

## Decreased tear lactoferrin concentration in patients with chronic hepatitis C

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### Abstract

**Background/claims**—Decreased tear volume in patients with chronic hepatitis C has been reported in the literature. Lactoferrin is abundantly present in human tears, the main source of which is the acini of the lacrimal glands. In this study tear lactoferrin levels were measured to investigate the dry eye condition of patients with chronic hepatitis C.

**Methods**—Lactoferrin in tears/fluid was measured by a radial immunodiffusion assay in 42 patients with chronic hepatitis C. The rate of lacrimal secretion was determined by the cotton thread test. Rose bengal staining of the ocular surface was also performed.

**Results**—Only three patients out of 42 complained of dry eye sensation and, in 31 patients, six showed positive results on the rose bengal staining test of the ocular surface. The lactoferrin concentration of tear

fluid in the chronic hepatitis C group (1.42 (SD 0.56) mg/ml) was significantly lower than in the control group (1.90 (0.62) mg/ml;  $p < 0.00048$ ). The cotton thread test results in the chronic hepatitis C group (12.9 (5.5) mm) were significantly lower than in the control group (17.9 (5.3) mm;  $p < 0.00048$ ). Also, in the chronic hepatitis C group, tear lactoferrin concentration correlated with the results of the cotton thread test ( $r = 0.35$ ,  $p < 0.05$ ).

**Conclusion**—Chronic hepatitis C patients showed both decreased tear volume, and decreased tear lactoferrin concentration. These findings suggest that there may be dysfunction of the lacrimal glands in patients with chronic hepatitis C, which may account for the mild dry eye.

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Hepatitis C virus (HCV) is a principal cause of chronic hepatitis worldwide.<sup>1</sup> It rapidly became evident that HCV infections were associated with disorders of various organs other than the liver, essentially through immunological mechanisms.<sup>2</sup> These include mixed cryoglobulinaemia and cryoglobulin associated vasculitis,<sup>2,3</sup> autoimmune disorders,<sup>2</sup> and lichen planus.<sup>4,5</sup> Also, HCV plays an active role in retinopathy,<sup>6,7</sup> episcleritis,<sup>8</sup> retinal vasculitis,<sup>9</sup> and Mooren-type corneal ulcer.<sup>10,11</sup> In addition, Haddad *et al*<sup>12</sup> reported that sialoadenitis was observed in chronic hepatitis C patients. In their report,<sup>12</sup> no patient complained of dry eye sensation, but of the six patients randomly assigned to ophthalmological examination, three had a low level of Schirmer's test, and one of these had a positive rose bengal test. Recently, Abe *et al*<sup>13</sup> and Ido *et al*<sup>14</sup> reported that tear volume was decreased in patients with chronic hepatitis C. The mechanism of decreased lacrimal secretion in patients with chronic hepatitis C is unknown. HCV-RNA is present in tears in patients with chronic hepatitis C.<sup>15</sup> There is therefore a possibility that lacrimal dysfunction is due to HCV.

Lactoferrin, an iron binding glycoprotein, is abundantly present in human tears. In studies by Fullard and Tucker,<sup>16</sup> lactoferrin levels remained relatively constant throughout the series of tear samples, from non-stimulated to the higher flow rate stimulated tears. Gillette and Allansmith<sup>17</sup> showed that tear lactoferrin is mainly secreted from the acini of the lacrimal glands. In this study, tear lactoferrin levels were measured to investigate the dry eye condition in patients with chronic hepatitis C.

Table 1 Clinical data from 42 patients with chronic hepatitis C

Case	Age (years)	Sex	Dry sensation	RB score	Cotton thread (mm)	Lactoferrin (mg/ml)	ALT (IU/l)
1	68	M	+	3	12.5	2.8	33
2	77	F	-	NT	12.0	1.1	45
3	59	M	-	NT	3.5	0.6	91
4	59	M	-	0	7.5	1.0	108
5	66	M	-	0	14.0	0.5	48
6	38	M	-	0	15.0	1.4	142
7	63	F	-	3	6.0	1.8	35
8	73	M	-	1	22.0	1.5	126
9	45	M	-	0	10.5	1.8	41
10	66	F	-	2	20.0	1.7	40
11	84	M	-	0	13.5	1.6	36
12	65	F	-	NT	7.0	1.1	32
13	49	M	-	0	4.0	1.0	40
14	38	M	-	NT	13.5	2.2	73
15	60	F	-	0	10.0	1.1	90
16	34	M	-	3	10.5	1.0	NT
17	61	M	-	2	16.5	1.7	54
18	55	F	-	0	12.0	2.2	20
19	74	F	-	NT	16.5	1.1	102
20	69	F	-	1	14.0	1.5	38
21	47	M	-	NT	7.0	2.1	90
22	62	F	-	0	12.0	1.8	60
23	65	F	-	NT	13.0	1.5	63
24	53	F	+	2	20.0	1.8	34
25	76	M	-	3	6.5	0.5	25
26	66	M	-	0	13.5	1.8	11
27	34	F	-	NT	22.5	2.2	NT
28	60	F	-	NT	14.0	0.5	18
29	64	F	-	0	21.0	2.0	27
30	65	M	-	0	17.5	1.3	153
31	39	M	+	1	13.5	0.8	157
32	62	M	-	0	16.5	1.8	23
33	70	M	-	NT	19.0	2.1	34
34	84	F	-	6	3.0	0.3	14
35	36	M	-	0	7.0	1.0	40
36	56	F	-	2	8.0	1.0	32
37	74	F	-	4	20.5	0.9	87
38	56	M	-	0	17.0	1.4	56
39	53	F	-	2	20.0	1.7	43
40	62	F	-	0	16.5	1.4	91
41	40	M	-	0	3.0	1.7	30
42	70	F	-	NT	9.5	1.9	NT

RB score=rose bengal score (0-9), ALT=level of serum alanine aminotransferase, NT=not tested.

Table 2 Clinical data from 42 control subjects

Case	Age (years)	Sex	Dry sensation	Cotton thread (mm)	Lactoferrin (mg/ml)
1	67	M	-	22.0	1.5
2	79	F	-	11.5	0.9
3	57	M	-	13.0	2.2
4	62	M	-	17.0	2.9
5	66	M	-	12.5	1.4
6	43	M	-	27.0	2.2
7	59	F	-	16.0	2.2
8	70	M	-	15.5	2.1
9	50	M	-	22.5	2.2
10	66	F	-	22.0	2.2
11	81	M	-	16.5	1.7
12	68	F	-	14.5	1.0
13	53	M	-	26.5	1.1
14	41	M	-	19.0	2.9
15	59	F	-	16.5	2.1
16	34	M	-	26.0	2.7
17	64	M	-	10.0	1.1
18	57	F	-	17.0	1.9
19	69	F	-	19.5	2.3
20	69	F	-	12.0	1.8
21	49	M	-	23.0	2.2
22	61	F	-	24.0	1.3
23	60	F	-	23.5	2.5
24	51	F	-	11.0	2.2
25	76	M	-	19.5	1.5
26	69	M	-	12.5	1.3
27	30	F	-	13.5	2.9
28	57	F	-	15.0	1.7
29	62	F	-	22.0	1.7
30	67	M	-	21.0	2.1
31	39	M	-	15.5	2.9
32	63	M	-	21.0	1.0
33	70	M	-	12.0	1.9
34	79	F	-	32.0	1.6
35	34	M	-	23.0	2.8
36	53	F	-	14.0	2.0
37	74	F	-	17.5	1.5
38	61	M	-	11.5	1.3
39	56	F	-	21.0	2.5
40	61	F	-	15.0	2.8
41	45	M	-	15.5	0.8
42	67	F	-	10.0	1.4

### Materials and methods

Forty two consecutive patients from October 1996 to September 1997 with chronic hepatitis C (22 men and 20 women) aged 34–84 years (mean 59.5 years) were included in the study (Table 1). The results were compared with a group of age (SD 5 years) and sex matched normal healthy control subjects (Table 2).

Patients with histories of external ocular diseases, ocular operations, contact lens use, and recent treatments with eye drops were excluded from the study. A question (have you had daily, troublesome dry eyes for more than one month?) for eye involvement was put to subjects. Lactoferrin in tear fluid was measured by a radial immunodiffusion (RID) assay; the Lactoplate test.<sup>18,19</sup> The tear volume was determined by the cotton thread test developed by Hamano *et al.*<sup>20,21</sup> The mean of both eyes was determined in all of the above mentioned examinations. Vital staining test of the ocular surface with rose bengal was performed, graded from 0 to 9, and based on the sum of the staining grades from the nasal conjunctiva, cornea, and lateral conjunctiva<sup>22</sup> in the right eye. A rose bengal score of  $\geq 3$  was regarded as significant according to Japanese diagnostic criteria of dry eye.<sup>23,24</sup> Levels of serum alanine aminotransferase (ALT) at the time of the eye test were also measured. The HCV subtype<sup>25</sup> and serum copy number of HCV-RNA (copies/ml)<sup>26</sup> were also investigated by polymerase chain reaction. The results were

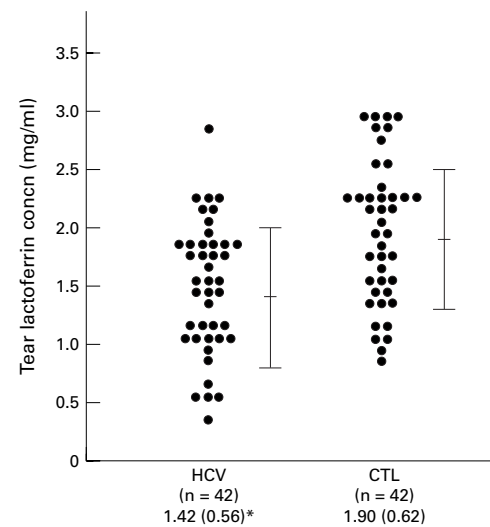


Figure 1 Tear lactoferrin concentrations in patients with chronic hepatitis C (HCV) and control group (CTL). Bars in graph and numbers under graph show mean values (SD). \*Mann-Whitney test;  $p < 0.00048$ .

expressed as mean (SD), and the Mann-Whitney test was used for statistical analysis of significance.

### Results

#### SYMPTOMS AND SIGNS

Three out of 42 patients (7.1%) complained of dry eye sensation.

In 31 patients, six (19.4%) showed positive vital staining test of the ocular surface, with a rose bengal score  $\geq 3$ .

#### LACTOFERRIN CONCENTRATION OF TEAR FLUID (FIG 1)

The lactoferrin concentration of tear fluid in the chronic hepatitis C group (1.42 (SD 0.56) mg/ml) was significantly lower than that of the control group (1.90 (0.62) mg/ml;  $p < 0.00048$ ).

#### THE COTTON THREAD TEST (FIG 2)

The cotton thread test score in the chronic hepatitis C group (12.9 (5.5) mm) was significantly lower than that of the control group (17.9 (5.3) mm;  $p < 0.00048$ ).

#### CORRELATION BETWEEN LACTOFERRIN AND OTHER FACTORS

##### Lactoferrin-cotton thread test

In the chronic hepatitis C group, the lactoferrin concentration of tear fluid correlated with the cotton thread test scores (Fig 3) (Pearson's correlation coefficient  $r = 0.35$ ,  $p < 0.05$ ). However, there was no correlation between these values in the control group ( $r = 0.18$ ,  $0.05 < p < 0.1$ ).

##### Other findings

In the chronic hepatitis C group, the lactoferrin concentration of tear fluid did not correlate with the rose bengal score (Spearman's correlation coefficient  $r_s = -0.18$ ,  $p > 0.05$ ), ALT ( $r = -0.16$ ,  $p > 0.1$ ), or the copy number of HCV-RNA ( $r_s = 0.11$ ,  $p > 0.05$ ) (data not

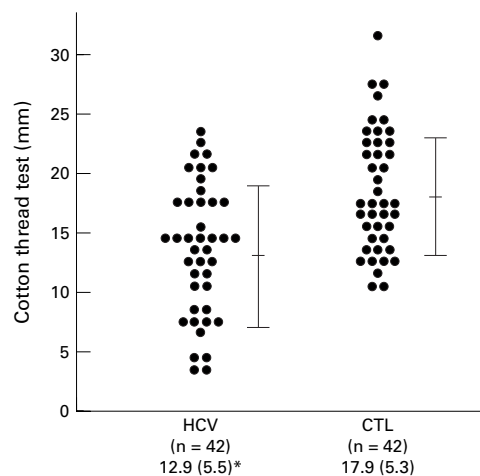


Figure 2 Cotton thread test in patients with chronic hepatitis C (HCV) and control group (CTL). Bars in graph and numbers under graph show mean values (SD). \*Mann-Whitney test;  $p < 0.00048$ .

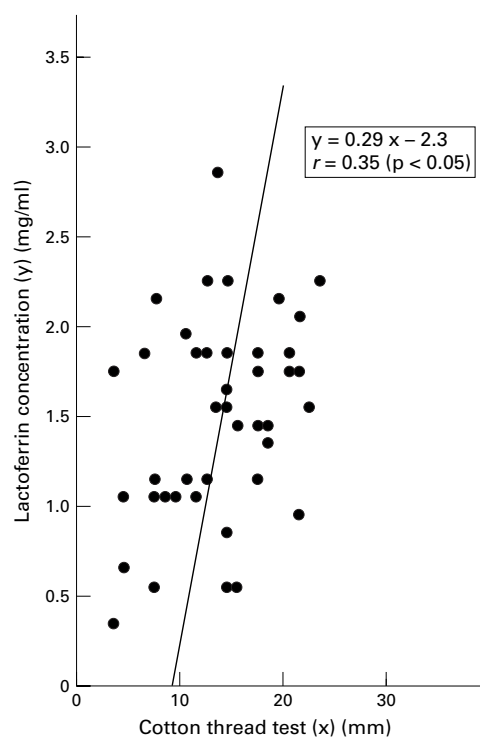


Figure 3 Correlation between lactoferrin and cotton thread test in patients with chronic hepatitis C.

shown). There was no difference in lactoferrin concentrations in tear fluid among HCV subtypes (data not shown).

### Discussion

The present study, together with previous reports,<sup>13 14</sup> demonstrated that patients with chronic hepatitis C showed decreased tear volumes. In this study, tear lactoferrin levels were measured to investigate the dry eye condition in patients with chronic hepatitis C. Patients with chronic hepatitis C also showed decreased tear lactoferrin concentrations. The main source of tear lactoferrin is the acini of the lacrimal glands.<sup>17</sup> Patients with chronic hepatitis C may have acinar cell dysfunction in the lacrimal gland, alterations in the circulation in

lacrimal tissue, and/or viral induced defects in ductal epithelial cells. It is also possible that lactoferrin binding to mucus could be enhanced in a dry eye state, and/or that lactoferrin binds to one of the HCV envelope proteins<sup>27</sup> in the tear film, thereby interfering with its measurement by RID. Possible low vitamin A levels associated with hepatic disease could contribute to lacrimal dysfunction in these patients.

As previously reported, HCV-RNA was present in tears<sup>15</sup> and saliva<sup>28</sup> in patients with chronic hepatitis C, and mild sialoadenitis was commonly observed in patients with hepatitis C.<sup>12 29</sup> Sjögren's syndrome was also reported during treatment with interferon  $\alpha$  for chronic hepatitis C.<sup>30</sup> Inflammation of the lacrimal gland was highly suspected in patients with chronic hepatitis C, but has not been definitely proved. Histological and virological examinations are necessary to confirm this.

In the present study only three of 42 patients complained of dry eye sensation. In addition, 19.4% of patients showed positive rose bengal staining. Previously, Haddad *et al*,<sup>12</sup> Abe *et al*,<sup>13</sup> and Ido *et al*<sup>14</sup> also reported that ocular surface disturbance in patients with chronic hepatitis C was mild. Ido *et al*<sup>14</sup> reported that Schirmer's test value was inversely proportional to ALT. However, in our study, cotton thread test values did not correlate with ALT. The reason for the discrepancy between our findings and those of Ido *et al*<sup>14</sup> is unknown, but may be due to different methods of lacrimal quantification, since Schirmer's test measures reflex flow and the thread test is more related to resting volume of tears.

In summary, chronic hepatitis C patients show decreased tear volumes and decreased tear lactoferrin concentrations. Chronic hepatitis C is the probable cause of mild dry eye in these patients.

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