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TOXOPLASMA GONDII IGG AVIDITY IN DISCRIMINATING BETWEEN ACUTE AND CHRONIC TOXOPLASMOSIS IN NEONATAL CHOLESTASIS

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Abstract

Toxoplasma IgM (IgM) is a sensitive indicator of primary toxoplasmosis; but with limited specificity, and it may occasionally be sustained in blood serum for extended periods, ranging from several months to years, following the first infection. The Toxo IgG avidity assay is a currently employed diagnostic method for accurately assessing the time it takes to acquire an infection and determining the initial *T. gondii* infection. This study evaluated *Toxoplasma* IgG avidity testing in new-borns diagnosed with cholestasis on 92 neonates presented with cholestasis, whose blood samples were assessed for the presence of toxoplasmosis using IgG and Toxo IgG Avidity ELISA tests. Biochemical changes including the lipid profile, liver and renal functions were also evaluated. ELISA test revealed Toxo IgG seropositivity at 52.2% (29.43±17.86IU/ml). Toxo IgG avidity tested low at 56.5%, high at 34.8%, and borderline at 8.7% (0.38 ±0.31) with a significant association (P<0.001) at a cut-off value of 0.315. IgG titre was significantly correlated with direct bilirubin (P=0.029), AST (P<0.001), ALT (P<0.001), ALP (P=0.041), PC (P=0.003), INR (P=0.041), urea (P=0.021), and triglyceride (P=0.022). Toxo IgG avidity showed a significant (P<0.001) negative association with total bilirubin, direct bilirubin, AST, ALT, PT, INR, and a significant positive association with PC (P<0.001), GGT (P=0.01).

Keywords: *T. gondii*, IgG avidity, Neonatal cholestasis, Lipid profile, Renal and liver functions.

Introduction

Toxoplasma gondii (*T. gondii*), a zoonotic protozoan parasite, belongs to Apicomplexa phylum of worldwide distribution (Dubey, 2021). It can lead to severe toxoplasmosis symptoms, particularly in individuals with weakened immune systems, pregnant women, and livestock (Deng *et al*, 2018;). Parasitic transmission can occur either horizontally or vertically (Milne *et al*, 2020).

Congenital toxoplasmosis is caused by *T. gondii* transplacental acquisition, manifested as prematurity, intrauterine growth restriction, jaundice, hepatosplenomegaly, myocarditis, pneumonitis, rash, chorioretinitis, hydrocephalus, intracranial calcifications, microcephaly, and seizures (Wang *et al,* 2017).

Toxoplasma infection is diagnosed primarily relied on detecting IgG, IgM, IgA, or IgE (Singh, 2003). An acute infection can be diagnosed by detecting IgM antibodies, but not IgG antibodies. A sub-acute level of elevation is achieved when both antibodies are detectable. However, in chronic infection, absence of IgM was necessary so that the host is solely IgG-positive (Oktenli *et al*, 2004).

Hedman *et al.* (1993) introduced a technique for distinguishing between the acute and latent stages of infection by assessing the antigen-binding avidity. This was defined as the potency of a combination of polyclonal IgG molecules reacting with several protein epitopes. Anti-*T. gondii* IgG binding affinity gradually increased following infection immunity, known as the maturation of humoral immune response (Teimouri *et al*, 2020). *Toxoplasma* IgG (T-xo IgG) avidity proved to be a dependable test, especially in high avidity in infection of at least 3 to 4 months duration (Barros *et al*, 2017).

Neonatal cholestasis (NC) is distinguished by conjugated hyperbilirubinemia in the new born and young baby with a prevalent manifestation in > 100 hepatobiliary and metabolic illnesses (Gottesman *et al*, 2015). If jaundice extends more than 2 weeks in full-

term infants or 3 weeks in preterm infants, serum bilirubin level should be fractionated into conjugated and unconjugated bilirubin to exclude NC diagnosis (Hartley, 2018). However, neonatal jaundice manifestations are multifactorial and include biliary atresia, neonatal hepatitis, metabolic disorders, infections, and other structural abnormalities (Fawaz *et al*, 2017).

Liver plays a crucial role in storing and transporting dietary xenobiotics and carbohydrates, facilitating detoxifying processes and communication with other organs within the body (Robinson and Klein, 2012) Toxoplasmosis has the potential to induce gradual hepatic impairment, which inevitably led to alterations in hepatic metabolism (Hussien *et al*, 2015).

The current study aimed to evaluate *T. gondii* IgG avidity in differentiation between acute and chronic toxoplasmosis in neonatal cholestasis and to evaluate some biochemical changes associated with cholestatic newborns.

Subjects and Methods

The study included 92 cholestatic neonates who attended the National Liver Institute from January to October 2023. Informed written consent was obtained from their guardians, and the study was approved by the Institutional Review Board (IRB) of the National Liver Institute, Menoufia University, Egypt (NLI IRB Protocol Number: 00504/2023). The research followed the principles and restrictions specified in the Declaration of Helsinki.

A volume of three ml of venous blood was carefully collected from each neonate. Sera were separated after centrifugation at 3000 rpm for ten minutes, and stored at -20°C until needed for analysis.

Sera were analysis using ELISA IgG test (BioCheck, Inc.) following the instructions of the manufacturers. The diluted sera were next transferred onto a microtiter plate covered with *T. gondii* antigen, followed by the addition of anti-human antibody conjugate. After incubation and multiple washes, chromogenic substrate was introduced, and optical densities were measured by an ELISA reader at a wavelength of 450nm. Results were interpreted by using instructions given by the manufacturers. IgG antibody values above 0.90 were classified as positive, while values below 0.90 were classified as negative (Zhang *et al*, 2016).

Sera were also subjected to Toxo IgG Avidity ELISA test (Institut Virion-Serion Gmbh, Germany Kit) following the kit instructions. Optical density (OD) was measured at 40nm using a microtiter plate reader after incubation and repeated washing. IgG avidity calculation involved determining the titter's percentage. This was expressed as the avidity index (%), calculated by dividing the OD of treated samples with the avidity reagent by the OD of the sample treated without the avidity reagent. Results were categorized into three groups: low (with an avidity index below 0.5 indicated an acute infection), borderline (with an avidity index between 0.5 & 0.6), or high (with an avidity index greater than 0.6) (Petersen *et al*, 2005).

Sera samples were sent to the laboratory for detection of lipid profile, liver functions, and renal functions.

Statistical analysis: Data were collected and analysed by using SPSS version 28.0 for Windows. Descriptive statistics presented the demographic, biochemical, and serological parameters. Spearman's coefficient, correlation analysis assessed associations between IgG avidity and each parameter. Gender-based differences in IgG avidity were examined using an independent t-test. Receiver Operating Characteristic (ROC) analysis evaluated the IgG avidity diagnostic performance, expressed as an Area under the Curve (AUC) with its confidence interval. Statistical significance was set at $P \leq 0.05$.

Results

Weight and age of neonates were 4±1 kg and 63±42 days respectively. As to liver functions, total bilirubin was 10.24±5.18mg/dl, direct bilirubin was 6.17±2.67mg/dl, total protein was 5.5±0.9g/dl, albumin was 3.7±

0.7g/dl, AST was 208.30±108.20U/L, ALT was 230.43±171.86 U/L, ALP was 484.00 ±204.07 IU/L, GGT was 341.43±312.32 IU/L, PT was 14.03±4.55 seconds, PC was 81.83±28.55, INR was 1.26±0.54.

Renal functions showed that urea was 17.92±5.99mg/dl and creatinine was 0.27± 0.12mg/dl. Cholesterol was 175.87±77.87 mg/dl and triglyceride was 168.74±76.33 mg/dl.

Toxo-IgG was positive in 52.2% (29.43± 17.86 IU/ml) and based on the results, IgG avidity was low in 56.5%, high in 34.8%, and borderline in 8.7% with (0.38±0.31). There was a significant association between IgG level and its avidity (P<0.001), Toxo IgG avidity was 0.64±0.21 with positive IgG level, while it was 0.10±0.03 with negative IgG level. Toxo IgG avidity showed that AUC value was significantly (P<0.001) high at 0.970, 95% confidence interval ranged from 0.938 to 1.000 and specified cut-off value was 0.315.

Liver functions in positive IgG level was significantly related with direct bilirubin (5.59±2.86mg/dl) (P=0.029), AST (164.08±-95.37 U/L) (P<0.001), ALT (155.25±132.04 U/L) (P<0.001), ALP (525.50±199.46IU/L) (P=0.041), PC (90.15±29.60) (P=0.003), INR (1.15±0.46) (P=0.041) as compared to total bilirubin, total protein, albumin, GGT, PT.

Renal functions in positive Toxo IgG level were significantly related to urea (18.58±-6.77 mg/dl) (P=0.021), and comparable with creatinine. As to lipid profile, there was a significant relation with triglyceride (186.08 ±78.66mg/dl) (P=0.022) and comparable to cholesterol. There was no significant association (P<0.001) between IgG avidity & total bilirubin, direct bilirubin, AST, ALT, PT, INR, and a significant positive association between IgG avidity and PC (P<0.001), GGT (P=0.01).

Details were given in tables (1, 2, 3, 4, 5 & 6) and figure (1).

Table 1: Demographic and biochemical parameters among neonates

Studied variables		Mean	SD
Demographics	Weight/kg	4	1
	Age/day	63	42
Liver functions	Total bilirubin (mg/dl)	10.24	5.18
	Direct bilirubin (mg/dl)	6.17	2.67
	Total protein (g/dl)	5.5	0.9
	Albumin (g/dL)	3.7	0.7
	AST (U/L)	208.30	108.20
	ALT (U/L)	230.43	171.86
	ALP (IU/L)	484.00	204.07
	GGT (IU/L)	341.43	312.32
	PT (seconds)	14.03	4.55
	PC	81.83	28.55
	INR	1.26	0.54
Renal functions	Urea (mg/dl)	17.92	5.99
	Creatinine (mg/dl)	0.27	0.12
Lipid profile	Cholesterol (mg/dl)	175.87	77.87
	Triglyceride (mg/dL)	168.74	76.33

Table 2: Toxo IgG level and its avidity among neonates

Studied variables		No.	Percentage
Toxo IgG	Negative	44	47.8%
	Positive	48	52.2%
Toxo IgG avidity	Borderline	8	8.7%
	High	32	34.8%
	Low	52	56.5%
Toxo IgG level mean ± SD (IU/mL)		29.43 ± 17.86	
Toxo IgG avidity mean ± SD		0.38 ±0.31	

Table 3: Association between Toxo IgG level and its avidity

Studied variables		Toxo IgG Avidity		P-value
		Mean	SD	
Toxo IgG level	Negative	0.10	0.03	<0.001
	Positive	0.64	0.21	

Table 4: ROC analysis of Toxo IgG avidity

Studied variable	AUC	P-value	95% C.I of AUC	Cutoff value
Toxo IgG avidity	0.970	<0.001	0.938- 1.000	0.315

Table 5: Association between Toxo IgG and biochemical parameters

Studied variables		Negative		Positive		P value
		Mean	SD	Mean	SD	
Liver functions	Total bilirubin (mg/dl)	10.72	4.00	9.80	6.07	0.211
	Direct bilirubin (mg/dl)	6.80	2.33	5.59	2.86	0.029
	Total protein (g/dl)	5.4	1.0	5.5	0.8	0.321
	Albumin (g/dL)	3.8	0.8	3.6	0.4	0.745
	AST (U/L)	256.55	101.33	164.08	95.37	<0.001
	ALT (U/L)	312.45	173.94	155.25	132.04	<0.001
	ALP (IU/L)	438.73	201.57	525.50	199.46	0.041
	GGT (IU/L)	254.18	247.54	421.42	345.16	0.693
	PT (seconds)	14.84	4.61	13.28	4.42	0.720
	PC	72.75	24.61	90.15	29.60	0.003
	INR	1.38	0.60	1.15	0.46	0.041
Renal functions	Creatinine (mg/dl)	0.28	0.11	0.27	0.13	0.11
	Urea (mg/dl)	15.73	4.63	18.58	6.77	0.021
Lipid profile	Cholesterol (mg/dl)	173.09	84.23	178.42	72.16	0.09
	Triglyceride (mg/dL)	149.82	69.76	186.08	78.66	0.022

Table 6: Correlation between Toxo IgG avidity and biochemical parameters

Studied variables		Spearman correlation coefficient	95% C.I		P-value
Toxo IgG avidity	Total bilirubin	-0.311	-0.485	-0.114	<0.001
	Direct bilirubin	-0.439	-0.590	-0.257	<0.001
	Total protein	0.157	-0.049	0.350	0.245
	Albumin	-0.062	-0.263	0.145	0.169
	AST	-0.501	-0.640	-0.330	<0.001
	ALT	-0.512	-0.649	-0.343	<0.001
	ALP	0.092	-0.115	0.291	0.568
	GGT	0.304	0.106	0.479	0.01
	PT	-0.368	-0.532	-0.176	<0.001
	PC	0.427	0.243	0.581	<0.001
	INR	-0.307	-0.481	-0.109	<0.001
	Urea	-0.009	-0.213	0.196	0.721
	Creatinine	-0.125	-0.322	0.082	0.241
	Cholesterol	-0.132	-0.328	0.075	0.764
	Triglyceride	0.185	-0.021	0.375	0.119

Discussion

The detection of *T. gondii* IgM is the traditional method to identify primary infection, but pitfalls of transient or diminished IgM (Fricker-Hidalgo *et al*, 2013) or persistent remain (Meylan *et al*, 2015). *T. gondii* IgG & IgM, with IgG-positive samples subsequently tested for IgG avidity regardless of IgM results (Teimouri *et al*, 2020). The correlation between infection duration and the interaction strength between the antibody and epitope was widely reported. Flori *et al.* (2008) found tthat IgG avidity ELISA was capability to quantify the avidity of particular IgG antibodies among acute and chronic stages of toxoplasmosis. The level of IgG avidity exhibits a decrease during the acute phase and an increase in chronic phase of toxoplasmosis. Thus, identifying a low IgG avidity serves as a dependable indicator for recent occurrences of toxoplasmosis, while a high avidity signifies that the infection had transpired within the preceding 3-5 months (Candolfi *et al*, 2007).

Cholestatic jaundice is highly prevalent in children, affecting one in 2,500 live births**.** The signature characteristic of cholestatic jaundice is serum-conjugated bilirubin (Moyer *et al*, 2004). But neonatal jaundice manifestations are multifactorial including biliary atresia, neonatal hepatitis, metabolic disorders, infections, and other structural

abnormalities. An early diagnosis of diseases might reduce healthcare costs and improve patient outcomes (Ohi, 2001).

In the present study, Toxo IgG was positive in 52.2% with 29.43±17.86 IU/ml and based on Toxo IgG results avidity testing, it was low in 56.5%, high in 34.8%, and borderline in 8.7% with 0.38±0.31. There was a significant association between Toxo IgG level and its avidity (P<0.001), Toxo IgG avidity was 0.64±0.21 with positive Toxo IgG level while was 0.10±0.03 with negative Toxo IgG level. The results of ROC analysis conducted on Toxo IgG avidity showed that the AUC value was significantly (P<0.001) detected at the cutoff value of 0.315.

Erdoğan *et al*. (2019) also, conducted a retrospective study to accurately diagnose toxoplasmosis in patients, irrespective of their IgM seropositivity. The study found that 0.7% of patients had low avidity, 6.5% had equivocal avidity, and 2.6% had poor avidity. The combination of ELISA and IgG avidity testing was considered a dependable method for assessing and validating the diagnosis of toxoplasmosis, particularly in cases where only a single serum sample is accessible (Naghili *et al*, 2017).

Employing an adjusted IgG avidity index threshold of 0.11 can effectively rule out infection during pregnancy with a satisfactory level of diagnostic precision in women with an IgG avidity index over 0.11. By clinical data, modification of the threshold for IgG avidity index was to accurately differentiate between low & moderate avidity, and acute or previous infection (Skvarč, 2022). The involvement of host cell cholesterol in the entrance and proliferation of intracellular microbial pathogens has been established. Nevertheless, a novel mechanism was elucidated of which is that host cholesterol regulates the intracellular pathogen. Cholesterol is present in parasitophorous vacuolar membrane (PVM) surrounding *T. gondii*. During cell entry process into host plasma membrane, cholesterol is integrated in developing PVM during invasion, utilizing a mechanism without depending on caveolae. Reducing cholesterol levels in host cell's plasma membrane hinders parasite internalization by decreasing creation of rhoptry proteins needed for invasion (Coppens and Joiner, 2003).

In the present study, lipid profile in positive Toxo IgG level showed a significant relation with triglyceride (P=0.022) comparable with cholesterol. Coppens *et al*. (2000) showed that *T. gondii* exploits host low-density lipoprotein receptor-mediated endocytosis for cholesterol acquisition. Whereas acyl-CoA: cholesterol acyl transferase and cholesterol esters play a crucial role in the optimal replication of *T. gondii* (Sonda *et al*, 2001). Flegr *et al.* (2014) reported that cholesterol increased level in the toxoplasmosis infected man. But, Al-Kuraishi *et al*. (2013) showed that the results of the lipid profile indicated a drop in cholesterol and triglyceride levels. There was no significant difference in some lipid profile parameters and *T. gondii* (Ali and Al-Warid, 2021).

The liver showed significant and gradual deterioration due to massive proliferation caused by infection that altered its metabolism (Al-Kaissy, 2010). The enzymes in sera had a notable predisposition for elevation following infection, potentially indicating the extent of liver impairment (Al-Kaysi *et al,* 2011). Liver damage can result in metabolic alterations that reduce the production of proteins in the liver. Besides, toxoplasmosis can lead to the infiltration of round cells in the portal areas, enlarged endothelial cells, cholestasis, and localized death of the liver (Mahmood and Dawood, 2012).

No doubt, the AST and ALT sera activities is a dependent indicator of hepatocellular injury, with ALT activity exhibiting greater specificity than serum AST in evaluating liver injury (Al-Jowari and Hussein, 2014).

In the present study, liver functions in positive IgG level showed a significant relation with the direct bilirubin (P=0.029), AST (P<0.001), ALT (P<0.001), ALP (P=0.041), PC (P=0.003), INR (P=0.041) and compara-

ble results with total bilirubin, total protein, albumin, GGT, PT. Also, there was a significant negative association (P<0.001) between Toxo IgG avidity and total bilirubin, direct bilirubin, AST, ALT, PT, INR, and a significant positive association between IgG avidity and PC (P<0.001), GGT (P=0.01).

The increase in the level of ALP agreed with earlier studies (Al-Kaysi *et al,* 2010; Al-Kaissy, 2011) and could be attributed to the existence of *T. gondii* parasites within the cells of the bile duct, as evidenced by the presence of hepatic ALP in both the canalicular and luminal regions of the bile duct epithelium (Mahmood and Dawood, 2012). *Toxoplasma* was associated with an increased total protein level (Portugal *et al*, 2004). *Toxoplasma* may infect and damage the kidney with increases protein excretion in urine leading to hypoalbuminemia with low concentration of albumin and damaged liver functions (Abdelrazek *et al*, 2013). Besides, there was a significant increase in the means of ALP, AST, and ALT activities in the serum of toxoplasmosis-infected group (Al-Jowari and Hussein, 2014). But, Ustun *et al*. (2004) didn't find significant differences in the severity of liver damage between *Toxoplasma*-infected and healthy rats

In the present study, renal functions in IgG positive cases showed significant a relation with urea level (P=0.021), and a comparable result with creatinine. The elevation in urea levels could be attributed to *Toxoplasma*'s detrimental impact on renal function, resulting in reduced elimination of urea and subsequently elevation of its concentration in the bloodstream (Gharadaghi *et al*, 2012). But, others showed an increase in both creatinine and urea levels among toxoplasmosis infected pregnant women compared to controls (Al-Kaysi *et al,* 2010; Al-Jowari and Hussein, 2014).

Conclusions

The early identification of congenital disorders is crucial to allow effective treatment, contributing to prospective implementation of health management strategies in newborns and infants. Toxo IgG avidity tests in prenatal and postnatal programs can mitigate congenital toxoplasmosis prevalence ce.

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Explanation of the figure

Fig. 1: ROC curve of Toxo IgG avidity.