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ORIGINAL ARTICLE

Evaluation of Thyroid Hormones in Chronic Hepatitis C Patients on Oral AntiviralTherapy

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ABSTRACT

Background: Hepatitis C virus (HCV) is a major cause of morbidity and mortality. About 71 million people around the world are chronically infected with HCV, however, in Pakistan, approximately 12 million people are suffering from this dreadful virus. In Pakistan, approximately 6.2 % of population is infected with HCV and its treatment rate is approximately 1 %. The aim of this research was to assess thyroid hormones level in chronic HCV patients which were taking the oral-antiviral therapy.

Methods: A total of 1650 suspects were screened by immunochromatographic technique. Then ELISA technique were used to confirmed the sample and PCR were also performed. All data were analyzed through Microsoft Excel and SPSS-21.

Results: Out of total ICT positive, 117 patients were positive for HCV. Subsequently, all positive samples were exposed to Enzyme linked Immunosorbant assay, out of 117 tested samples 112 (6.7 %) were found positive. However, the final confirmation was done by using Real Time PCR, which showed 109 (6.6 %) patients positive for HCV. Among these 109 HCV patients, the age-wise prevalence of HCV patients was observed maximum 35 (32 %) in age group (55-74 years). Furthermore, these 109 patients were tested for the thyroid dysfunction, before and after anti-viral therapy (Sofosbuvir and Daclatasvir therapy) which was recommended by World Health Organization. Out of 109 HCV positive patients the frequency, 92 (84.6 %) showed euthyroidism, 6 (5.5 %) hypothyroidism and 11 (10 %) hyperthyroidism. Thyroid dysfunction was high (n=17, 15.6 %) in patients with age above 50 years. Six (35.2 %) of thyroid dysfunction patients revealed hypothyroidism and hyperthyroidism was observed in 11 (64.7 %) patients. Mean values of thyroid function test before antiviral therapy were TSH = 3.88 ± 1.0, TT3 = 1.15 ± 0.58, and TT4 = 7.7 ± 01.99 and mean values after three months therapy were TSH = 2.49±1.72, TT3 =1.38 ± 0.69 and TT4 = 8.78 ± 2.29.

Conclusion: It is concluded that HCV infected individuals on oral antiviral drugs have an effect on the thyroid gland, therefore, HCV patients should be frequently screened for thyroid disorders before and after treatment to ensure euthyroid status.

Keywords: Thyroid hormones, Hepatitis, Oral Anti-viral therapy, Thyroidism

INTRODUCTION

Hepatitis C virus (HCV) is responsible for hepatitis C infection and it is considered a global health problem. No sign symptoms with gradual progression from acute to severe chronic and ultimately lead to chronic infection, liver cancer, liver fibrosis and eventually death, creating health, economic and social burden[1]. HCV related death enormously increased between 1990 to 2013[2]. The progression of fibrosis can lead to increase the probability of morbidity and mortality in CHC patients because of complications caused by cirrhosis or hepatic-cellular-carcinoma of HCV which can even lead to death[3].

Prevalence of HCV is also high in India, china, Indonesia but notably lower in Europe, Japan and Australia[4]. In Pakistan, around 6.2 % of population infected from HCV and predominant genotype is 3a 63.4 %, province wise prevalence revealed that in Sindh its 2.6 %, Khyber[5]. The prevalence of HCV in district Buner KP was 4.6 %[6]. The Blood and their products are the major source for the HCV infection transmission. Unscreened blood transfusion, organ transplantation, contaminated medical instruments, needles, lancets, syringes, catheter and intravenous drug user[7]. Among all these routes of transmission intravenous drug user are quite higher. Numerous studies reported that IFN based treatment increased the risk of thyroid disorder[8].

Thyroid dysfunction had been observed 15% in those patient whose are receiving interferon INF based treatment for HCV infection and its mechanism either inflammatory or autoimmune however little is known about effect of oral antiviral therapy on thyroid function[9]. This study was designed to evuluate the effect of new treatment on thyroid function. Historically, interferon alpha (IFNα) and ribavirin therapy were used for HCV treatment and it was well documented that both are associated with thyroid dysfunction the most common autoimmune disorder associated with IFN-α[9]. Tomer et al. (2007) conducted a study which shown that 15 % of HCV patients were receiving IFNα, developed thyroid disease and 40 % HCV infected individuals become thyroid positive antibodies[10].

The conventional treatment for the chronic hepatitis C PegIFN is replaced by DDAs agent Sofosbuvir (sovaldi) and its effectiveness was confirmed[11]. The Development of the DAAs revolutionized the therapy for Chronic HCV infection and the DDAs targeting HCV viral proteins such as NS3/4A protease, NS5B-polymerase accompanied by nucleotide, non-nucleotide inhibitors and NS5A viral replication complex. An uprising in HCV infection treatment was bringing off in the last 7 years by the launching of the DAAs. Among DAAs agent the Sofosbuvir is a DAAs the backbone of oral antiviral therapy. This is a uridine nucleotide analogue which is selectively hamper NS5B polymerase and has powerful activity[12]. The study aims to evaluate the effect of oral antiviral therapy on thyroid function and To check the magnitude of thyroid dysfunction among HCV patients on oral antiviral therapy.

PATIENTS AND METHODS

This study was carried out in district Mardan and patients visiting to Mardan Medical Complex Mardan (MMC) for Hepatitis screening were enrolled after taking informed consent. This was prospective cohort study. The duration of this study was from September 2019 to December 2019. A total of 109 HCV patients diagnosed by Real Time PCR were included. Written contest from all the patients included in the study were taken.

Patients with age above 18 years, positive for HCV RNA by PCR and those who were willing to take part in this study were enrolled in this study. Those patients who were platelets count less than 50,000/µl. Those patients who were Suffering from hepatocellular carcinoma, Extra hepatic malignancy, Pregnant, Co-infected with HBV, renal, cardiac failure, Patients taking another antiviral therapy and patients who were not willing to participate in this study were excluded from this study. Centrifugation at 1000 rpm was carried out for the separation of plasma from blood.

Accurate kit was used for initial HCV screening it is also called one step test device. Enzyme Linked Immune Sorbent

Assay: The samples of the chronic hepatitis C individuals screened by ICT were subjected to HCV antibodies on 3rd generation ELISA using (EMP-CR-201 ELISA kit) following manufacturer instructions which has more sensitivity and specificity. Final conformation was done by Qualitative PCR. RNA was extracted from the serum sample and following protocol was followed.

For examination of thyroid function tests (TFTs), Architect 1000 was used. It was an automatic chemistry analyzer for which blood samples were collected in gel tubes and by centrifugation serum was separated from blood and then serum was placed in the trays for screening and processing of TFTs. Three tests including TSH, Total T_3 (TT_3) and Total T_4 (TT_4) were performed to access thyroid function tests.

The data was analyzed by Statistical package for social sciences (SPSS)1BM version 21 and Microsoft Excel.

RESULTS

Initial diagnosis by ICT and ELISA: In the present research, a total of 1,650 suspected HCV patients have been diagnosed using the ICT method. The physicians referred the patients to research lab after screening. The most common symptoms of enrolled patients were weight loss, jaundice, fever, depression, jaundice, liver tenderness, myalgia and arthralgia. All the patients screened with RDT-coated recombinant HCV antigen devices. Anti-HCV antibodies were found and statistics showed that HCV-positive patients were 117 (7.1 %), while ICT-negative were 1533 (92.90 %). Of all 117 ICT-positive samples, ELISA was found negative for the HCV infection in 122 patients, while rest of 5 patients were remained positive, comprising the HCV prevalence by ELISA method, around 6.7 %. Diagnosis by RT-PCR: ELISA confirmed 112 samples were exposed to real time PCR for HCV RNA detection, among these 109 (97.3 %) samples were positive for HCV RNA while 3 (2.7 %) were negative (Table 1)

Table 1: Prevalence of HCV determined by different method

Diagnosis Method	Number of patients	Percentage (%)
ICT	117/1650	7.1
ELISA	112/1650	6.7
PCR	109/1650	6.6

Patients from age above 18 were enrolled. Frequency of male and female were 71 (61.5 %) and 45 (38.5 %). Among these positive patients the status of TFTs before therapy were as follows; normal TFTs 105 (96.33 %), hypothyroidism 3 (2.75 %) and hyperthyroidism 1 (0.91 %) while TFTs after therapy was normal TFTs 92 (84.40 %), hypothyroidism 6 (5.50 %) and hyperthyroidism 11 (10.09 %) (Table 2).

Table 2: Gender-wise distribution of HCV Patients

Gender	Number	Percentage (%)
Male	72	61.5
Female	45	38.5
Total	117	100

Patients above age 18 years were included in the current study. These patients were categorized into four groups; 18-34 years, 35-54 years, 55-74 years and above 75 years age. The highest prevalence of HCV in this research was 35 (32.1 %) detected in age groups 55-74 years. The second highest infectious rate in the 34-54 age group was 30 (27.5 %). The third-highest rate of HCV infection was 26 (23.8 %) in the 31-40 years age group, while 18 (16.5 %) in the 18-34 age group were found for low HCV infections (Table 3).

Table 3: Age-wise distribution of HCV patients

Age groups	No. of patients (%)
18-34	18 (16.5)
35-54	30 (27.5)
55-74	35 (32.1)
> 75	26 (23.8)

All HCV-positive patients diagnosed with RT PCR n=109 were tested for thyroid function and mean value was recorded in new HCV patients as TSH (3.88 ± 1.07), TT3(1.15 ± 0.58), and TT4 (7.7 ± 01.99). TFTs of all HCV-positive patients are given in Table 4.4 after three months of treatment. TSH (2.49±1.72), TT3(1.38±0.69) and TT4 (8.78±2.29) Table 4.4 were noted as the mean ± SD value of HCV patients after therapy (Table 4).

Table 4: Mean values of TSH, T3 and free T4 before/ after treatment

No. Patients n=109	TSH Mean ± SD (IU/mL)	TT_3 Mean± SD (ng/mL)	TT_4 Mean± SD (ug/dL)
Before therapy	3.88±1.74	1.15±0.58	7.7±1.99
After therapy	2.49±1.72	1.38±0.69	8.78±2.29
Reference ranges	0.3500-4.9400	0.58-1.59	4.87-11.72

Before HCV treatment of 109 HCV-positive patients, 105 (96.3 %) were normal TFTs, while 4 (3.6 %) had abnormal TFTs. Of these four patients, 3 (75 %) had hypothyroidism (TSH increase) while 1 (25 %) had hyperthyroidism. 92 (84.4 %) had euthyroidism, 6 (5.5 %) had hypothyroidism (TSH increase) and 11 (10.09 %) had hyperthyroidism (Table 5).

Of 11 patients with hyperthyroidism, 7/109 (6.4%) had overt hypothyroidism, and 4/109 (3.6%) had subclinical hypothyroidism. Among 5/109 (4.5%)- had overt hypothyroidism and 1 had (0.9%) subclinical hypothyroidism (Table 6).

To categorize four age groups of HCV thyroid dysfunction patients, the incidence of TFTs was high in patients over 50 years of age. There was no high variance among different age groups; however, the highest hyperthyroidism patients were found in the age group of 35-54 years while the highest hypothyroidism patients were found in the age group of > 75.5 years. Age group 18-34 years found the lowest number of thyroid patients. Among age groups, age groups likewise 18-34 year had 2 (11. %) hyperthyroidism, 1 (3.3 %) had hypothyroidism, and 4 were hypothyroidism (13.3 %). Hypothyroidism was 55-74, 2 (5.7 %) and hyperthyroidism 3 (8.5 %) and hypothyroidism 75-3 (11.5 %) and hyperthyroidism 2 (7.6 %) (Table 7).

Table 5: Hypo/Hyperthyroidism among HCV patients before and after therapy

TFTs Level	Normal (%)	Hypothyroidism (%)	Hyperthyroidism (%)
Before Treatment	105 (96.3)	3 (2.85)	1 (0.95)
After Treatment	92 (84.4)	6 (5.5)	11 (10.1)
P-value	0.05	0.02	0.001

Table 6: Categories of thyroid dysfunction in HCV patients after therapy

No. Patients	Euthyroidism (%)	Hyperthyroidism (%)		Hypothyroidism (%)	
n=109	92 (84.5)	Over	Subclinical	Over	Subclinical
		5/109 (4.6)	2/109 (1.8)	3/109 (2.7)	7/109 (6.4)

Table 7: Age-wise distribution of thyroid dysfunction among HCV patients

Age	No. of Patients (%)	Hypothyroidism (%)	Hyperthyroidism (%)
18-34	18 (16.51)	0	2 (11.1)
35-54	30 (27.52)	1(3.3)	4 (13.3)
55-74	35 (32.11)	2(5.7)	3(8.5)
>=75	26 (23.85)	3 (11.5)	2 (7.6)

DISCUSSION

HCV infectivity rate is very high in Pakistan. In the current study the prevalence of HCV was 6.6 %, similar findings were recorded in another study conducted by Umer et al., 2016 in Pakistan[1]. The prevalence of HCV varies from provincially, likewise Punjab at 5.%, Sindh at 2.6 %, KPK at 6.07 %, FATA at 3.37 % and Balochistan at 25.8 %[13]. The HCV infectivity rate of the present study was different from the research conducted in Mardan in 2014, which

recorded a 12 % rate of HCV infectivity[14]. Similarly, another Mardan study showed a prevalence of HCV of 9 %, higher than the present findings[15]. Contrary to the current study, A, Khan et al., 2013 reported about 20% HCV prevalence in Quetta city[16]. Furthermore the prevalence of HCV was observed, which also shows diversity in various areas; Shangla 10.4 %, Buner 6.6 %[17]; Islamabad 31% and Swat 2.3%[18].

In the line of previous studies, the RT-PCR method considered as a gold standard for the detection and monitoring of viral load during treatment. A comparative study in Peshawar city was done on three hundred samples for detection of HCV infection using ELISA, of which 14 samples were positive. These 14 samples were subsequently re-analyzed using the RT-PCR method that detected the HCV infection in only 5 samples[19]. Similarly, Kwenti et al. (2011) reported that the maximum sensitivity and specificity of the RT-PCR[20].

HCV infection prevalence is also based on the diagnostic method 's specificity and sensitivity. On comparative analysis, the prevalence of HCV on the same samples was reported to be 15 % by ICT, 11 % by ELISA and 10 % by RT-PCR[21]. A further study on 3,1560 samples tested with HCV showed positive results in 16 % of which were based on ELISA, while the methodology used in PCR was 13%[22]. Therefore, RT-PCR technique is highly recommended to be used to detect and monitor hepatitis C virus because it is high specificity and sensitivity. Exposure to the different HCV risk factors is specially gender specific in Pakistan mostly due to our social culture. Exposure of males to some HCV risk factors is higher than female. Ali et al. (2011) concluded a study, in infectivity rate in male was 72.3 % higher than female 37.2 %[23].

In the present study, of 117 HCV-positive patients with qualitative PCR, the male infectivity rate was higher than 72 (61.5 %) compared to 45 females (38.5 %) with a gender-based distribution of HCV. In accordance to a study conducted in Southern KP, where 54 % of males were HCV-infected and 46 % of females were HCV-infected[6]. Contrary, current results is not supported a previous study conducted in Mardan, which reported the high prevalence of HCV in females as compared to males[24]. One more study also found support for another study in which the rate of males was higher 58.3 % than females 41.7 %[25].

The highest prevalence among 55-64 years was observed by Ahmad et al., 2018[26]. Farooq et al. (2019) observed the highest 14.2 % prevalence above 45-year group that was very similar to that[25]. Disagreed with a study that showed no substantial difference between the ages of different age groups[24]. This high prevalence of HCV infection among older people; hence how the self-protective strategies are restructured to reduce the load and prevent this infection from spreading further across the healthy people.

Thyroid disorder in both interferon-based therapy as well as in DNA-based therapy, according to Wahid et al., 2019[27]. This concluded prevalence of hyperthyroidism was found in both treatment plans. However, hyperthyroidism was found to be significantly higher in those patients treated with interferon-based treatment compared to interferon-free regimens[27]. According to Shaaban et al., 2019, the value of TSH (P = 0.580) and free T4 (P = 0.279) after DAAs was not statistically elevated[28].

A similar study by (EL Feki et al., 2016) reported that 10–15 % thyroid dysfunction was shown during interferon-free HCV treatment[29]. A latest 2019 study reported that patients receiving new direct antiviral for chronic hepatitis C. In the study, 100 chronic hepatitis patients completed normal thyroid functions before starting DAAs. After completion of therapy evaluation of analyzed patients receiving DAAs. There is no statistically significant difference before and after[28]. The incidence of thyroid dysfunction was 26.8 %, which was significantly higher than the healthy population. Our study finding compared to a study in which median age groups were 36, and thyroid dysfunction was high in patients above the median age 36[30].

CONCLUSION

In our finding, DAAs affect the thyroid gland, so these patients should be screened regularly for thyroid disorders and properly treated to maintain euthyroid status. While HCV-based interferon-treated patients are more likely to develop thyroid dysfunction, the results suggest that sofosbuvir-treated patients also have a high frequency. This finding highlights the importance of periodic screening of thyroid hormones in HCV-infected people. Levels of hypothyroidism or hyperthyroidism can alter the treatment outcome against HCV.

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