DIFFERENCES IN THE DESCRIPTION OF CEREBROSPINAL FLUID AND ROUTINE BLOOD ANALYSIS BETWEEN PATIENTS WITH SUSPECTED TOXOPLASMOSIS AND NON-TOXOPLASMOSIS

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ABSTRACT

Background: Toxoplasma gondii is an obligate intracellular parasite, which can infect the central nervous system. Based on data in the Neuroinfection Department of RSUPNCM, Jakarta, the incidence of cerebral Toxoplasmosis was 31%. In several studies show the analysis of the cerebrospinal fluid obtained by increasing the number of cells and proteins in patients with Toxoplasmosis. While on routine blood, obtained an increase of leukocytes in patients of Toxoplasmosis.

Objective: To determine differences in the description of cerebrospinal fluid analysis and complete blood count in patients suspected of Toxoplasmosis and non-Toxoplasmosis

Methods: Retrieval of data from the results of examination of all patients in Central laboratory of RSSA Malang, where data was collected from the period January - July 2019. Data analysis used SPSS version 25 (α <0.05).

Result: From the 60 subjects, there were 15 (25%) subjects with anti-Toxoplasma IgG positive and there were 45 (75%) subjects with anti-Toxoplasma IgG negative. Median (25-75 percentile) cerebrospinal fluid protein in patients with suspected Toxoplasmosis was lower than non Toxoplasmosis 60 (39.7-172) (p = 0.887, α> 0.05). Similarly, the median MN cells in cerebrospinal fluid in suspected Toxoplasmosis 75% (0-100%) were higher than in non-Toxoplasmosis 35% (0-100%) (p = 0.358, α> 0.05). In this study also obtained a median value of leukocytes in peripheral blood is higher in the group of non-Toxoplasmosis subjects that is 13460 (7705-18185).

Discussion: In the Toxoplasmosis group, the median value of protein was lower compared to the non-toxoplasmosis group. According to previous studies, the value of cerebrospinal fluid protein in the group with toxoplasmosis was lower than the Cryptococcus group. Whereas research in Turkey, which reported that peripheral blood leukocyte values in Toxoplasmosis patients were found to be low.

Conclusion: Parameters of analysis of cerebrospinal fluid and complete blood cannot be used directly to describe the suspicion of Toxoplasmosis

Keywords: CSF Analysis, Blood Complete, Toxoplasmosis

I. INTRODUCTION

Toxoplasmosis, a disease caused by Toxoplasma gondii, is a parasitic disease in animals that can be transmitted to humans.1 This parasite includes protozoa which are obligate intracellular. Approximately 30% - 65% of the world's population is estimated to have chronic Toxoplasmosis. The prevalence of toxoplasmosis in Indonesia namely 36.9% of the general population (1982-1994), 64% of people in East Java (1992-1993), 7% in Irian Jaya (1972), 3.1% of children and adolescents in Bali, 9.7% to 51% in the rural of South Kalimantan (Kalimantan), 40% of women and 50% of women over the age of 10 in Surabaya, 70% of adults in Jakarta, 8,4% of HIV- positive in Jakarta.2
Toxoplasmosis on the central nervous system occurs due to reactivation of the disease in patients who are given immunosuppressive or cytotoxic therapy or in patients with HIV infection. In America, the occurrence rate reaches 30% -50%, while in Europe it reaches 50% -70%. Based on research in the neuroinfection section of RSUPNCM, the occurrence rate of cerebral toxoplasmosis was 31%. To diagnose Toxoplasmosis in the central nervous system, it is necessary to have supporting examinations such as analysis of cerebrospinal fluid and imaging with MRI.

II. METHODS

This research is an analytic observational study with a cross-sectional research design. Data collection was carried out at the Central Laboratory of the RSSA Malang, from January - July 2019.

Subject
The research subjects were 60 patients who were tested for toxoplasmosis serology at the Central Laboratory of Saiful Anwar General Hospital Malang (RSSA Malang), who met the inclusion and exclusion criteria of the study. The inclusion criteria in this study were patients who were hospitalized at the General Hospital of Saiful Anwar Malang, had anti-Toxoplasma IgG serology checked, cerebrospinal fluid analysis, and routine blood on the same day as cerebrospinal fluid analysis. Whereas the exclusion criterion was tap trauma at the time of cerebrospinal fluid taking.

Complete Blood Examination
Complete blood examination, calculate the number of leukocytes and erythrocytes using Sysmex XN-1000 from venous blood with EDTA.

Cerebrospinal Fluid Analysis
Examination of cerebrospinal fluid analysis using a plain tube of approximately 2 tubes of 1 mL each using manual macroscopic and microscopic methods and chemical examination. Macroscopic examination consists of examining color, clarity, and clotting. Meanwhile, microscopic examination consists of counting the number of leukocytes, MN cells, and PMN cells with a counting chamber.

Qualitative examination of pandy and nonne proteins was carried out manually by adding reagents. Chemical tests for quantitative protein, LDH, and glucose tests using Cobas 501 (Roche).

Statistical Analysis
The data processing of the research results were analyzed using the Software Statistical Product and Service Solution PS (SPSS 25) for Windows with a significance level of 0.05 (p <0.05) and a confidence level of 95% (α = 0.05). The differences test conducted on the data using Mean Whitney in 2 groups. The data normality test used the Saphiro Wilk test.

III. RESULTS

Characteristics of Research Subjects
From the 60 subjects, there were 15 (25%) subjects with positive anti-Toxoplasma IgG and 45 (75%) subjects with negative anti-Toxoplasma IgG. Based on the research results of positive anti-Toxoplasma IgG, the value of a median age namely 39 (23-60), while on the subject of negative anti-Toxoplasma IgG namely 19 (1-42) (Table 1). It shows that there is a significant difference in age parameters between the 2 groups of subjects p = 0.005 (α< 0.05).

From the 15 subjects, there were 5 women (33.3%) and 10 men (66.7%) with positive anti-Toxoplasma IgG. It showed that the highest number was 40 (66,67%) of female subjects with positive anti-Toxoplasma IgG and negative anti-Toxoplasma IgG.
Table 1. Demographical Data (Age and Gender) of the Subjects

<table>
<thead>
<tr>
<th>Age Median (IQR)</th>
<th>Susp. Toxoplasmosis N = 15</th>
<th>Non-Toxoplasmosis N = 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>39 (23-60)</td>
<td>19 (1-42)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gender Total (Percentage)</th>
<th>Susp. Toxoplasmosis</th>
<th>Non-Toxoplasmosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 10 (66.7%)</td>
<td>W 5 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>W 35(77.8%)</td>
<td>P 10 (22.2%)</td>
<td></td>
</tr>
</tbody>
</table>

P : pria/male ; W : wanita/female

Clinical Laboratory Data

The laboratory parameters used were peripheral blood leukocytes, cerebrospinal fluid LDH, cerebrospinal fluid glucose, cerebrospinal fluid protein, and cerebrospinal fluid leukocytes. This study will be divided into 2 groups, namely data from subjects with suspected toxoplasmosis and non-toxoplasmosis. The values of Median (percentile) of cerebrospinal fluid protein 60 (39.7-79), cerebrospinal fluid glucose values 55 (36-79), cerebrospinal fluid leukocytes 20 (0-80), and cerebrospinal fluid LDH 74 (33-208) in subjects with suspected Toxoplasmosis. Likewise, the median of MN cells in cerebrospinal fluid in suspected Toxoplasmosis was 75% higher than in non-Toxoplasmosis 35%. In this study, it was also found that the median value of leukocytes in peripheral blood was higher in the group of non-Toxoplasmosis subjects, namely 13460 (7705-18185) (Table 2).

Table 2. Laboratory Parameters of Cerebrospinal Fluid and Complete Blood

<table>
<thead>
<tr>
<th>Cerebrospinal Fluid</th>
<th>Susp. Toxoplasmosis N = 15</th>
<th>Non-Toxoplasmosis N = 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes, median (IQR)</td>
<td>20 (0-80)</td>
<td>10 (0-85)</td>
</tr>
<tr>
<td>Percentage of MN Cells, median (IQR)</td>
<td>75% (0-100%)</td>
<td>35% (0-100%)</td>
</tr>
<tr>
<td>Glucose, median (IQR)</td>
<td>55 (36-79)</td>
<td>68 (43-81.5)</td>
</tr>
<tr>
<td>LDH,median (IQR)</td>
<td>74 (33-208)</td>
<td>88 (46-436)</td>
</tr>
<tr>
<td>Protein,median (IQR)</td>
<td>60 (39.7-172)</td>
<td>64.8(27.1-154.15)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complete Blood</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes, median (IQR)</td>
<td>10470 (8520-13690)</td>
<td>13460 (7705-18185)</td>
</tr>
</tbody>
</table>

Table 3. Characteristics of Nonne and Pandy Cerebrospinal Fluid

<table>
<thead>
<tr>
<th>Nonne</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (Frequency)</td>
<td>10</td>
</tr>
<tr>
<td>Negative (Frequency)</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pandy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (Frequency)</td>
<td>11</td>
</tr>
<tr>
<td>Negative (Frequency)</td>
<td>4</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

The results showed the prevalence of T. gondii IgG in Saiful Anwar Hospital was 25%. The highest toxoplasmosis seroprevalence in this study was found at a median age of 39 (23-60) (Table 1). This is in line with Laksmi's research in Karangasem Regency, Bali. This is also in line with other studies in India and Korea and several countries in Asia, America and Europe.5
Factors that affect the prevalence of IgG T. gondii in humans vary, among others geography, immunological status, socio-economic conditions, and community lifestyle.6 Toxoplasmosis has infected 15-18% of the world's population. People with a habit of eating raw or undercooked meat are generally found to have a fairly high prevalence of toxoplasmosis7. The low prevalence of toxoplasmosis seropositive is found 4.1% in Thailand to a high 75% in Brazil8.

The highest toxoplasmosis seroprevalence in this study was found at a median age of 39. This is in line with other studies in India and Korea which stated that the higher the age, the higher the possibility of accidentally being exposed to or ingesting oocysts so that the prevalence of positive Toxoplasma IgG was higher.8

Seroprevalence of anti-toxoplasma IgG equal to 25% in this study was not much different from studies in other Asian countries, namely 10.6-17.5% in Malaysia, 15% in India, 0.33-11.97% in China.5 However, this result is actually lower than the study in East Java which received seroprevalence results of 64.9%

The variety of toxoplasmosis seroprevalence in humans is also influenced by dietary habit, ethnicity, age, sex, place of residence, cat ownership, environmental conditions, and socioeconomic status. The sensitivity and specificity of the measuring instrument used, the type of antibody used to detect and the type of kit used also greatly affect the results obtained.10

In this study, the median value (IQR) of cerebrospinal fluid analysis protein was lower in the group of subjects with suspected toxoplasmosis, namely 60 (39.7-79) (Table 2), However, there was no significant difference between the subject groups with the value (p = 0.887, \( \chi^2 > 0.05 \)). This is in accordance with the results of the Ganiem study at Hasan Sadikin Hospital, which showed that the value of cerebrospinal fluid protein in the group with toxoplasmosis was lower than the Cryptococcus group. Cerebral toxoplasmosis enforcement without further radiological examination and isolation of pathogens will be difficult to distinguish from meningitis caused by other pathogens, both symptoms and analysis of cerebrospinal fluid in these two groups have similarities11.

On routine hematological examination, the median value of leukocytes in the peripheral blood in the non-toxoplasmosis group namely 13460 higher than that in the toxoplasmosis group (Table 2), with significant differences in the two groups of subjects (p = 0.033, \( \chi^2 < 0.05 \)). This is consistent with a study in Turkey, which reported that the value of peripheral blood leukocytes in toxoplasmosis patients was low in the population of pregnant women due to the leukocyte cells infected by the toxoplasma parasite, where these infected cells are an important factor in activating the natural immune response and found in pregnant women who infected with toxoplasmosis.4

In the analysis of cerebral fluid, the high number of PMN and MN cells indicates an infection in the brain, either meningitis or encephalitis. In Andriyani's study regarding brain fluid analysis in HIV patients with meningitis, it was found that MN cells were increased at high anti-Toxoplasma IgG levels compared to low and negative levels. Probably because in toxoplasmosis, MN cells, especially macrophages, are more important in eliminating T. gondii infection.12 It is also proven by this study that the median value of MN cells in the group of suspected toxoplasmosis subjects namely 75% (0-100%), higher than the non-toxoplasmosis group (Table 2).

V. CONCLUSION

The description of cerebrospinal fluid and hematology routine analysis in toxoplasmosis patients varies greatly so that these parameters cannot be used directly in establishing Toxoplasmosis. This study can only describe early central nervous system abnormalities in patients with suspected toxoplasmosis. Other investigations are needed, such as clinical, radiological and biomolecular features to establish cerebral toxoplasmosis.

This study presents an overview of the characteristics of the cerebrospinal fluid analysis in subjects with anti-toxoplasmosis serologic tests, but no biomolecular examination was carried out to prove the presence of the T.gondii parasite in the cerebrospinal fluid. For this reason, it is necessary to carry out further research using PCR and biomolecular methods.

DISCLOSURES

The HEADING line must begin with the words DISCLOSURES boldfaced. UPPERCASE, Align left, TNR 12, 1.5 spaces, bold, 1 column
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Author Contribution
Provide detailed contribution of each authors for example. AA and BB involved in concepting, designing and supervising the manuscript. CC and DD conduct the study. AA and CC analyses the data. All authors prepare the manuscript and agree for this final version of manuscript to be submitted to this journal.

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REFERENCES