CASE REPORT OLGU SUNUMU

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Molecular Detection and Genotyping of Active Toxoplasmosis from Blood Sample

Kan Örneğinden Aktif Toksoplazmozis Moleküler Tespiti ve Genotiplemesi

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ABSTRACT *Toxoplasma gondii*, which is an obligate intracellular protozoan, and its primer infection during pregnancy can cause several problems. This study presents a case report of a woman who delivered a baby with multi-organ failure and was diagnosed with congenital toxoplasmosis. During the gestation period, there was no history of febrile, enlargement of lymph nodes, or common cold symptoms, but there was a history of miscarriage. The serology test of anti-*Toxoplasma* immunoglobulin (Ig)G and IgM were reactive. The IgG avidity test was 90%, and positive SAG2. The sequencing and analysis of polymerase chain reaction product assumed to have the Type I T. gondii, which must be confirmed using other loci apart from SAG2. This case report aims to raise awareness of the risk of congenital toxoplasmosis in asymptomatic mothers with a history of miscarriage. Routine and serial screening for the disease is recommended in high-risk pregnant women to prevent congenital transmission.

Keywords: Congenital toxoplasmosis; avidity test; SAG2 gene

ÖZET Zorunlu bir intraselüler protozoa olan *Toxoplasma gondii* ve hamilelik sırasında primer enfeksiyonu, pek çok soruna sebep olabilir. Bu çalışma, çoklu organ yetmezliği olan bir bebeğe doğum yapan ve konjenital toksoplazmozis tanısı konan bir kadının olgu sunumudur. Gebelik süresi boyunca ateş, lenf nodu büyümesi ya da yaygın soğuk algınlığı semptomları öyküsü olmamıştır, ancak düşük öyküsü olmuştur. Anti-*Toxoplasma* immunoglobulin (Ig)G ve IgM seroloji testleri reaktiftir. IgG avidite testi %90 ve SAG2 pozitiftir. Tip I T. gondii bulunduğu varsayılan polimeraz zincir reaksiyonu ürününün dizilenmesi ve analizi, SAG2 dışında bir lokus kullanılarak doğrulanması gerekmiştir. Bu olgu sunumunun amacı, düşük öyküsü olan asemptomatik annelerde, konjenital toksoplazmozis riski hakkında farkındalık yaratmaktır. Yüksek riskli hamile kadınlarda konjenital bulaşmayı önlemek için hastalığın rutin ve seri taramaları yapılması önerilmektedir.

Anahtar Kelimeler: Konjenital toksoplazmozis, avidite testi, SAG2 geni

Toxoplasma is an intracellular protozoan, which causes asymptomatic toxoplasmosis in immune-competent individuals, but immunodeficient people often experience severe manifestations. Furthermore, it has infected approximately one-third of the world's population. Seropositive results were found in people who had a history of close contact with cats, consumed undercooked meat or unwashed raw fruits and vegetables, had low-level education, or lived in rural areas.[1,2] In a meta-analysis by Foroutan-Rad et al, its prevalence rate among pregnant women was 41%, with 38% immunoglobulin (Ig)G positive and 4% positive IgM.[3] The risk of transmission from mother to fetus is approximately 10-15%, 30%, and 90% during the first, 2nd, and 3rd trimesters, respectively.[4,5]

The laboratory diagnosis of toxoplasmosis has several approaches, and the type of technique used depends on the patient's immune status and clinical manifestations. This study presents a case report of diagnosis of toxoplasmosis from a woman who was asymptomatic during pregnancy and delivered a baby with multi-organ failure.

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CASE REPORT

An asymptomatic 30-year-old woman who delivered a baby with multi-organ failure 3 months ago was referred to the Centre of Clinical Parasitology Laboratory, Faculty of Medicine Universitas Indonesia, to get a whole assessment of the possibility of toxoplasmosis. The baby was diagnosed with congenital toxoplasmosis with positive anti-toxoplasma IgM at 1.9 INDEX (reactive >1.00) and positive IgG titer at 109.6 IU/mL (reactive >10.0 IU/mL). Meanwhile, the anti-human immunodeficiency virus (HIV), HBsAg, and anti-HCV were non-reactive. There was a history of miscarriages 2 years ago at ten weeks gestation. At that time her blood glucose level was 321 mg/dL and ketonuria 3+, but the infection marker was not checked. The patient has the habit of eating raw vegetables, such as basil and cabbage, but no pets at home or close contact with cats. Furthermore, the patient received antenatal care only in the first trimester and the result was normal. There was no history of febrile, enlargement of lymph nodes, and common cold symptoms during gestation. Written informed consent was obtained from the patient for publishing all data.

Complete blood count showed 13.7 g/dL hemoglobin, 8,500/mm^3 total leukocyte count, 326,000/mm^3 platelet count, 47 mm erythrocyte sedimentation, 345 mg/dL (70-110 mg/dL) fasting blood sugar, and 12.9 hemoglobin A1c (HbA1c) (<7%). The kidney parameters and liver profile were within the normal limit. The serological anti-toxoplasma IgM and IgG titer by ELISA were positive at 2.4 INDEX (reference reactive >1.00) and 164 IU/mL (reference reactive >10.0 IU/mL), respectively, while the IgG avidity test was 90% (>60% AVL: high avidity). Other TORCH examinations were nonreactive. Serology tests of TP rapid, HbsAg, and anti-HIV (rapid) were non-reactive.

Blood polymerase chain reaction (PCR) for the toxoplasma *SAG2* gene was positive. The patient's DNA was extracted from the buffy coat of 3 mL venous blood and then processed using predetermined methods.[6] PCR product was separated with electrophoresis in a 2% agarose gel, followed by staining with *Atlas Sight DNA Stain* (BioatlasTM) and 100 bp ladder (Thermo Scientific). After the amplification, the 241 bp and 221 bp products, which represent a fragment of the 5'-SAG2 and 3'-SAG2, respectively were amplified, as shown in Figure 1A. Every sample amplification was carried out along with positive control using *Toxoplasma gondii* RH strain collected from the Indonesian Research Centre for Veterinary Science and negative control containing sterile water.

The patient was given anti-*Toxoplasma* therapy, 960 mg cotrimoxazole twice a day, 25 mg pyrimethamine thrice a day, and 5 mg leucovorin once a day for 4 weeks. After the treatment, the PCR result obtained was negative, as shown in Figure 1B. Purified PCR products were sequenced using the same primers, the result was then aligned

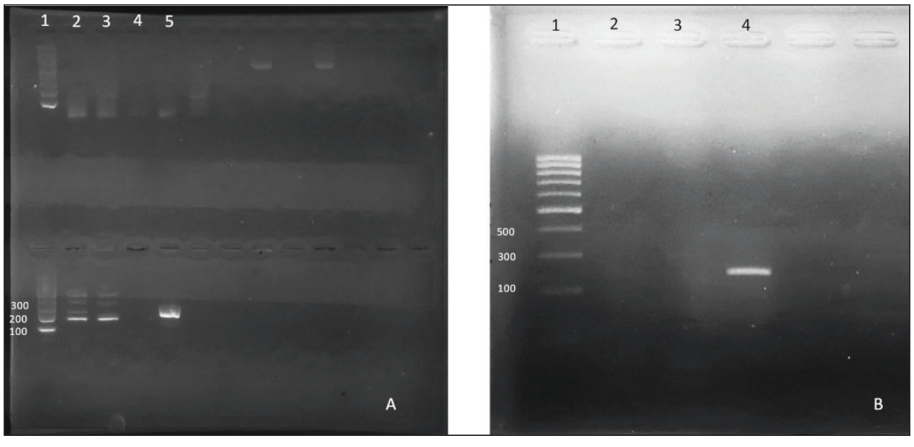

FIGURE 1: PCR product of T. gondii amplified by 5'-SAG2 and 3'-SAG2 primers. **A)** Pre-treatment. Lane 1. Ladder, Lane 2. Patient, Lane 3. Another sample, Lane 4. Negative control. Lane 5. Positive control. **B)** PCR 4 weeks after treatment. Lane 1. Ladder, Lane 2. Patient, Lane 3. Negative control, Lane 4. Positive control.
PCR: Polymerase chain reaction.

and analyzed using the Molecular Evolutionary Genetics Analysis X (MEGA X) software, version 10.2.20 (United States).[7] The phylogenetic tree result of both targets showed that the sample was a SAG2 Type I strain of *T. gondii*.

DISCUSSION

The prevalence of toxoplasmosis varies globally based on eating habits, level of education, sanitation, and environment. The infection often occurs through the ingestion of tissue cysts in uncooked meat, unwashed vegetables, or water/hand contaminated with cat feces containing sporulated oocysts. It can also be transferred through transplacental mother-to-child, blood transfusion, or organ transplant.[2,5] The patient in this study was exposed to *T. gondii* by eating raw or unwashed vegetables.

There was also a 2 years history of diabetes, which was reinforced by an abnormal HbA1c value and the data of blood glucose level when she got a miscarriage. Ozcelik et al revealed that 53% of diabetes mellitus (DM) sufferers as well as 27% of non-diabetic controls were positive for the Toxoplasma IgG antibodies.[8] Meanwhile, positive IgM antibodies were present in 13% of the people with DM and 1.0% in healthy control. Uncontrol DM patients are more susceptible to toxoplasmosis compared to others.[9]

During pregnancy, toxoplasma infection is a serious risk factor for fetuses due to congenital toxoplasmosis. Its transplacental transmission in the first trimester can cause severe clinical manifestations, such as hydrocephalus, microcephaly, deafness, ocular disorder, and abortion. Congenital infections in the 2nd trimester often lead to milder complications including anemia, epilepsy, splenomegaly, hepatomegaly, cerebral calcifications, pneumonitis, thrombocytopenia, and retinochoroiditis. For infection in the 3rd phase, more than 80% of the fetus is asymptomatic, but retinochoroiditis and neurologic deficits can occur in their childhood stage.[4,5] The patient's baby was delivered with several disorders, such as acute renal failure, intracranial hemorrhage, hepatomegaly, anemia, as well as positive anti-*Toxoplasma* IgG and IgM. This indicates that fetal infection occurred during pregnancy, presumably in the 2nd or 3rd trimester.

The risk of transplacental transmission during gestation is higher in women exposed to primary toxoplasmosis after conception compared to others exposed before conception.[10] The patient had a history of a miscarriage, which suggests the reactivation of latent infection or acquired reinfection in the 2nd trimester. Furthermore, diagnosis of *T. gondii* infection can be made through several methods, including directly and indirectly. Indirect testing of IgG antibodies, which is often carried out before pregnancy and in asymptomatic patients helps to identify people at risk of reactivation.[11] There was no data about the antibodies in this patient before conception. Assays for functional affinity have become standard to differentiate between recently acquired and more chronologically distant infections.[10] The presence of high avidity antibodies in the current case can exclude infection acquired in the past 4 months, and the high titer of IgM indicates an active form of the disease.

The majority of *T. gondii* isolates from human and animal sources are clustered into clonal Types I, II, and III. Furthermore, the Type I genotype is considered the most virulent, with a high level of parasitemia that increases the risk of transplacental transmission, and produce severe symptoms in the fetus or newborn.[12] The patient was assumed to have Type I *T. gondii,* which must be confirmed using other loci apart from SAG2. For reproductive females, special precautions and appropriate detection of the parasites can prevent the disease's progression and transplacental transmission during pregnancy.

In conclusion, an active case of toxoplasmosis in a woman without any symptoms during pregnancy and a history of delivering a baby with the congenital form of the disease was presented in this study. Routine and serial screening is recommended in high-risk pregnant mothers to prevent congenital transmission.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or mem-

bers of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Sri Wahdini, Ika Puspa Sari, Agnes Kurniawan;* ***Design:*** *Agnes Kurniawan;* ***Control/Supervision:*** *Agnes Kurniawan;* ***Data Collection and/or Processing:*** *Sri Wahdini, Ika Puspa Sari;* ***Analysis and/or Interpretation:*** *Sri Wahdini, Ika Puspa Sari;* ***Literature Review:*** *Ika Puspa Sari;* ***Writing the Article:*** *Sri Wahdini;* ***Critical Review:*** *Agnes Kurniawan;* ***References and Fundings:*** *Agnes Kurniawan.*

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