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The prevalence and clinical consequences of TORCH infection in biliary atresia and non-biliary atresia cholestatic disorders

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Abstract:

Background: Infection with Toxoplasma, Rubella, Cytomegalovirus (CMV), and Herpes simplex virus (HSV) types 1 and 2 have been accused over the past years as causative factors for neonatal cholestasis, including biliary atresia (BA). This assemblage of organisms could generate severe manifestations, especially in immunocompromised hosts, which have the potential to be prevented and treated.

Objective: The current research aimed at identifying the immunity to TORCH infection in biliary atresia and non-biliary atresia cholestatic disorders and identifying the most accompanied complications.

Subjects and Methods: This cross-sectional, analytical comparative study involved the enrollment of 100 pediatric patients using systemic random sampling methodology; 50 infants were diagnosed as having BA, and others suffered from other cholestatic from National Liver Institute (NLI) attendees. These individuals were examined for TORCH immunity using imaging, serological biomarkers, and molecular diagnosis of CMV infection.

Results: The two groups showed similar TORCH infection rates (P>0.05). Among the patients, 24% tested positive for CMV IgM, 23.3% tested positive for CMV by polymerase chain reaction (PCR), 16% tested positive for Rubella, 41% tested positive for Toxoplasma, 28% tested positive for HSV 1 and 19% tested positive for HSV 2 IgM. The most frequently observed complications were developmental delay (32%), microcephaly (11%), ocular issues (11%), low birth weight (9%), congenital heart diseases (6%), and hydrocephalus (4%). However, there was no statistically significant disparity between the two groups' distribution of these reported complications. Female infants and a history of positive consanguinity were higher in the BA cholestatic infants.

Conclusion: Immunity to toxoplasmosis was the highest in the present study, followed by CMV, HSV, and Rubella. Among the accompanied complications, developmental delay followed by microcephaly was the most presented. Therefore, screening for TORCH complex in diagnosed with cholestasis should be routinely performed.

Keywords: *TORCH, NLI, Neonatal cholestasis, Biliary atresia, Pediatrics, Egypt.*

1. Introduction

Neonatal cholestasis is a condition in which newborns and early infants have high levels of conjugated bilirubin in their blood, a common symptom in more than 100 liver, bile ducts, and metabolism-related diseases. Different disorders can cause Neonatal cholestasis. Some must be diagnosed quickly and treated promptly to prevent permanent damage to other organs and ensure the best possible biliary atresia treatment results (Feldman and Sokol 2020).

The incidence of neonatal cholestasis was estimated to be one in 2500 live births (Sokol et al., 2007; Fawaz et al., 2017). Biliary atresia accounts for 25-45% of these cases. Elements of the TORCH complex were accused of etiology, although with fewer percent, they are curable causes (Satrom and Gourley 2016).

The TORCH complex involves a collection of infectious organisms, including *Toxoplasma gondii*, syphilis, hepatitis B, HIV, *varicella-zoster* virus, *parvovirus* B19, *Rubella* virus, *cytomegalovirus*, and *herpes simplex* viruses (HSV type 1 and 2) (Mocanu et al. 2021). *Toxoplasma gondii*, the etiological agent responsible for developing toxoplasmosis, is a protozoan parasite that exhibits an obligatory intracellular lifestyle, infecting several warm-blooded organisms, including humans. *Toxoplasma gondii* exhibits a global distribution, with an estimated prevalence of latent toxoplasmosis found in approximately one-third of the global population (Halonen & Weiss, 2013; Fallahi et al., 2018). The classical trio of congenital toxoplasmosis comprises chorioretinitis, hydrocephalus, and cerebral calcifications (Jones, Lopez, and Wilson 2003). The clinical manifestations associated with the condition include microcephaly, hepatosplenomegaly, jaundice, the presence of a maculopapular or petechial rash, myocarditis, pneumonitis, respiratory distress, and convulsions. Other occasional abnormalities include hearing impairments, a presentation resembling erythroblastosis, low platelet count (thrombocytopenia), high lymphocyte count (lymphocytosis), high monocyte count (monocytosis), and nephrotic syndrome (McAuley 2014).

Cytomegalovirus is classified as a member of the *herpes* virus family, characterized by its enclosed structure and possession of a double-stranded DNA genome (McAuley, 2014; Kagan & Hamprecht, 2017). Congenital CMV infection, with an estimated birth frequency of roughly 0.6%, is the predominant congenital viral infection in developed nations. CMV infection typically presents as either asymptomatic or with moderate symptoms in individuals across many age groups, including newborns, children, and adults (Swanson and Schleiss 2013). Nonetheless, congenital infection can have severe consequences, such as neurological complications. The clinical manifestations associated with congenital infection encompass a range of symptoms. These may consist of intrauterine growth restriction, fetal hydrops, generalized petechiae, purpura, thrombocytopenia, jaundice, hepatosplenomegaly, pneumonitis, microcephaly, periventricular calcifications, seizures, chorioretinitis, sensorineural hearing loss, bone abnormalities, abnormal dentition, and hypocalcified enamel. Sensorineural hearing loss is the most common consequence (Swanson and Schleiss 2013).

Rubella is caused by the *Rubella* virus, classified as an enveloped, single-stranded, positive-sense RNA virus. It belongs to the *Togaviridae* family and is the only member of the *Rubivirus* genus (Leung, Hon, and Leong 2019). *Rubella* is a benign, self-restricted ailment accompanied by a distinctive rash. The occurrence of *Rubella* virus infection during pregnancy has been associated with the development of congenital Rubella syndrome (CRS), as well as the increased risk of spontaneous abortion and stillbirth (Dontigny, Arsenault, and Martel 2018). The typical trifecta, particularly when the infection occurs throughout embryogenesis, is characterized by cataracts, cardiac defects and hearing loss (Bouthry et al. 2014). Nevertheless, additional temporary, enduring, or delayed abnormalities may be observed. The enduring impairments encompass microphthalmia, cataracts, retinopathy, sensorineural deafness, patent ductus arteriosus, pulmonary artery hypoplasia, mental or psychomotor retardation, language delay, and microcephaly (Bouthry et al. 2014). Given that infections caused by *Toxoplasma gondii*, CMV, and the *Rubella* virus typically result in nonspecific or subclinical manifestations of disease, it is plausible for children and neonates to be particularly susceptible to these infections, particularly in the context of hepatology departments. Most research endeavors focus on examining the seroprevalence of a solitary pathogen, with only a limited number of studies investigating concurrent immunization against two or three pathogenic agents. This study aimed to ascertain cholestatic neonates with and without biliary atresia who exhibited immunity or vulnerability to infection caused by the TORCH complex and the clinical consequences.

2. Subjects and Methods

2.1. Study Setting, and Subjects:

In this Analytical comparative cross sectional study, we included 100 infants who visited the Pediatrics Department of the National Liver Institute hospitals between January 2021 and November 2021. Each participant met the inclusion criteria of having cholestatic disorder accompanied either caused by biliary atresia or other causes. A similar number of patients with cholestasis caused by biliary atresia (n = 50) and factors other than BA (n = 50) were recruited

within a similar period and referred to as the non-BA group. The participation of these infants was contingent upon obtaining consent from their guardians. Pertinent data pertaining to patient demographics, including age, gender, and presenting symptoms, as well as comprehensive findings from the clinical examination, was documented.

2.2. Sample Technique and Sample Size Calculation

A systematic random sampling methodology was used to conduct our study. In our hospital, we selected a child randomly (1, 4, 7, and 10...etc.) till the target sample size was met.

To estimate the sample size, we used the Epi info program version 7 and according to: The frequency of cholestasis was 2.2% in neonates who were born near term of gestation (Tufano M et al., 2009). The annual live newborns in Egypt are 2.7 million children (Pugliese-Garcia M et al., 2020). Design effect 1 - 97 % confidence level- Margin of error 5%. Sample size should be at least 49. So we enrolled **50** neonate with biliary atresia and **50** without biliary atresia.

2.3. Study Tools/Instruments

2.3.1. Laboratory investigations

Blood and serum samples were withdrawn from the participants to fulfill diagnosis of cholestasis. Complete blood picture was done. Measurements of serum levels of direct bilirubin, AST, ALT, total protein, GGT, and albumin were performed. Prothrombin time (PT) and concentration (PC) were also assessed. Viral markers including HBsAg, HBcIgM and HBcIgG, and Ab were checked.

2.3.2. TORCH elements diagnosis

A volume of 5 milliliters of venous blood was obtained from each infant included in the study. The blood samples were conveyed to the central laboratory. At first, the blood samples were subjected to centrifugation at a speed of 4000 revolutions per minute for 5 minutes. Afterward, the sera were separated and stored at a temperature of -20°C until they could be further analyzed. The TORCH panel screen was conducted using the Cobas e 411 immunoassay analyzer from Roche Diagnostics, Germany. This analyzer utilizes electrochemiluminescence (ELC) technology to detect IgG and IgM antibodies for *Toxoplasma*, *Rubella,* CMV, and HSVs. The kits used for this analysis were obtained from COBAS (Roche Diagnostics) with Elecsys according to the manufacturer's recommendations.

CMV-DNA polymerase chain reaction (PCR) was performed with the COBAS AMPLICOR monitor test, version 2.0, on the COBAS AMPLICOR Analyzer manufactured by Roche (Boom et al. 1999). The kit's detection limit was 200 copies per milliliter.

Diagnostic imaging methods employed in this study included abdominal ultrasonography (USG) and echocardiography. Ocular examination was conducted on all participants in the study to detect metabolic disorders or syndromes.

2.4. Ethics approval and consent to participate

This study was approved by the Damietta Faculty of Medicine IRB, Al-Azhar University, Damietta (IRB 00012367-21-01-024), and approved date: 5-1-2021. All methods and tools of the study were carried out following the relevant guidelines and regulations of the Declaration of Helsinki. The study's objectives and the potential detrimental effects of puncture were communicated to all participants' guardians, and their informed consent was obtained and documented.

2.5. Statistical analysis

Data was collected and entered to the computer using the SPSS (Statistical Package for Social Science) program for statistical analysis (version 21; IBM Corporation, Armonk, NY, USA). Quantitative data were shown as mean± standard deviation (SD). Qualitative data were expressed as frequency and percent (%). The Chi-square test and Fisher exact test were used to measure the association between qualitative variables, as appropriate. The student's t-test was used to compare continuous parametric variables between 2 groups. The Mann–Whitney U test was used for continuous non-parametric variables between 2 groups. P (probability) value was of statistical significance if it is ≤ 0.05.

3. Results

The study included 100 infants with cholestatic liver disorders: 50 BA infants and 50 infants with other cholestatic disorders. In terms of age, the study found no significant difference between infants with BA and those with non-BA cholestasis (2.95 ± 1.1 months vs. 9.38 ± 2.8 months, p=0.137). A significantly higher proportion of female infants was observed in the BA group compared to the non-BA cholestasis group (36.0% vs. 56.0%, p=0.045). Consanguinity was also more prevalent in the BA group compared to the non-BA cholestasis group (16.0% vs. 36.0%, p=0.023). Additionally, stool colour was significantly different between the two groups, with the majority of infants with BA presenting with clay-colored stools (98.0% vs. 26.0%, p=0.000). Moreover, hepatomegaly was observed in all infants with BA compared to 86.0% in the non-BA cholestasis group (p=0.006). Other factors such as splenomegaly, Dextrocardia, and Situs Inversus did not show significant differences between the groups (Table 1).

Concerning the laboratory investigations; the hemoglobin, platelets, WBCs, total and direct bilirubin, AST, total protein, serum, and albumin were comparable between both groups (P> 0.05). The ALT, PT and PC were significantly higher in the non-BA group (P< 0.05), while the ALP and GGT were significantly higher in the BA group (P< 0.05). HBsAg, HBc IgM and HBc IgG Viral markers were negative in 100% of the patients. HCV Ab was positive in just 1 case of BA infants. The incidence of TORCH infection was comparable between both groups (P>0.05). 24% of the patients had positive CMV IgM, 23.3% were positive for CMV by PCR, 16% were Rubella IgM positive, 41% were *Toxoplasma* IgM positive, 28% had HSV 1 IgM positive and 19% had HSV 2 IgM positive (Fig. 1, Table 2,3).

Ultrasound imaging revealed that; the mean of the liver span in the BA infants was 7.82±1.061 cm and that in the non-BA group was 7.95±1.59 cm, with no significant difference between both groups (P = 0.948). The mean of the splenic size in BA infants (6.14±1.30 cm) was comparable

to that in the non-BA group (6.20±2.17cm) (P = 0.510). Ascites was present in 6% and 4% of BA and non-BA infants, respectively (P = 0.646). Non-contractile gallbladder was present in 62% of BA infants and 30% of non-BA infants (P<0.001). 32% of BA infants had atretic gallbladder. 4% of BA infants had polysplenia. TC sign was present in 30% of BA group and 10% of non-BA group (P = 0.012). Hepatic sub capsular flow was present in 40% of BA group and 18% of non-BA group (P = 0.015). IHBRD was seen in 2% of BA infants.

The commonest complications recorded in our patients were developmental delay (32%), microcephaly (11%), eye problems (11%), Low birth weight (9%), congenital heart diseases (6%) and hydrocephalus (4%), with no significant difference regarding the distribution of the reported complication in both groups (Table 4).

4. Discussion

Neonatal cholestasis represents the clinical presentation of several pathological conditions. The timely identification of etiology is of utmost importance, as the prognosis of children with biliary atresia is significantly influenced by treatment administered early in life (Robino, Machado, and Montano 2013). The present study aimed to determine the TORCH immunity in cholestatic and to evaluate different biomarkers, imaging techniques that could help in diagnosis of BA.

Newborn infected with congenital toxoplasmosis may exhibit asymptomatic manifestations of, or it may present with mostly neurological, ophthalmological, or gastrointestinal symptoms such as hepatomegaly and cholestatic jaundice. Toxoplasmosis is recognized as a treatable but possibly lethal disease (Peyron et al. 2019). The occurrence of neonatal cholestasis as a result of congenital toxoplasmosis is not commonly documented (Robino, Machado, and Montano 2013). Unfortunately, if a primary infection occurs during pregnancy, it might result in irreversible fetal consequences. This specific protozoan parasite has the capacity to appear in persons who have weakened immune systems (Peyron et al. 2019). The positive association between *Toxoplasma* infection and chronic liver diseases was documented by El-Sayed et al. (2016) as the liver plays a crucial role and is significantly impacted during the parasitemia stage of *T. gondii* infection, and most chronic liver diseases (CLD) were characterized by a suppression of both humoral and cell-mediated immunity, resulting in a significant incapacity to effectively combat invading microorganisms (Geng et al. 2000). In the current study 41% of cholestatic cases showed recent infection with toxoplasmosis with non-significant differences regarding biliary atresia. The current high reported seropositivity to toxoplasmosis disagreed with previous reports by Mahmud et al. (2016) who found that infection with toxoplasmosis was the least representative among the TORCH assemblage in infants with cholestatic disorders. Nevertheless, recent global reports recorded that the estimates of IgM or IgG seroprevalence have not declined over time (Bigna et al. 2020), which aligned with the current results.

Infection with *Toxoplasma, Rubella,* CMV, and HSV types 1 and 2 is a causative factor for NC, including biliary atresia (BA) (Carvalho et al., 2007; Betalli & Davenport, 2019). Comparing recent infection marked by IgM seropositivity to the TORCH complex between the two examined groups, no significant difference was recorded (P > 0.05), which disagreed with Sira et

al. (2016) who found that the incidence of IgM antibodies was markedly greater in the non-BA group than in the BA group (23% vs. 8.5%; P = 0.006). In the current work *Toxoplasma* IgM was the most prevalent causative agent followed by CMV which exhibited IgM seropositivity in 24% of cases being greater in BA group than non- BA. However, CMV DNA detected by PCR were substantially greater in the non-BA group 29.3% compared to BA group which accounted for 17.8%. Additionally, no notable statistical disparities were observed among the other examined markers.

Quak et al. (2008), found in their study that CMV being the most prevalent causative agent TORCH infections which accounted for 22% of cases of newborn cholestasis. Also Sira et al. (2016) in their research claimed CMV as the prevalent causative element of TORCH in neonatal cholestasis. In most cases, the significant occurrence of IgG antibodies in TORCH infections indicates the transfer of these antibodies from the mother to the fetus through the placenta. This transfer provides passive immunity to the fetus (Silasi et al. 2015). Several patients who tested positive for CMV DNA did not test positive for CMV IgM. One potential reason is that the newborns either had not yet developed IgM or had been infected for a prolonged period, resulting in the absence of ongoing viral IgM production (Fischler et al. 1998).

One of the significant findings in this research was the presence of higher positive consanguinity higher in the non-BA cholestatic infants (36%) than that in the BA group (16%) (P = 0.023), which agreed with Ağın et al. (2016).

According to conventional teaching, a typical case of BA is characterized by a full-term infant who appears healthy, exhibits elevated GGT cholestasis, and presents with pale stool. Nevertheless, recent research indicated that newborns with BA may be born prematurely and may exhibit typical GGT cholestasis in approximately 12-14% of instances (Chiu et al., 2013; Durkin et al., 2017; Van Wessel et al., 2017 ;Shankar et al., 2020). These data align with results of the current work where GGT levels were significantly higher in the BA group.

Triangular cord (TC) sign and non-contractile gall bladder were suggestive for biliary atresia diagnosis in the current research which align with earlier research by Li et al. (2008). However, absence of these criteria shouldn't rule out BA diagnosis (R Fawaz, Baumann, and Ekong 2017).

5. Conclusion

Toxoplasmosis was the most common infection among newborns diagnosed with cholestasis, followed by CMV, with rubella as the least presented infection. Screening for TORCH infection should be routinely required in newborns diagnosed with cholestasis to avoid clinical consequences of treatable infections.

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Data availability: The datasets are available upon reasonable request.
Consent for publication: Not applicable.

Abbreviations: CMV: Cytomegalovirus HCP: Hydrocephalus HSV: Herpes Simplex Virus T. Gondii: *Toxoplasma gondii* TORCH: *Toxoplasma* Gondi, Rubella, CMV, Herpes

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Table 1: Clinico epidemiological characters of the study infants

	BA N=50	**Non-BA cholestasis N=50**	**P**
Age (Month)	2.95 ± 1.1	9.38 ± 2.8	0.137
Sex			
Male	18 (36.0%)	28 (56.0%)	0.045*
Female	32 (64.0%)	22 (44.0%)	
Consanguinity			
Positive	8 (16.0%)	18 (36.0%)	0.023*
Negative	42 (84.0%)	32 (64.0%)	
Stool color			
Yellow	1 (2.0%)	37 (74.0%)	0.000*
Clay	49 (98.0%)	13 (26.0%)	
Hepatomegaly	50 (100.0%)	43 (86.0%)	0.006*
Splenomegaly	22 (44.0%)	20 (40.0%)	0.685
Dextrocardia	3 (6.0%)	0 (0.0%)	0.079
Situs Inversus	1 (2.0%)	0 (0.0%)	0.998

* Significant value (P< 0.05)

Table 2 Comparison of the laboratory parameters in the BA and non-BA cholestatic infants.

	BA N=50 Mean± SD	**Non- BA cholestasis N=50 Mean± SD**	**P**
T. bilirubin	11.4±3.94	10.6±4.49	0.230
D. bilirubin	8.31±3.13	7.40±3.12	0.194
AST	194±98.3	287±232	0.100
ALT	131±120	186±140	0.013
Alk. Phosphatase	568±245	459±244	0.012
GGT	933±697	320±462	<0.001*
T. proteins	5.5±0.60	5.51±1.01	0.477
Serum albumin	3.5±0.45	3.40±0.63	0.362
PT	11.9±1.25	13±2.06	0.003*
INR	1.02±0.11	1.09±0.17	0.025
Hb	9.71±1.14	10.1±1.30	0.088
WBCs	12.2±4.70	12.5±5.91	0.679
Platelets	453±172	401±191	0.220

* Significant value (P< 0.05)

Table 3 Positive TORCH infection in BA and non-BA cholestatic patients

	BA **N=50** **N (%)**	**Non- BA cholestasis** **N=50** **N (%)**	**P-value**
CMV IgM	14 (28.0%)	10 (20.0%)	0.349
CMV IgG	42 (84.0%)	40 (80.0%)	0.603
PCR.CMV (N=86)	8 (17.8%)	12 (29.3%)	0.114
Rubella IgM	10 (20.0%)	6 (12.0%)	0.275
Rubella IgG	24 (48.0%)	20 (40.0%)	0.420
Toxoplasma IgM	18 (36.0%)	23 (46.0%)	0.309
Toxoplasma IgG	38 (76.0%)	41 (82.0%)	0.461
HSV1 IgM	12 (24.0%)	16 (32.0%)	0.373
HSV1 IgG	31 (62.0%)	36 (72.0%)	0.288
HSV2 IgM	10 (20.0%)	9 (18.0%)	0.799
HSV2 IgG	22 (44.0%)	23 (46.0%)	0.841

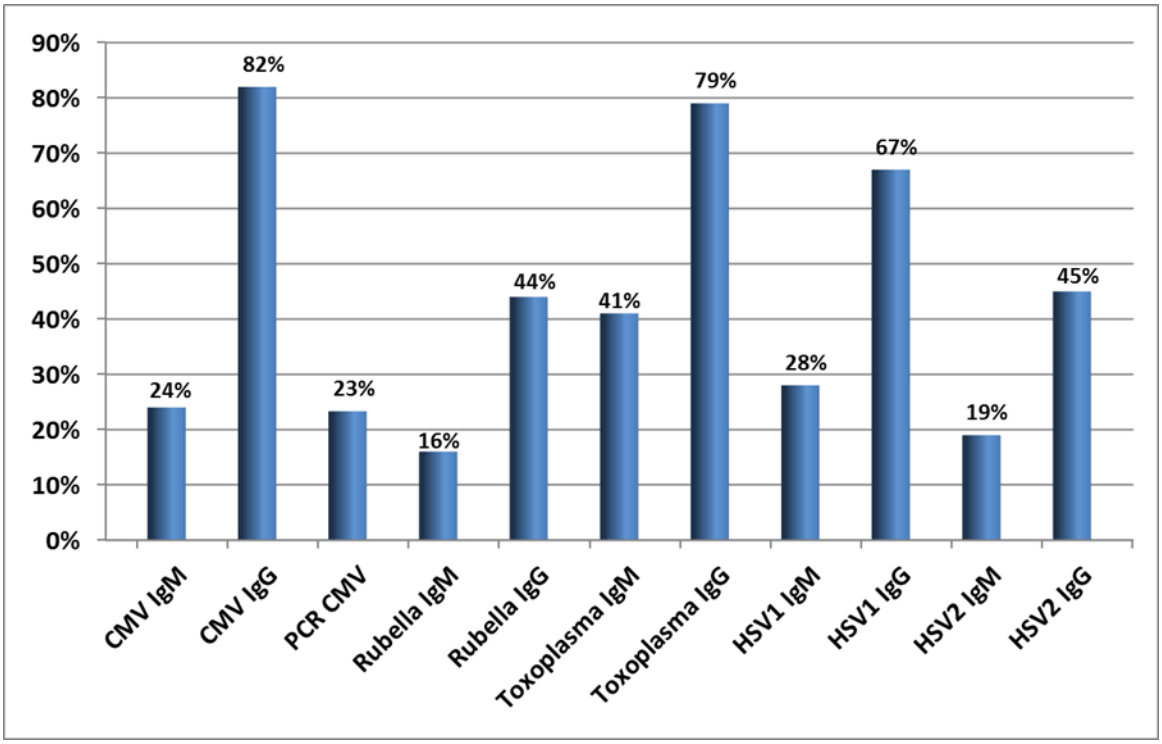

Fig. 1 Bar chart showing the incidence of TORCH infection in the cholestatic children

Table 4 The recorded complications in the cholestatic infants

	BA **N=50** **N (%)**	**Non- BA cholestasis** **N=50** **N (%)**	**P**
Congenital heart diseases	5 (10.0%)	1 (2.0%)	0.204
thrombocytopenia	0 (0.0%)	2 (4.0%)	0.495
Hydrocephalus	2 (4.0%)	2 (4.0%)	1
Microcephaly	3 (6.0%)	8 (16.0%)	0.110
Low birth weight	3 (6.0%)	6 (12%)	0.318
Auditory Problems	1 (2.0%)	1 (2.0%)	1
Developmental delay	15 (30.0%)	17 (34.0%)	0.668
Eye Problems	4 (8.0%)	7 (14.0%)	0.338
Brain Calcification	2 (4.0%)	1 (2.0%)	1