Histopathological Findings of Autoimmunity in Thyroid, Pituitary and Adrenal Diseases in Chronic Hepatitis C Post-Mortem Cases

Huy A Tran, FACE, FRCPA 1*, Glenn EM Reeves, FRCPA, FRACP 1, Tim J Lyons, MD, FRCPA 2, John R Attia, MD, PhD, FRACP 3,4

1 Hunter Area Pathology Service, Locked Bag Number 1, Hunter Mail Region Centre, Newcastle, New South Wales 2310, Australia

2 Department of Forensic Medicine Services, Hunter New England Health Service, Newcastle 2310, New South Wales, Australia

3 Centre for Clinical Epidemiology and Biostatistics, University of Newcastle, Newcastle 2310, New South Wales, Australia

4 Department of General Medicine, John Hunter Hospital, Locked Bag Number 1, Hunter Mail Region Centre, Newcastle, New South Wales 2310, Australia

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All authors contributed equally to this work

* Corresponding author: HAT, huy.tran@hnehealth.nsw.gov.au

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ABSTRACT

Objective: In chronic hepatitis C infection, there is a heightened immune response resulting in many autoimmune diseases. The commonest endocrinopathy in association with this chronic infection is thyroid disease. Other endocrinopathies include hypophysitis, adrenalitis and diabetes. The vast majority of reported studies classify thyroid diseases according to biochemical, serological and imaging studies. So far, there is only one solitary (non-English) report of histological findings in chronic hepatitis C patients. The aim of the study is to assess the histologic prevalence of immune-mediated thyroid, pituitary and adrenal diseases in postmortem cases with hepatitis C.

Methods: One hundred and eight cases of chronic hepatitis C patients in which a full postmortem was performed were reviewed. All microscopic and histologic slides of the thyroid, pituitary and adrenal reports were reviewed and assessed for evidence of autoimmune diseases. These were compared with 100 non hepatitis C cases.

Results: There were 14 (13%) cases with evidence of thyroiditis. No cases of pituitary and adrenal diseases were found. The mean age was 52 (range: 22 to 67). This compared with 7 (7%) in the control group.

Conclusion: Thyroid disease was the only major endocrinopathy present in hepatitis C infection at postmortem with a prevalence of ~13%. This was comparable with other serological and non-histologic ante-mortem findings. There was no evidence of pituitary or adrenal involvement.
INTRODUCTION

Endocrinopathies are the commonest extra-hepatic manifestation of hepatitis C. The majority of epidemiology studies involve the use of serology, antibody profile and imaging techniques to define and categorise the condition, particularly thyroid disease (1,2,3). The definitive method to define the condition is to perform histological assessment of tissue samples. Thus far, there has been one solitary non-English report in the literature looking at the ‘gold standard’ in the histologic involvement of the thyroid (4). In order to fully assess the involvement of the three major endocrine organs, histological slides were reviewed retrospectively for evidence of inflammation of the thyroid, pituitary and adrenal gland.
METHODS

One hundred and eight postmortem cases in which chronic hepatitis C was documented were reviewed. In addition, 100 cases without hepatitis C were also reviewed to serve as controls. All cases come from the database of the Forensic Medicine department in a major tertiary referral hospital, performing ~1,000 post mortem forensic cases annually. Paediatric and pregnant cases were excluded, including controls. None of the patients had received interferon-based therapy for their hepatitis C at the time of their demise.

The demographic data is represented in Table 1.

All cases were documented to have hepatitis C which had been confirmed serologically ante-mortem. The serology was confirmed by immunochemiluminescence using the Architect (Abbott) assay. In addition, all liver histologic slides were reviewed and were found to be consistent with chronic viral hepatitis infection although clearly not diagnostic.

No immunohistochemistry was performed on any of the histologic samples, including autoantibody staining.

Histological definitions

1. Autoimmune thyroiditis is defined as the presence of interfollicular infiltration with lymphocytes and plasma cells. The presence of germinal centres, destruction of the follicles and fibrosis may be added to the diagnosis but are not considered essential (5,6).
2. Hypophysitis is defined as the presence of predominantly lymphocytic infiltrate. Plasma cells, lymphoid follicles with germinal centers may be present but are not critical to the diagnosis. (5,7).
3. Adrenalitis is defined as the presence of chronic cellular infiltrates including lymphocytes, plasma cells and histiocytes (5).
Statistical methods

Data are presented as Mean ± Standard Error of Mean (SEM). Fisher’s exact test was used to compare the prevalence of thyroid disease in the two groups.

RESULTS

Of 108 consecutive cases analysed, the prevalence of thyroid disease was ~13% (95% Confidence Interval (CI): 7-19%). There were no cases of hypophysitis, pituitary or adrenal disease. The mean age was 52 years (range, 29–68). The causes of death were predominantly due to trauma, suicide or end-stage liver disease, Table 1. No cases of unexplained sudden death or deaths due to chronic liver failure were recorded.

In control cases, the prevalence of thyroid disease was ~7% (95% CI: 2-12%); this was not different to the prevalence in the case group (p=0.13). Similarly, no adrenal or pituitary abnormalities were noted.

Biochemico-histological correlation:

There were 33 (out of 108) cases where thyroid function tests, including thyrotropin (TSH), free tetra-iodothyronine (fT4) and tri-iodothyronine (fT3) levels, were available. All thyroid parameters were performed within 12 months of the postmortems and all were normal. The mean TSH, fT4 and fT3 levels with standard error of the means were 1.58 ± 0.13 mU/L, 16.4 ± 2.1 and 3.4 ± 1.8 pmol/L respectively. The reference ranges for these were 0.4–4.0, 10.1–25.4 and 3.5–5.5 respectively.

DISCUSSION

This study is the first report in the English literature that documents the histologic and probably ‘gold-standard’ in the assessment of endocrine organ inflammation in the pituitary, thyroid and adrenal glands in the setting of hepatitis C. The finding confirms that the thyroid is the predominant endocrine organ affected with autoimmune lymphocytic infiltration with a prevalence of ~13%. This is consistent with previous reports, ranging from 5.3 to 19% (8,9). Given the relative small number of subjects, it is not
surprising that there is no significant difference compared with the control group. The predominant histologic appearance involved cluster of lymphocytes. No distinct germinal centres or fibrosis were found. This does not necessarily translate to active thyroid disease as there are no corresponding serological thyroid function studies to validate. It is appreciated however that these represent various stages in the evolution of autoimmune thyroid disease prior to the clinical development and expression of active thyroid disease. It is probable that additional factors such as interferon therapy will activate these primed clusters of lymphocytes to clinical thyroid disease. Our post-mortem thyroid disease prevalence is lower than previous reports (10,11) likely due to the age difference in our relatively young cohort.

There is no histologic evidence of autoimmunity or inflammation in the adrenal and pituitary glands in this setting, although the confidence interval around this zero estimate reaches up to 3-4%. This is consistent with the fact that there are few case reports of hypophysitis and adrenal disease. The first case was described by Sakane et al (12) in 1995 in which the endocrinopathies developed 1 month after stopping interferon therapy. This case was shown to have pituitary antibodies against GH3 cells, a rat pituitary tumor cell line that secretes growth hormone and prolactin. In 2003, Concha et al (13) reported a second similar case. The proposed panhypopituitarism was detected 1 year after the completion of therapy and there was no evidence of antipituitary antibodies. Chan et al (14) described a case of panhypopituitarism but in the presence of hepatitis B infection. The patient developed amenorrhoea whilst on treatment and displayed permanent panhypopituitarism thereafter. Ridruejo et al (15) in 2006 reported a possible case of reversible or spontaneously recovered hypophysitis whilst on combination interferon and ribavirin therapy. The diagnosis was based on the pituitary hormonal profile, a non-specific pituitary magnetic resonance finding and the absence of thyroid and other autoimmune markers. Pituitary antibody tests were not performed. Given the rare and unconvincing nature of these cases, it is not surprising that no evidence of lymphocytic infiltration of the pituitary was detected in our report. Together with the absence of adrenal disease, it was highly plausible that no cause of death was recorded as sudden or unexplained. Similarly, the absence of death due to chronic liver failure was a reflection of this relatively young cohort.
**Clinical Application**

Our study has several limitations. This is a snapshot of the autoimmune endocrinopathies in patients with hepatitis C and does not address the progression of the disease, especially the thyroid condition. Although the detection of lymphocytic infiltration in thyroid tissue is definitive of chronic thyroid inflammation this may not indicate active disease. Graves’ disease (GD) also has lymphocytic infiltration and thus could not be excluded fully in the absence of additional ante-mortem autoantibody results. Although GD had been described previously in association with interferon therapy, our experience has been exclusively that of bi-phasic thyroiditis (16). The availability of biochemical thyroid function tests and/or autoantibody profile would have been invaluable to assess the biochemico-histological relationship.

Despite the aforementioned case reports on the development of adenohypophysitis, most observe the development of the condition in association with and some time after the cessation of IFN therapy. These reports pointed towards an association without any definitive proof of causality. In addition, there is no report on the development of hypophysitis in association with hepatitis C alone, without interferon therapy. Similarly, there is no evidence of adrenalitis consistent with the paucity of reports in the general literature. There is only one report in the literature (17) but with reversible subclinical hypoadrenalism influenced by ribavirin and interferon-α.

These findings are reassuring in that on occasions when treatment for hypothyroidism is required, there appears to be, on the basis of our data, little need to assess the adrenal status. This consideration refers to the theoretical possibility that excessive thyroxine therapy or thyrotoxicosis can accelerate cortisol metabolism, resulting in transient hypocortisolaemia or precipitate an actual Addison crisis in patients with occult hypocortisolaemia (18,19).
CONCLUSION

Although this investigation confirms the histologic evidence of lymphocytic infiltrative thyroid disease in patients with chronic hepatitis C infection, the prevalence is not above that which occurred in the general population. This suggests that additional factors such as interferon are required to completely evolve to active thyroid disease. There is no histologic evidence of pituitary or adrenal involvement, despite anecdotal case reports.
COMPETING INTERESTS

There are no competing interests pertaining to any of the authors, either financial or non-financial.

AUTHORS’ CONTRIBUTIONS

HAT conceived the study, participated in its design, assisted with data collection and statistical analysis, and coordinated and drafted the manuscript. TJL gathered, provided the data, and participated in the discussion and drafting of the manuscript. JRA and GEMR contributed to the statistical and analytical methods. All authors participated in the discussion, read and approved the final revised manuscript.

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### Demographics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Hepatitis C group (n=108)</th>
<th>Controls (n=100)</th>
</tr>
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<tbody>
<tr>
<td>Median Age (yr, range)</td>
<td>52 (29–68)</td>
<td>49 (33–67)</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>62:46</td>
<td>59:41</td>
</tr>
<tr>
<td>Causes of Death</td>
<td>Motor vehicle accident – 68</td>
<td>Motor vehicle accident – 50</td>
</tr>
<tr>
<td></td>
<td>Ischaemic heart disease – 12</td>
<td>Ischaemic heart disease – 9</td>
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<tr>
<td></td>
<td>Asphyxiation – 20</td>
<td>Asphyxiation – 26</td>
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<tr>
<td></td>
<td>Subarachnoid haemorrhage – 2</td>
<td>Overdose – 12</td>
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<tr>
<td></td>
<td>Overdose – 4</td>
<td>Miscellaneous – 3</td>
</tr>
<tr>
<td></td>
<td>Miscellaneous – 2</td>
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<tr>
<td>Thyroid disease (n/%)</td>
<td>14 (13%)</td>
<td>7 (7%)</td>
</tr>
<tr>
<td>Pituitary disease (n/%)</td>
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<td>0 (0%)</td>
</tr>
<tr>
<td>Adrenal disease (n/%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

**Table 1.** The demographic characteristics of the two cohorts and the respective prevalence of endocrinopathies in post-mortem examinations.