyroid dysfunction can affect the oxidative state and lipid peroxidation. There was no significant correlation between total bilirubin and MDA, indicating that the presence of hepatitis C or thyroid dysfunction (therapy-induced) have no relation with bilirubin synthesis.

The present study showed the importance of establishing an association between HCV treatment and thyroid disorders. The immunological basis of TD in chronic hepatitis C patients who have undergone IFN therapy remains unclear. Further studies are required to fully elucidate the pathogenesis of IFN-induced TD. The role of genetic, viral and environmental factors in its etiology should also be further investigated. An increase in the knowledge of these aspects will not only enhance our understanding of the disease, but it will also lead to an improvement in patient care, diagnosis of thyroid side effects at early stages and more appropriate therapeutic approach to IFN-induced TD.

CONCLUSION

IFN therapy leads to an increase in liver enzymes, particularly the AST, ALT, ALP and MDA levels, in patients with thyroid dysfunction. Therefore, patients treated with IFN therapy should be pre-informed of their risk of developing thyroid dysfunction. Additionally, using a panel of antibodies to screen for thyroid function should be conducted before and during HCV treatment.

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