Study of Cough Reflex Sensitivity in a Group of Egyptian Patients with Chronic Hepatitis C Virus Infection

Kronik Hepatit C Virüs Enfeksiyonlu Mısırlı bir Hasta Grubunda Öksürük Refleksi Duyarlılığı Çalışması

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ABSTRACT

Objective: The aim of this work was to study the effect of chronic hepatitis C viraemia on cough reflex sensitivity.

Material and Method: Cough reflex sensitivity was tested by Capsaicin cough provocation in 57 patients (33.66±6.809 years old [M±SD]) with chronic HCV infection. Patients were divided into 3 subgroups: group I: 22 with normal Alanine aminotransferase (ALT) and no evidence of liver cirrhosis on ultrasound, group II: 19 with elevated ALT and active hepatitis but no cirrhosis on liver biopsy (8 on treatment) and group III: 16 with elevated ALT and positive cirrhosis on liver biopsy (6 on treatment). 23 normal age matched subjects were tested as controls.

Results: log C5 was significantly lower in the three groups of patients than controls ($p<0.05$). Patients with liver cirrhosis showed a significantly lower log C5 than those without cirrhosis and normal ALT ($p <0.05$). No significant difference was found between the two groups with high ALT or the two groups without cirrhosis ($p>0.05$ for both). Females showed significantly lower log C5 values than males in normal controls but not in patients ($p<0.05$ and $p>0.05$ respectively. Patients on interferon therapy showed a significantly lower log C5 values than untreated patients ($p<0.01$). In all patients, the relation between viral load (RNA PCR) and provocative concentration of capsaicin was inverse and significant ($r=-0.717$, $p<0.001$).

Conclusion: Both hepatitis C viraemia and interferon therapy enhance cough sensitivity in chronic hepatitis C patients and more studies are needed to explain how this occurs.
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Key words: Capsaicin, cough, hepatitis c, sensitivity

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ÖZET

Amaç: The aim of this work was to study the effect of chronic hepatitis C viraemia on cough reflex sensitivity.

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Anahtar sözcükler: Kapsaisin, öksürük, hepatit C, duyarlılık

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ABBREVIATIONS

HCV = Hepatitis C Virus
M = Statistical mean
SD = Standard Deviation of mean
SE = Standard Error of mean
PCR = Polymerase Chain Reaction
C5 = Capsaicin concentration that produce 5 coughs
BAL = Bronchoalveolar lavage

INTRODUCTION

Over the last decade, an increasing number of reports have suggested that chronic hepatitis C virus (HCV) infection is associated with both direct and indirect effects on pulmonary tissues [1]. Asthmatics and COPD patients infected with the virus have shown a more rapid decline in lung function than those not infected [2,3]. Hepatitis C virus is a small single stranded RNA virus of the Flaviviridae

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family that can pass the immune system and causes chronic liver inflammation [4]. The disease is not uncommon and is present worldwide with an especially high prevalence in Egypt [5,6]. We have seen many chronic hepatitis C patients referred from hepatology and gastroenterology clinics for treatment of their cough, they did not have asthma or COPD and responded well to cough suppression therapy. We thought of the possibility of enhanced cough sensitivity as a cause in these patients and carried out this study to detect any possible effect of the disease on cough sensitivity.

MATERIAL and METHOD

In compliance with Ethical Committee for Scientific Research at our locality, we selected 57 patients (33.66±6.809 years old [M±SD], 39 males and 18 females) with proved chronic hepatitis C virus infection by quantitative polymerase chain reaction (PCR) assay (COBAS® AmpliPrep/COBAS® AMPLICOR HCV Test, v2.0, Roche Diagnostics, USA) from those attending the outpatient clinic in the Hepatology and Tropical Medicine department of our institute. PCR assays older than 3 months were repeated and recent assays (within 3 weeks) were done for patients on interferon therapy. Patients had no history of pulmonary disease, or recent (within 4 weeks) symptoms suggestive of respiratory tract infection or seasonal allergies. Also, they were non smokers and not receiving any medication known to affect cough reflex sensitivity [7]. Diabetics, renal failure patients as well as those with systemic collagen vascular diseases were also excluded due to possible effects on the lungs and airways. All patients were subjected to full clinical evaluation, general lab testing with special attention to liver functions, abdominal ultrasound and pulmonary function testing (using the dry spirometer; Spirosift, Fukuda, Japan) to confirm exclusion criteria. Patients with active liver disease already had their liver biopsy stained slides with reports (done at outpatient clinics). Patients were divided according to liver status into 3 groups, group I: 22 patients (34.076±4.224 years [M±SD],15 males and 7 females) with normal serum Alanine aminotransferase (ALT) and no liver cirrhosis on ultrasound, group II: 19 patients (32.842±4.179 years [M±SD], 13 males and 6 females) with high serum ALT and active hepatitis but no cirrhosis on liver biopsy, and group III patients (34.083±5.844 years [M±SD], 11 males and 5 females) with high serum ALT and positive cirrhosis on liver biopsy. 8 of group II and 6 of group III were on interferon therapy. 23 normal, non smoker volunteers (33.434±4.413 years [M±SD], 15 males and 8 females) were studied as controls (group IV). The study was carried out from August 2008 to February 2009, and all subjects agreed to be enrolled in the study.

Cough challenge testing with inhaled capsaicin was performed as previously described [8]:

30.5 mg of Capsaicin (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in 1mL ethanol and 1 mL polyoxy-ethylenesorbitan monooleate 80 (Tween 80) and then dissolved in 8 mL physiologic saline solution to make a stock solution of 0.01M. This solution was further diluted with saline solution to make serial doubling concentrations ranging from 0.98 to 1,000 mol/L. Fresh dilutions were prepared on each day of testing.

Subjects inhaled single breaths of capsaicin solution from a compressed-air driven dosimeter nebulizer (model MB Mefar, Electromedicali, Korea). This nebulizer was modified by the addition of an inspiratory flow regulator valve that regulated inspiratory flow rate to a consistent 0.5 L/s regardless of inspiratory force, thereby guaranteeing a consistent and reproducible amount of aerosol delivered with each breath. Under these conditions, the output of this nebulizer was determined to be 1.007 mL/min. The duration of aerosol delivery was programmed at 1.2 s, thereby providing 0.02 mL per breath. Single breaths of capsaicin were given in incremental concentrations, with inhalations of saline solution randomly interspersed to increase challenge blindness, until the concentration inducing five or more coughs (C5) was attained. Breaths were delivered at 1-min intervals. Subjects were unaware that the end point of the study was the number of coughs induced.

All studies were performed by the same investigator, using the same nebulizer dosimeter, and settings. log C5 in μM was used for interpretation. For all, the timing of performing the test was between 9-11am.

Statistical Analysis

Collected data were analyzed using the NCSS statistical software for Windows (version 2007). Measured values were presented as mathematical mean±standard deviation of mean (M±SD). Multiple comparisons were done by the one way ANOVA test and significance confirmed by the Bonferroni method, while unpaired comparisons were done by the student t test. Correlation statistics were done by linear correlation and regression analysis. P values less than 0.05 were considered significant, while values greater than 0.05 were non significant.

RESULTS

Important clinical data of studied groups are shown in Table 1. With multiple comparisons, the differences in the median values among the studied groups were statistically significant (P<0.001, Table 2). All the three groups of patients attained C5 at significantly lower concentrations of capsaicin than normal controls (p<0.05 for all). Also, group III patients showed significantly lower log C5 values than group I (p<0.05). A non significant difference was found between group I and II and II and III (P>0.05 for both) (Table 3). Females showed significantly lower log C5 values than males in normal controls but not in patients (p<0.05 and p>0.05 respectively, Table 4). log C5 was significantly lower in patients receiving antiviral therapy than in those not on treatment (p<

Table 1. Clinical characteristics of patients and controls included in the study

		Patients Group I	Patients Group II	Controls Group III	
Number		22	19	16	23
Age in years (M±SD)		34.076±4.224	32.842±4.179	34.083±5.844	33.434±4.413
Sex	Males Females	15 (6.8.18%) 7 (31.82%)	13 (68.4%) 6 (31.6%)	11 (68.75%) 5 (31.25%)	15 (65.22%) 8 (34.78%)
Smoking		No	No	No	No
PCR(x103IU/ml)[M±SD]		679.269±123.242	1146.947±229.262	1683.333±331.87	Negative
Liver	ALT US + Biopsy	Normal No AH	High AH but no cirrhosis	High Cirrhosis present	Normal
Interferon therapy		No	8	6	-
log C5(μM)((M±SD)		1.373±0.241	1.08±0.25	0.789±0.124	1.77±0.15

US: ultra sound, AH: active hepatitis

Table 2. Comparison of median log C5 among studied groups*

Group	Number	Missing	Median	25%	75%	DF	P
I	22	0	1.350	1.230	1.490	3	< 0.001**
II	19	0	1.110	0.915	1.225		
III	16	0	0.760	0.700	0.820		
IV	23	0	1.810	1.652	1.910		

DF: degrees of freedom, *One way ANOVA on Ranks, **Significant

Table 3. Paiwise comparison of log C5 among different groups*

Comparison	Diff of Ranks	Q	P<0.05**
G IV vs G III	57.077	7.545	Yes
G IV vs G II	38.819	5.388	Yes
G IV vs G I	23.541	3.397	Yes
G I vs G III	33.537	4.392	Yes
G I vs G II	15.279	2.099	No
G II vs G III	18.258	2.316	No

*One way ANOVA on Ranks (Dunn's Method), **Significant

0.01, Table 4). Correlation between the virus load (PCR) and log C5 was negative and significant (r=-0.717, p<0.001) (Table 5, Figure 1).

DISCUSSION

In this study, the three patient groups showed enhancements in their cough reflex sensitivity compared to controls as indicated by their significantly lower log C5 values. Also, cough sensitivity was found to be higher in patients with cirrhosis and abnormal liver function than those without.

After extensive search in related medical literature and journals we found no published data about cough sensitivity in chronic hepatitis C patients. Moreover, effects of hepatitis C virus on the lungs and airways were not fully explored in the clinical research up to the time of this report. In view of these limitations we cannot have a satisfying explanation to such cough enhancement in these patients.

Depending on the few published preliminary reports on the effects of this disease on lungs and its associated systemic effects [9], we can propose some possible mechanisms. Firstly; patients have chronic hepatitis C viraemia [10] and it is possible that the virus affects the airways in the same manner as respiratory viruses but in a less severe form, a postulation that needs supportive studies.

Respiratory viruses are well known to have deleterious effects on the airways and the lungs [11]. Cough sensitivity to citric acid was found to be enhanced following influenza infection [13]. Many mechanisms were described by which respiratory viral infections increase cough sensitivity. One important mechanism is by an

Table 4. Comparison of log C5 between males and females of all groups and between interferon treated and untreated patients

Comparison	M±SD	p
Males : all patients	1.166±0.168	> 0.05*
Females: all patients	1.123±0.221	
Normal controls (males)	1.857±0.124	< 0.05**
Normal controls (females)	1.533±0.132	
On interferon therapy (groups II,III)	1.191±0.153	< 0.01**
Not on interferon therapy (groups II,III)	1.744±0.251	

*Non significant ** significant

Table 5. Correlation between log C5 and HCV PCR in all patients

	M±SE	r	p
PCR (x10^3IU/ml)	1046.543± 228.125	- 0.717	< 0.001**
log C5 (μM)	1.152± 0.134		

** Significant

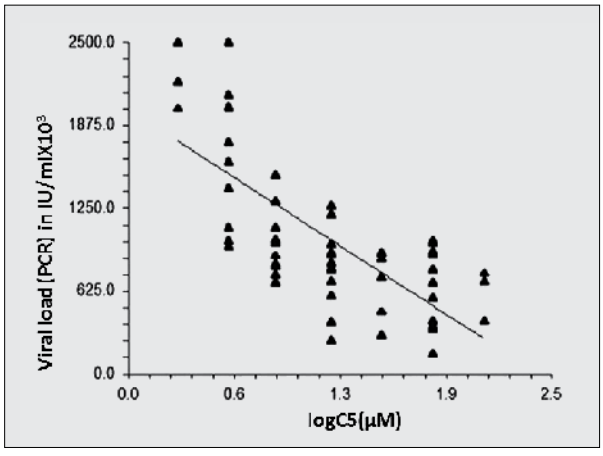

Figure 1. Correlation between viral load (PCR) and log C5 in all patients

increase of tachykinins in patient blood, possibly mediated by neurotrophins produced in response to viral infection. Other ways for a rise of tachykinins in these patients include the reduced effect of neutral endopeptidase, an enzyme that is important in degrading and inactivating tachykinins. Moreover, viral infections activate eosinophils and releasing proteins that stimulate them to secrete tachykinins. Not only do viral infections increase blood tachykinins, but they also increase expression of their receptors [12].

Another important mechanism for enhanced cough sensitivity with viral infections is by decreasing expression of M_2 muscarinic receptors, which normally decrease the sensitivity of sensory nerves [13].

Epithelial shedding and increased mucus production are also considered by some as important ways of enhanced cough sensitivity in viral infections of the lungs [14]. Whether hepatitis C virus causes these changes in the airways or not is not known. Moreover, if it causes these changes in the airways, it is not known whether this occurs due to a direct effect of the virus or due to the systemic immune response associated it [15]. Hepatitis C virus RNA has been detected in bronchoalveolar lavage (BAL) from patients with interstitial pulmonary fibrosis [16] meaning that the virus is also present in airway secretions, which gives some support to the above postulates.

Some studies showed an increase in eosinophil number in the lungs of these patients [17] and eosinophils are well known to produce powerful inflammatory mediators [18] that could be provoked by viral infections [19]. It is also possible that these cells may have a role.

In this study, females of the normal control group showed significantly higher cough sensitivity (i.e. lower log C5) than males. In a group of healthy adult population, Dicpinigaitis and Rauf [8] had found cough sensitivity to be significantly lower in males than females, which agrees with our results. Surprisingly, this significant difference between the two sexes in normal controls was not seen in patients. This discrepancy between patients and controls as regards the effect of gender on cough sensitivity needs further studies to explain it. Simply, we could suppose the possibility of masking the physiological effect of gender by pathological changes of the disease.

In the present work we found a significant negative correlation between the virus load (i.e. HCV PCR) and log C5, indicating that cough sensitivity increases as the virus load increases. These results provide some support to the above speculation about a direct role of the virus and its associated systemic inflammatory response in enhancing cough sensitivity. From 40-76% of patients infected with HCV develop at least one extra hepatic manifestation during the course of the disease [20]. The relation between the virus load and the associated systemic inflammatory and immune response has been excellently reviewed by Galossi et al. [21].

In our study, patients on antiviral therapy have attained C5 at lower concentrations of capsaicin than

those not on treatments, denoting an enhancement of cough reflex due to interferon. Many reports about the use of interferon in treatment of chronic hepatitis C infection showed cough to be one of the important side effects of the drug, occurring in about 6% of treated patients [22-24] although the mechanism is not yet explained. In a case report from Turkey, one patient who was treated by interferon for hepatitis B infection had an intractable cough that mandated drug withdrawal [25].

Pulmonary sarcoidosis was reported to occur with chronic hepatitis C infection [26] and to worsen with interferon therapy [27]. Endobronchial sarcoidosis might be present and might have a role in this cough enhancement.

Surprisingly, and in contrast, Isler et al. [28] reported that cough is a rare complication of peglated interferon therapy, which is not in agreement with the above reports that support our result.

Finally, we think that the effects of chronic hepatitis C viraemia on human airways constitute a wide and virgin area for investigation and we await future studies that might prove or disprove our propositions.

Both hepatitis C viraemia and interferon therapy enhance cough sensitivity in chronic hepatitis C patients and more studies are needed to explain how this occurs.

Conflict of Interest

No conflict of interest is declared by the authors.

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