Alteration of Troponin T Levels and Histological Change of Myocardial Tissue in Infected Mice with *Trichinella spiralis*

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**ABSTRACT**

Trichinellosis is the zoonosis disease resulting in tissue injury and serious complication as myocarditis leading cause of death. This study aimed to determine Troponin T (Trop-T) levels and myocardial tissue injury after infection of Trichinella spiralis. This study was performed in infected mice with 300 T. spiralis larvae. The evaluation of Trop-T levels in plasma based on the quantitative immunological test and histologic study from myocardial tissue based on hematoxylin and eosin staining on day 5th, 10th, 15th and 21st. The results revealed abnormal increased Trop-T levels in the experimental groups when compared to the control groups all study period, 88.89% to 100%, statistically significant (p < 0.01) and the experimental groups showed myocardial cell necrosis when compared to the control groups on day 10th, 15th, and 21st DPI. 11.11% to 100%, a statistically significant difference (p < 0.01). The correlation between Trop-T levels and myocardial tissue injury in the experimental group on day 10th, 15th and 21st DPI revealed increasing in abnormality and myocardial cell injury, respectively (p < 0.01). In conclusion, this study indicates that T. spiralis - infected mice revealed myocardial tissues injury consequence of the myocardial infarction and the abnormal of Trop-T levels. Therefore, should be realize in patients after infected with T. spiralis even though in early period as 1st week and apply to useful for Trop-T test for investigating myocardial cell injury for prevent and reduce serious complications.

Key words: Trichinellosis, Troponin T, myocardial cell injury, Histology, Hematoxylin and Eosin staining.

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INTRODUCTION

Trichinellosis is an important zoonotic disease and is considered one of the major global public health problems and infection by consumption of uncooked meat containing first stage larvae (L-1) of Trichinella species (Anunnatsiri, 2004; Miterva and Jasmer, 2006; Kaepitoon et al., 2008). Trichinellosis occur almost every year in Thailand. The majority was occurring in the Northern and in all age groups of patients from cases report (Keawpitoon et al., 2006). The mortality rate of Trichinellosis is about 1% (Technical appendix, 2010). The pathogenesis of disease in the human and mammalian animals by ingestion of raw or inadequately cooked meat-products containing of encysted first stage larvae, pass through the stomach and digested with pepsin and hydrochloric acid, resulting in the first stage larvae are released into the small intestinal tissue. Thereafter, the larva molts four times, transforming into the adult (Harley and Gallicchio, 1971). Mating ensues within 36 hours and an adult female worm will be breeding the newborn larvae within 4-7 days post infection. It is estimated that 500 to 1,500 newborn larvae and shed over the life span per a female worm within 4-6 weeks (Tara, 2009). According to the mechanism of invading newborn larvae into various tissues and internal organs, resulting in immune responses by host such as inflammatory responses in consequence of clinical features as fever, abdominal pain, diarrhea, myalgia (98%) and serious complications such as myocarditis (13%), pneumonitis (6%) and encephalitis (50%) that the leading cause of death (Anunnatsiri, 2004; Miterva and Jasmer, 2006). Hence, an interesting point of this research is attention to myocardial tissue injury and biochemical marker that could be the detection of myocardial tissue injury as Troponin T. The Troponin is a complex proteins and divided to three subunits (troponin C, troponin I, and troponin T) that is integral to muscle contraction in skeletal muscle and cardiac muscle, but not show smooth muscle. The Troponin T (TnT: 37 kDa) binds the complex to Tropomyosin strand of the thin filament in the process cardiac contraction (Wu, 1998), that is widely used for screening and detection in myocardial tissue injuries such as arrhythmia, myocarditis, myocardial infarction (MI) and congestive heart failure (CHF). Hence, this study focused on alteration of Troponin T combine to the morphological change of myocardial tissue injury and/or myocardial infarction was expected to apply for predicting and monitoring in myocardial tissue injury and/or myocardial infarction in patients after infected with Trichinella spiralis that expect to further proper treatment and prevent serious complications.
MATERIALS AND METHODS

Research Design

The research is an experimental design and was performed by 72 males ICR mice, age 9-11 weeks old, body weight 25-40g which divided into two groups; 36 infected mice by *Trichinella spiralis* as the experimental group and 36 uninfected mice as a control group.

Mice and Parasite

Male ICR mice, 9-11 weeks old, body weight 25-40g obtained from the National Laboratory Animal Centre, Mahidol University and ethical clearance No. 2012/018 was approved by the Animal Care and Use Committee (FTM-ACUC), Faculty of Tropical Medicine, Mahidol University. The source of *Trichinella spiralis* larvae was supported by Department of Parasitology and Entomology, Faculty of Public Health, Mahidol University, Thailand.

Maintaining parasites

The experiment was performed by following the steps I to V; Step I: Male ICR mice were infected by 300 *Trichinella spiralis* larvae via oral feeding by oral gastric curved gavages No.18 and cared under conventional control for 45 days at the Faculty of Tropical Medicine, Mahidol University. Step II: All infected mice were euthanized by CO₂ inhalation in chamber 7x11x5 inches in size until completely dead. Step III: dissection of mice by blade No.11 and gross examination were done. Step IV: detection of encysted larvae in muscle by crushing technique under stereomicroscopic examination, after that selected positive encyst-muscle and cut into multiple small pieces, digested by 1% pepsin-HCl solution (Pepsin 3g, HCl 7 ml, DW (distill water) to 1000 ml) at 1:10 (W/V) and incubated at 37°C for 4-6 hours (Belosevic and Dick, 1979). Step V: the alive larvae were washed by 0.85% NaCl and administrated 300 live *Trichinella spiralis* larvae per mouse via the oral feeding with oral gastric curved gavages No.18 (Wang and Tong, 2009) and the total mice were treated under conventional condition.

Troponin T levels analysis

The heparinized blood sample was collected from tail clip of total 72 mice to determine the plasma Troponin T levels. The plasma levels of Troponin T were evaluated as an index of cardiac cellular damage by using a quantitative rapid assay (Roche Diagnostics, Cobas h 232). Briefly: removed the test strip from the foil pouch and held the test strip area to face up, inserted it in the test strip guide of the cobas h 232. The thermometer symbol showed that the test strip was warmed up and used the microcapillary heparin tube to draw exactly 150 μL of fresh blood, then applied the entire sample to the application area of the test strip. After processing the sample, the test result was shown on the display within 8 - 12 minutes. However, exposure to suspicious biases was prevented by double
blind. The Troponin T levels were graded as follows; negative (low risk) <0.03 ng/ml, positive grading as, (medium risk) 0.03-0.1ng/L, (high risk) >0.1- 2 ng/ml, (highly severe) > 2 ng/ml compared to the control group.

Histopathological analysis

The total 72 male mice of the experimental groups and the control group were dissected after determination of plasma Troponin T. After that the euthanized mice were performed and the general gross examination was done. The tissue processed under histology with H&E staining techniques was performed as following; the tissue was fixed with 10% phosphate-buffered formalin, dehydration by ethyl alcohol and acetone, infiltration (paraffin), embedding (paraffin block) and 4-6 µm sections of tissue were processed routinely into hematoxylin and eosin (H&E) staining. The histological criteria for grading the severity of necrotic cells in cardiac myocytes was graded as follows: score 0: normal (0% no involvement noted of the histologic section), score 1: mild (<25% involvement of the histologic section), score 2: moderate (>25-50 involvement of the histologic section) and score 3: severe (>50-75% involvement of the histologic section) and score 4: highly severe (>75% involvement of the histologic section) (Guta and Garg, 2003; Walsh et al, 2009; Zhan et al, 2012).

Statistical analysis

The statistical analysis of Troponin T levels grading in the experimental groups compared to the control groups with Mann-Whitney U-Test and the correlation between Troponin T levels and myocardial infarction grading was analyzed by Spearman Rank Correlation. We considered the P-value less than 0.05 (p<0.05) considered significant.

RESULTS

Troponin T levels

The Troponin T levels grading in the experimental groups showed three levels of risk (low, medium, and high) on 5th DPI and reached high risk on the dates of 10th and 15th DPI. Low, medium, and high risk levels were shown on 21st DPI (Table 1). The statistical analysis of Troponin T levels grading in the experimental groups was compared to the control groups by Mann-Whitney U-Test with statistically significant difference (p < 0.001).

Myocardial tissue injury/Myocardial infarction

The data analysis of myocardial tissue injury was based on myocardial tissue injury criteria (Aretz et al, 1987; Maisch and Factor, 1999; Masisch and Richter, 2005; Karatolios et al, 2006) and evaluated by histology (H&E) staining under microscopic examination. The results were that necrotic cells in cardiac myocytes in the experimental groups (33.33% to 100%) on the dates of 5th, 10th, 15th and 21st DPI (Table1) characterized by hypereosinophilia of cytoplasm, contraction bands of myocardial fibers, and nucleus (pyknosis, karyorrhexis and karyolysis).
The correlation between of Troponin T levels and myocardial tissue injury/myocardial infarction increased on the dates of 10th, 15th and 21st DPI in the experimental groups. The Troponin T test showed that risk grading raised from medium to high (33.33% to 100%) on the dates of 10th and 15th and 21st DPI. The histology pointed out myocardial infarctions from mild to severe grading (22.22% to 66.67%) on the dates of 10th and 15th and also indicated myocardial infarctions at the mild (55.55%) and moderate (33.33%) levels on the date of 21st DPI.

**Table 1**: The correlation between of Troponin T levels and myocardial infarction grading in experimental groups on day 5th, 10th, 15th and 21st DPI.

<table>
<thead>
<tr>
<th>Day post-infection (DPI)</th>
<th>No. of mice (%)</th>
<th>No. of mice (%)</th>
<th>The correlation between Trop-T levels and MI grading</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trop-T levels grading</td>
<td>Myocardial tissue injury (MTI) grading</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low risk</td>
<td>Medium risk</td>
<td>High risk</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>Experiment</td>
<td>1 (11.11%)</td>
<td>7 (77.78%)</td>
<td>1 (11.11%)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>9 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>10</td>
<td>Experiment</td>
<td>9 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>15</td>
<td>Experiment</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>21</td>
<td>Experiment</td>
<td>1 (11.11%)</td>
<td>2 (22.22%)</td>
<td>2 (22.22%)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*Note: n= the number of mice and percentage in groups (n=9), Trop-T = Troponin T levels, MI = myocardial tissue injury, statistically significant at p<0.05, Trop-T levels grading: Low risk = <0.03μg/ml, Medium risk = 0.03-0.01μg/ml, High risk = >0.01-2μg/ml and Highly severe = >2μg/ml, experiment group= infected mice with T. spiralis control group= non infected mice.
DISCUSSION AND CONCLUSION

The result of this study based on the infected mice with *Trichinella spiralis* on the dates 5th, 10th, 15th and 21st as a consequence of the parasites migrate into various tissues and internal organs. It was detected abnormality of Troponin T levels in blood circulation 88.88% to 100% in all study periods of DPI. The histology showed the acute and chronic inflammatory myocarditis or myocardial infarction 11.11% to 100% on 10th, 15th and 21st DPI. In addition, the correlation between Troponin T levels and histology increased together in the abnormality of Troponin T levels with histology in myocardial tissue as acute and chronic myocarditis (P<0.001). The infected mice with *Trichinella spiralis* for the longer period of DPI in this study pointed out to the abnormality increasing troponin T levels.
levels and severity of histologic myocardial injury (Table 1). This appearance could be explained based on the theory of *Trichinella spiralis* life cycles. The phase of the life cycle after infection with *Trichinella spiralis* on the dates 5th, parasites localized into the gastrointestinal tract. After that, they invaded into various tissues and internal organs via lymphovascular system. In the parenteral phase of the life cycle, the parasites could be identified in the internal organs such as heart, lung and especially in the muscle that occurred encysted larvae (Compton *et al.*, 1993). From the above mentioned theory, the infection of parasites which invaded to heart reflected to myocardial tissue injury responding which was investigated in 5th, 10th, 15th, and 21st DPI. And also detected abnormality increasing of Troponin T levels and histopathology in myocardial tissue with acute and chronic myocarditis or myocardial infarction. That was a consequence of immune response between host and parasites interaction in the various tissues and internal organs injury resulting in the inflammatory reaction, especially myocardial tissue injury in infected mice with *Trichinella spiralis* (Fu *et al.*, 2009). Therefore, the results of this study revealed the increasing of abnormality Troponin T levels in the early period as the 5th DPI demonstrated that the cardiac Troponin T (37 kd) used the following myocardial damage, cardiac Troponin T (cTnT) was released into blood within 2 to 6 hours after the event and detected with immunoassays based on specific antibodies (Collinson and Bao, 2001; Kendall *et al.*, 2004; Wu, 2008). While the histological features of myocardial tissue showed myocarditis and myocardial infarction in this study on dates 10th DPI. It means that this study can show the result to demonstrate in the mechanism of inflammatory reaction occurring within 24-72 hrs after infection (Kumar, 2005). Thus, in this study can detect abnormality Troponin T levels in the early period, which is more rapidly than detected histological change of myocardial tissue and myocarditis or myocardial infarction as mentioned above.

In conclusion, this study showed that the detection of Troponin T test for myocardial tissue injury was detected more rapidly than the histiological features in acute myocarditis or myocardial infarction. The histology is quite difficult to diagnose because of histological technique under myocardial tissue biopsy for suspected case in harmful and aggressive method. Therefore, The Troponin T test can be applied with routine diagnosis in patients suspected myocarditis or myocardial infarction condition and also monitoring and prediction myocardial tissue damage for reducing serious complication in severe case with acute myocarditis of Trichinellosis.

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