HISTOPATHOLOGICAL VARIATION OF THYROID GLAND IN INFECTED MICE WITH TRICHINELLA SPIRALIS

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ABSTRACT

Trichinellosis is a zoonotic disease with worldwide distribution and remains public health problem. The disease causes inflammatory reaction and can manifest as periorbital swelling, myalgia, which can lead to serious complications such as myocarditis and encephalitis. Larval concentration of Trichinella spiralis were reported to localize in the neck muscle and the adjacent tissue to thyroid gland. This study in focused on the alteration of histopathology in the thyroid gland. Experiment in animal model was performed in female ICR mice infected with 450 larvae of T. spiralis. Histopathological study was evaluated by hematoxylin and eosin staining technique and the histopathological changed was observed by microscopic examination on 2nd, 5th, 13th, 18th and 21st day post infection (DPI). The results of this study showed an acute inflammatory reaction in the thyroid gland of infected mice on 13th, 18th and 21st DPI, which occurred in 9.09% to 100% (p-value < 0.05). The reaction was found in perithyroidal capsule (100%), thyroid capsule (81.81%), interstitial tissues (50%) and the thyroid follicles (9.09%). Encysted larvae in the intracytoplasmic of the striated muscular fiber of neck adjacent to the thyroid glands was identified. In conclusion, this study showed that the alterations of the thyroid glands was observed in early period on 13th DPI. The phenomenon probably disrupts thyroid glands to produce thyroid hormones which are essential to normal metabolism and homeostasis. Therefore, the results of this study demonstrated that inflammation of the thyroid gland and thyroid gland function should be realized during the first week of T. spiralis infection. Further work on the effect of thyroid hormone, its metabolism and homeostasis in T. spiralis infection can delineate the pathogenesis of T. spiralis infection in thyroid gland.

Keywords: Trichinella spiralis, thyroid gland, inflammatory reaction, histopathological change, histology

INTRODUCTION

Trichinellosis is a zoonotic infection caused by ingestion of uncooked meat with the encysted larvae stage of Trichinella spiralis. The disease has worldwide distribution and remains an importance problem in global public health (Cui et al, 2006). Once T. spiralis encysted larvae are ingested, they are digested by digestive enzymes in the stomach. The larvae then spread to the lymphovascular tract and carried throughout the body (Lindquist et al, 2017). The clinical of trichinellosis is characterized by two phases, namely the enteral phase and the parenteral phase. In the enteral phase, infected patients are usually asymptomatic or manifest mild symptoms of mild diarrhea and nausea. In parenteral phase, the newborn larvae travel though the bloodstream and enter the
tissues. Patients usually presented with diffuse myalgia, aparalysis-like state, periorbital and/or faciæ dema, conjunctivitis and fever (Capo and Despommier, 1996). The main pathogenesis is inflammatory reaction caused by immune response from host and parasite interaction after newborn larvae invade into internal organs and various tissues via lymphovascular system (Gottstein et al, 2009). The parasites invade and induce inflammatory cells infiltrate in the thyroid gland and has effects on inducing either hypothyroidism or hyperthyroidism. Hypothyroidism is a result of previous inflammation of the thyroid gland, which leaves a large number of the damaged cells and incapable of producing sufficient hormone while hyperthyroidism is a condition in which the thyroid gland is overactive and makes excessive amounts of thyroid hormone (Shahid and Sharma, 2018). The present work aimed to study the histopathological changes (inflammatory reaction) in the thyroid gland after infection with T. spiralis. It is hope that understanding the changes of T. spiralis infection in thyroid gland can prevent subsequent thyroid metabolic complications, as well as acute myocarditis which could be fatal.

**Sample size calculation**

The sample size was calculated by using the formula below. The power of the test was set at 90%, significance level of 5%, the appropriate standard deviation and mean were obtained from a review of the public literature (Glaharn et al, 2014). Sample size was calculated using “Comparison of two mean” formula (Riffenburgh, 1999 and Sakpal, 2010). ($n = \text{total sample sizes per group for each case and control, } u = \text{power} = 90\%, v = \text{significance level} = 5\%, \mu_1 = \text{means of experimental groups from reviewed literature}, \mu_0 = \text{means of control groups from reviewed literature}, \sigma_1 = \text{standard deviation of experimental groups from review literature}, \sigma_2 = \text{standard deviation of control groups from review literature}$).

$$n = \frac{(u + v)^2(\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_0)^2}$$

The sample size per group calculated from this study is 9 mice, plus 20% increase for unexpected sample loss during the experimental period, resulting in 11 mice per group or 110 mice as shown in the experimental design of this study (Figure 1).
Parasite maintenance
Female ICR-mice 8-12 weeks old, 25-40 grams were obtained from the Nomura Siam International Co.ltd. The ethical clearance was approved by Faculty of Tropical Medicine Animal Care Mahidol University (Number FTM-ACUC 019/2017). All ICR mice (8-12 week old) were maintained with 200 \textit{T. spiralis} larvae per mouse under conventional conditions for 45 days according to a standard protocol from the National Laboratory Animal Center, Mahidol University. All infected mice with \textit{T. spiralis} were euthanized via CO\textsubscript{2} inhalation and dissected with crushing technique under standard protocol (Gail et al, 2013) to identify the encysted larvae in muscle of mice. The striated muscle with positive encysted larvae was digested with pepsin-HCl solution (pepsin 1 g/ HCl 1 ml and distill water 100 ml) at 37ºC in incubator for 12-17 hours (Siriyasatien et al, 2003).

Experiment procedure
All 110 mice were divided equally into control and experimental groups. The experimental groups of 55 mice were infected with 450 \textit{T. spiralis} live larvae per mouse. Oral feeding of \textit{T. spiralis} was performed using stainless curved NG-tube gavage No.18. Fifty five uninfected mice served as a control group. All 110 mice in each group were euthanized via CO\textsubscript{2} inhalation until dead on 2nd, 5th, 13th, 18th and 21st DPI (Gail et al, 2013) followed by dissection, gross examination and histopathological study of thyroid gland.

Histological techniques
Selected thyroid gland were removed immediately after mice death and fixed for 24-48 hours in 10\% neutral buffered formalin. The tissue processing was performed under principle techniques of dehydration, infiltration, paraffin embedded, sectioning at 5 \textmu m in thickness by microtome and stained with H&E staining at Department of Pathology, Chulalongkorn University, Bangkok, Thailand.

The inflammatory reactions graded criteria of thyroid gland
The modified criterion of inflammatory reactions in the thyroid gland tissue was based on previous study (Wang et al, 2002) (Table 1). The interpretation of inflammatory reactions was evaluated under microscopic in consecutive pattern at least 52 high power field (400 magnification or 101.92 mm\textsuperscript{2}) per sectioned of thyroid glands. The grading of inflammatory reaction is as following; 0= normal, 1= mild, 2= severe and 4= extensive.

<table>
<thead>
<tr>
<th>Score</th>
<th>Grading</th>
<th>Inflammatory reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>Normal histology</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
<td>Interstitial tissues accumulation of inflammatory cells distribution around one or two follicles</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>One or more foci of inflammatory cells reaching at least the size of one follicle</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
<td>10 – 40% of thyroid gland tissue replaced by inflammatory cells</td>
</tr>
<tr>
<td>Extensive</td>
<td>4</td>
<td>&gt; 40% of thyroid gland tissues replaced by inflammatory cells</td>
</tr>
</tbody>
</table>

Table 1  Histological inflammatory reaction grading of the thyroid gland.
RESULTS

Histopathological changes (Inflammatory reactions) of thyroid gland

The inflammatory reactions in the thyroid glands were identified in 110 mice (control and experimental groups) on 2\textsuperscript{nd}, 5\textsuperscript{th}, 13\textsuperscript{th}, 18\textsuperscript{th} and 21\textsuperscript{st} DPI. 9.09% to 100% of infected mice had mild and moderate acute inflammatory reaction in the thyroid gland on 13\textsuperscript{th}, 18\textsuperscript{th} and 21\textsuperscript{st} DPI which was characterized by polymorphonuclear cells and mononuclear cell infiltration in perithyroidal capsule (100%), thyroid capsule (81.81%), interstitial tissues (50%), thyroid follicles and colloid material (9.09%). No inflammatory reaction was noted in 55 uninfected mice. There was a statistically significant difference between the inflammatory reactions in the thyroid gland of the experimental group and control group ($p$-value $< 0.05$) on 13\textsuperscript{th}, 18\textsuperscript{th} and 21\textsuperscript{st} DPI. No difference in inflammatory reactions was noted on 2\textsuperscript{nd} and 5\textsuperscript{th} DPI (Table 2). There was a significant difference of the inflammatory reactions among the experimental group on 18\textsuperscript{th} and 21\textsuperscript{st} DPI ($p$-value $< 0.05$) No significant difference was observed on 2\textsuperscript{nd}, 5\textsuperscript{th} and 13\textsuperscript{th} DPI.

Fig. 2- The photomicrographic images (400x) of thyroid gland from infected mice with T. spiralis; (A) Thyroid gland in the control group showing normal architecture on day 13\textsuperscript{th} DPI; (B) Thyroid gland shows mild acute inflammatory reaction characterized by predominant polymorphonuclear cells infiltrates in thyroid capsule (arrow) on day 13\textsuperscript{th} DPI; (C) Thyroid gland shows mild acute inflammatory reaction characterized by inflammatory cells infiltrate in the interstitial tissues (arrow) on day 18\textsuperscript{th} DPI; (D) Moderate acute inflammatory reaction in the thyroid gland showing numerous polymorphonuclear cell and mononuclear cell infiltrates in the interstitial tissues (arrow) on day 21\textsuperscript{st} DPI.
Fig. 3- The photomicrographic images (100X) of thyroid gland and adjacent tissues from infected mice with *T. spiralis*; (A) Serial sections of the thyroid gland showing an inflammatory cell infiltrates in the thyroid capsule (arrow) and encysted larvae of *T. spiralis* in the intracytoplasmic of muscle fiber (arrowhead) on 13\(^{th}\) DPI; (B) The thyroid gland showing inflammatory cell infiltrates in the perithyroidal capsule, thyroid capsule, interstitial tissues, (arrow) and encysted larvae in the intracytoplasmic muscular fiber (arrowhead) on 18\(^{th}\) DPI; (C) shows numerous inflammatory cell infiltrates in the interstitial tissues of thyroid gland (arrow) on 21\(^{st}\) DPI and (D) shows encysted larvae in intracytoplasmic muscular fiber (arrow) on 21\(^{st}\) DPI.
Table 2  The comparison of inflammatory reaction grading in the thyroid gland between the experimental group and control group on 2\textsuperscript{nd}, 5\textsuperscript{th}, 13\textsuperscript{th}, 18\textsuperscript{th} and 21\textsuperscript{st} DPI.

<table>
<thead>
<tr>
<th>DPI</th>
<th>Groups</th>
<th>n</th>
<th>Number of mice (%) and inflammatory reactions grading of thyroid gland tissues</th>
<th>Compare mean between experimental group and control group ((p)-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Control</td>
<td>11</td>
<td>11(100)</td>
<td>0 0 0 1.000</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>11</td>
<td>11(100)</td>
<td>0 0 0 1.000</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>11</td>
<td>11(100)</td>
<td>0 0 0 1.000</td>
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<tr>
<td></td>
<td>Experimental</td>
<td>11</td>
<td>11(100)</td>
<td>0 0 0 1.000</td>
</tr>
<tr>
<td>13</td>
<td>Control</td>
<td>11</td>
<td>11(100)</td>
<td>0 0 0 0.317</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>11</td>
<td>9(81.81)</td>
<td>2(18.18) 0 0 0.001*</td>
</tr>
<tr>
<td>18</td>
<td>Control</td>
<td>11</td>
<td>11(100)</td>
<td>0 0 0 0.001*</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>11</td>
<td>4(36.36)</td>
<td>7(63.63) 0 0 0.001*</td>
</tr>
<tr>
<td>21</td>
<td>Control</td>
<td>11</td>
<td>11(100)</td>
<td>0 0 0 0.001*</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>11</td>
<td>3(27.27)</td>
<td>5(45.45) 3(27.27) 0 0.001*</td>
</tr>
</tbody>
</table>

Note: Asterisks (*) denotes; the significant \(p\)-value < 0.05; the data show the number of mice (%) and inflammatory reactions grading; N= number of mice per group; DPI= Day Post Infection.

**Fig 4**: The graph shows severity of an inflammatory reaction in the thyroid gland in experimental groups (among groups) and days post infection (DPI) during study periods on 2\textsuperscript{nd}, 5\textsuperscript{th}, 13\textsuperscript{th}, 18\textsuperscript{th} and 21\textsuperscript{st} DPI. The experimental groups (infected mice) with the highest inflammatory reaction in the thyroid gland were observed on 21\textsuperscript{st} DPI and the least inflammatory reaction was observed on 13\textsuperscript{th} DPI and no inflammatory reaction was noted on 2\textsuperscript{nd} and 5\textsuperscript{th} DPI.
DISCUSSION AND CONCLUSION

The present study showed occurrence of inflammatory reactions in the thyroid gland of female ICR mice on 2nd, 5th, 13th, 18th and 21st DPI after infection with 450 T. spiralis larvae. There was mild to moderate acute inflammatory reactions in the perithyroidal capsule, thyroid capsule, interstitial tissues and thyroid follicles. This study demonstrates that infected mice with T. spiralis 450 larvae could induce immune responses between host and parasite interaction in 9.09% to 100% of infected mice in perithyroidal capsule and thyroid gland capsule on 13th, 18th and 21st DPI. In addition, acute inflammatory response occurs in 9.09% to 50% in the thyroid gland follicles and interstitial tissues on 21st DPI. This period of infection coincides with the parenteral phase in the life cycle of parasite, where the newborn larvae were disseminated in lymphovascular tract and carried throughout the body tissues (Salvana et al, 2014) to striated muscle where they become encysted larvae. Previous research documented that inflammatory response induced by T. spiralis infection was observed in internal organs of various tissues, such as heart, lung, and adjacent tissues of the neck (Sovyra, 2008 and Ribicich et al, 2001). Our study revealed mild to moderate acute inflammatory reaction in thyroid gland on 13th, 18th and 21st DPI and also mild to severe acute inflammatory reaction in the muscle of neck adjacent to the thyroid gland was observed on 13th, 18th and 21st DPI. In conclusion, our study indicated that an inflammatory reaction in the thyroid gland detected in the early period on day 13th after infection. The occurrence of inflammatory response posted a concern to the damage of the thyroid tissue which may subsequently lead to the alteration in thyroid hormone and thyroid gland function, such as metabolism and homeostasis. In addition, abnormality of the cardiovascular system such as palpitations and arrhythmia within 1-2 weeks after infection may occur in severe cases. Further study in the thyroid hormones and homeostasis post T. spiralis infection could be investigated to understand the pathogenesis of T. spirals infection to help prevent and control fatal complication, such as myocarditis.

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