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CLINICAL STUDY

Correlation of toxoplasmosis with small-for-gestational-age newborns

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ABSTRACT

BACKGROUND: *Toxoplasma gondii* infection in pregnant women could lead to significant changes during the pregnancy, affect the outcomes of pregnancy and the timing of labour. Small-for-gestational-age (SGA) newborns are defined by birthweight below the 10th percentile for gestational age. We tested an association between latent toxoplasmosis in pregnant women and deliveries of SGA babies.

MATERIAL AND METHODS: For testing, we included 1,647 women who gave birth to a singleton baby at ≥ 37 weeks of gestation. The complement-fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) tests for IgG and IgM were used. The latent form of toxoplasmosis was defined as a CFT titre of 1:8 or higher, together with index positivity IgG ELISA > 1.1 and negative IgM.

RESULTS: There were 406 (24.7 %) women positive, and 1,241 (75.3 %) women negative for latent toxoplasmosis. Of all deliveries. 190 were SGA-positive and 1,457 were SGA-negative. Our study found a statistically significant association between latent toxoplasmosis and SGA foetuses born at term. The Pearson chi-square model was statistically significant ($\chi^2(1) = 7.365$, $p = .007$). The odds ratio was 1.567.

CONCLUSION: Pregnant women with latent toxoplasmosis giving birth at ≥ 37 weeks of gestation have a 1.567 times higher risk of delivering an SGA baby *(Tab. 2, Fig. 1, Ref. 30)*. *Text in PDF www.elis.sk*

KEY WORDS: *Toxoplasma gondii*, toxoplasmosis, low birthweight, small for gestational age, SGA.

Introduction

Small-for-gestational-age (SGA) newborns are smaller than normal for their gestational age. Their size is primarily defined by a weight below the 10th percentile relative to their gestational age (1, 2). Except for rare genetic traits, SGA is the result of intrauterine growth restriction (3). There is a difference between intrauterine growth retardation, which refers to poor growth *in utero*, and prematurity, a broad term defining neonates born before 37 weeks of gestation (4). Some babies with intrauterine growth retardation could be born as SGA (5, 6). The 10th percentile of weight was first used in the 1960s. This straightforward definition was expanded several times to incorporate additional parameters, which however, obscured the central issue. Therefore, in 1995, the World Health Organization published recommendations that defined SGA by birth weight below the 10th percentile, utilising localized anthropometric newborn curves. Despite some general revisions, this definition remained unchanged until recently. Some studies were successful in demonstrating that toxoplasma can lead to prematurity, low birth weight of newborns, or both (7). *Toxoplasma gondii (T. gondii),* the causal agent of toxoplasmosis is one of the most common parasites, an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa and subclass coccidia (8). *T. gondii* infects about one-quarter/third of the world's population. Infected are warm-blooded vertebrates such as humans, livestock, birds, and marine mammals. The life cycle of T. gondii includes two hosts: the intermediate host (such as mammals and birds), where asexual stages occur, and the final host, cats, where the sexual stage occurs. The genome of *T. gondii* is haploid, except for sexual division in cats (9). There are several stages of development: oocyst, tachyzoite, and sporozoite. Tachyzoites represent the vegetative form that can affect all human cells except erythrocytes, and they are the dominant form during the acute phase of infection. In this phase, the production of antibodies is triggered. Upon the rise of the immune response, tachyzoites convert into bradyzoites, forming tissue cysts. Bradyzoites persist inside the tissue cysts for the entire life of the host and are morphologically identical to tachyzoites but multiply more slowly. Bradyzoites can get released from cysts, transform back into tachyzoites and reactivate the infection in immunodeficient

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patients. Cysts form infective stages in intermediate and definitive hosts (10). The definitive hosts of *T. gondii* are cats. The sexual reproduction of the parasite occurs in their intestine, leading to the production of oocysts (11). Millions of oocysts are shed in the faeces of cats for 7–21 days during acute infection. After sporulation, between 1 and 21 days, oocysts (containing sporozoites) are infective when ingested by mammals (including humans) and give rise to the tachyzoite stage, thus closing the cycle of intermediate and definitive hosts. T. gondii infection is considered a major risk for spontaneous abortion, prematurity and low birth weight in animals. Studies of *T. gondii* infection focused on this area in humans are rare (6, 7, 12–16).

Acute *T. gondii* infection during pregnancy often leads to spontaneous abortion, severe sequelae, or both (10, 17, 18). Latent T. gondii infection in pregnant women could cause less obvious but still important changes during the pregnancy and affect the outcomes of pregnancy and the timing of labour (19, 20).

Our aim is to investigate whether there is a connection between latent toxoplasmosis in pregnant women and SGA neonates born at term.

Material and methods

A retrospective study design was used. The study participants were pregnant women who consecutively gave birth to their children and underwent regular antenatal biochemical screening between the 14th and 16th week of pregnancy. Only mature deliveries, i.e., parturitions at ≥ 37 weeks of pregnancy were included. The participants were offered screening that included ultrasound examinations for congenital diseases and pregnancy dating, human chorionic gonadotropin (HCG) test, alpha-fetoprotein (AFP), estriol and toxoplasma serological profile test. There was no specific selection of participants. One blood sample was used for all tests.

Participants' data were obtained from the databases of the teaching hospital of 1st and 3rd faculties of medicine, State organization for statistics and informatics in health care, genetic center Gennet, and National Reference Laboratory for Toxoplasmosis (NRL, TOXO) in the National Institute of Public Health in Prague. The study period was from 1995 to 2015. All data were treated anonymously, i.e., identification was accessible only to the principal authors of the study. The study was approved by the Ethical Committee of the Teaching Hospital, Charles University Prague (18.8.2020/9720/ EK-Z). Patients' written consent was waived as all data were retrospectively collected, treated from databases only and rendered anonymously.

Definition of small for gestational age newborn

According to World Health Organisation criteria, SGA newborns are smaller than average for their gestational age, primarily defined by weight below the 10th percentile for their gestational age (1, 2).

Serological profiles

The complement-fixation test (CFT) provided serological profiles for toxoplasmosis that expresses the overall levels of toxoplasma immunoglobulins of all classes (14, 21). This test has been used in our system for decades and entirely ensures comparativeness. Enzyme-linked immunosorbent assay (ELISA) tests for IgG and IgM were used simultaneously. IgM positivity helped to distinguish the acute and latent stages of toxoplasmosis. These results were expressed in the form of index of positivity (IP). In its latent form, the positivity for toxoplasmosis was defined as a CFT titre of 1:8 or higher alongside IgG ELISA > 1.1. For toxoplasmosis test to indicate the latent stage, IgM needs to be negative. When the result is dubious, retesting is performed. Pregnant women in our study were coded as toxoplasma-positive with latent toxoplasmosis (T+) or toxoplasma-negative (T–). As for SGA, the coding was either SGA-positive (SGA+) or SGA-negative (SGA–). Serological profiles of HCG, AFP, estriol and ultrasound examinations fell outside the scope of this study and were not investigated. Other factors linked to SGA, such as socio-economic status and maternal smoking, were not assessed. These factors would confound the analysis only if they were associated with acute toxoplasma infection.

Statistical analysis

SPSS IBM statistical software was used for the primary analysis. The Pearson Chi-Square model was performed to ascertain the effects of latent toxoplasmosis on the likelihood that participants gave birth to SGA newborns.

Results

For testing, we included 1,646 women from a larger database who gave birth to a singleton baby at ≥ 37 weeks of gestation. Multiple pregnancies were not included. Earlier delivery and lower birth weight are common in multiple pregnancies and are likely to bias the data. Women with a suspected acute form of toxoplasmosis (IgM-positive) were omitted. The mean age of the final sample was 29.6 years (range 17–46 years) at the time of delivery. From these pregnant women, 406 (24.7 %) were diagnosed as latent toxoplasma-positive (T+) and 1,241 (75.3 %) as toxoplasma-negative (T–). Participant characteristics are presented in Table 1. The counts of SGA cases were acquired for each week of gestation and adjusted for gender and country. There were 190 SGA+ deliveries and 1,457 SGA– deliveries. The prevalence rates are listed in Table 1 and Figure 1.

The Pearson chi-square fit was statistically significant ($\chi^2(1) = 7.365$; $p < .007$) (Tab. 2).

Tab. 1. Main characteristics of patients in the study.

	Count	Age	SGA +	SGA –
Toxo +	406	29.7	62	344
% of Total	24.7		32.6	23.6
Toxo –	1,241	29.6	128	1,113
% of Total	75.3		67.4	76.4
Total	1,647		190	1,457

Toxo +/–: women with/without latent toxoplasmosis; SGA +/–: women with/without small-for-gestational-age baby

Tab. 2. Pearson chi-square test and risk ratio for SGA.

Pearson Chi-Square	Value	df	Asymptotic significance (2-sided)
	7.365	1	0.007
Odds Ratio	Value	95% Confidence Interval	
	1.567	Lower 1.131	Upper 2.172

0 cells (0.0 %) have an expected count of less than 5

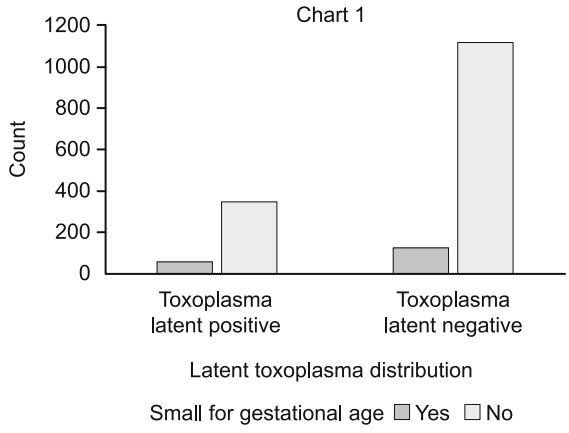

Fig. 1. Prevalence rates.

The model ascertains that latent toxoplasma is associated with SGA appearance of neonates born at term. The odds ratio for this effect was 1.567 (95% confidence interval [CI] = 1.131–2.172). When delivered at term, women with latent toxoplasmosis have 1.567 times higher odds of delivering a baby with SGA (Tab. 2).

Discussion

In our study, we assessed the impact of latent toxoplasmosis on the birthweight in the last month of pregnancy. The Pearson chi-square model was statistically significant in proving the association of latent toxoplasmosis with SGA. According to available databases, the effect of latent toxoplasmosis on giving birth to SGA newborns delivered at term has not been targeted yet.

The main opinion was that birthweight and prematurity were linked together. Sometimes, the clinical experience does not follow this opinion and low birthweight appears more frequently than expected (22, 23). SGA babies are linked with increased perinatal mortality, lung disease (6), hypotension, necrotizing enterocolitis, poor thermoregulation, hypoglycaemia, and polycythaemia. Long-term risks of small infants are insulin resistance associated with type II diabetes mellitus (5), cardiovascular disease, chronic kidney disease, poor neurodevelopmental and cognitive impairment (24) and short stature in adulthood (3).

Hypoglycaemia is especially common in asymmetrical SGA babies, i.e., those with normal-sized head/brain and hypotrophic bodies. The larger brain burns more calories at a faster rate than available from their fat stores (25).

Toxoplasmosis infection can be assessed through serological testing. The diagnosis of primary toxoplasmosis in pregnant women is mainly based on specific IgM and IgG. Since IgMs are antibodies that are produced during the first week after infection, they are the earliest to appear in acute infection. IgGs are the last antibodies to emerge, namely a few weeks after IgMs. The diagnosis of confirmed infection is based on the appearance of IgG. The specific IgA test is used by some laboratories to diagnose acute infection. The IgG avidity and Western blot tests could provide broad additional information about the severity of infection (9, 20).

It is believed that in humans after primary infection, the infection persists in the body in a chronic, latent form (17). It is a lifetime condition for humans who are not immunocompromised. In humans with autoimmune diseases (17, 26), chronic corticosteroid applications (27), bone-marrow transplants, or AIDS (28), it can progress into an active acute form leading to consequential impairment of the brain, eyes, senses and other structures (29). Many pregnant women with acute infection experience no obvious symptoms or signs. A large number of mothers who gave birth to congenitally infected offspring cannot recall experiencing an infection-related illness during their pregnancy or identify any of epidemiological risk factors (8).

The influence of the processes of toxoplasma infection on the foetus and delivery is not known (10). We think that one possible explanation could be based on the effect of inflammatory mediators on the placenta, foetus, and onset of labour. It could also influence the nutrition of the foetus, which could result in poor development seen in SGA newborns. One study (30) presented an enzyme-linked assay, by way of which it is possible to distinguish type II and non-exclusively type II [NE-II]) of *T. gondii* parasite, namely by detecting antibodies in human sera that recognize allelic peptide motifs of distinct parasite types. Type II and type NE-II parasites were supposed to cause congenital toxoplasmosis in North America. NE-II serotypes were more prevalent in certain demographics and associated with prematurity and severe disease at birth. The strength of this study is that the method of screening used has been employed for decades and enables long-term comparability. All the patients' data on infection/non-infection and delivery are available. Regarding the available databases, no similar study has been performed or published up to now. One potential limitation of our study is that the pregnant women were mostly from urban areas, and evidence suggests (19) that the prevalence of infection is lower in urban areas compared to the rural environment.

Conclusion

Our study found a statistically significant association between latent toxoplasmosis and SGA foetuses born at term/mature. The Pearson chi-square model fit was statistically significant ($\chi^2(1) = 7.365$; $p = .007$). The odds ratio was 1.567. Women with latent toxoplasmosis giving birth at ≥ 37 weeks of gestation have a 1.567 times higher risk of delivering an SGA baby. Although the strength of the association in our large sample is relatively moderate, the combination of latent toxoplasmosis with other adverse factors could have serious sequelae in newborn's development.

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