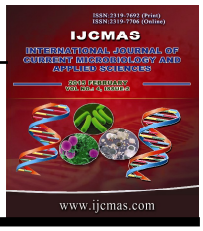
Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 314-321

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 2 (2015) pp. 314-321**
http://www.ijcmas.com



Original Research Article

Investigation of Toxoplasmosis in Cord Blood of Newborns at Al-Najaf Province, Iraq by Searching for IgG and IgM Antibodies

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Keywords

Congenital toxoplasmosis, Cord blood, Newborns

A B S T R A C T

Toxoplasmosis is a world-wide health problem that has been considered a major health hazard to pregnant women and their newborns. Three-hundred neonate (one hundred sixty- five male and one hundred thirty- five females) selected in systematic random sampling were included in our study. Samples of cord blood were taken immediately after birth from neonates delivered at Al-Zahraa teaching hospital in a period from the 1st of August 2010 to the 30th of April 2011. Blood samples were analyzed for *Toxoplasma* specific IgG and IgM by a capture ELISA. Among Three-hundred newborns, we found that one-hundred five neonates were positive for IgG (35%) and only one neonate with a positive IgM (0.33%). Congenital toxoplasmosis is not uncommon in our society, early serological diagnosis by ELISA is difficult with the use of specific IgG and IgM alone.

Introduction

Toxoplasmosis is one of the most widespread infections in animals and humans. It is caused by an intracellular obligatory parasite, *Toxoplasma gondii*. Infection is usually acquired orally or transplacentally, rarely by inoculation in a laboratory accident, by blood or leukocyte transfusion, or from a transplanted organ; multiplies only in living cells (Halonen, 2013; McLeod and Remington, 2007). Cats and other felines are considered as only known definitive hosts for this parasite (McLeod and Thulliez, 2013).

When a human ingests infected meat that contains tissue cysts, the stomach acid destroys the cyst wall and facilitates the release of bradyzoites. These transform rapidly to tachyzoites, which reside briefly in the intestinal epithelium before disseminating throughout the body (Nicole and LeSaux, 2003).

If an immunocompetent pregnant woman acquires the organism for the first time during pregnancy or an immunologically compromised pregnant woman is infected

chronically, the parasite can be transmitted to the fetus in uterus (McLeod and Thulliez, 2013). Congenital infection is least frequent but most severe when the mother acquires and transmits infection in the first trimester and most frequent but with fewer clinical signs at birth when it is acquired in the third one (Fabiani *et al.,* 2013).

Pregnant women with acute acquired infection do not experience obvious symptoms or signs A minority may experience malaise, low-grade fever, and lymphadenopathy (McLeod and Thuilliez, 2005; Boyer *et al.,* 2005).

Manifestations of congenital infection are protean, varying from a mild or asymptomatic infection to a generalized infection dominated by signs of irreversible CNS damage (Halonen, 2013). From 25% to >50% of infants with clinically apparent disease at birth are born prematurely. Intrauterine growth retardation, low Apgar score and temperature instability are common.

Neurologic abnormalities have ranged from subtle findings to severe encephalitis. Hydrocephalus may be the only clinical manifestation of congenital toxoplasmosis; Chorio-retinitis is usually a sequelae of infection acquired in uterus (Helieh, 2014; Torgerson and Mastroiacovo, 2013). Sensorineural hearing loss, both mild and severe, may occur (McLeod and Remington, 2007).

The clinical diagnosis of toxoplasmosis should be considered doubtful unless supported by appropriate laboratory test results (Hsu *et al.,* 2014). The diagnosis of toxoplasmosis most often rests on serologic confirmation.

The Sabin-Feldman dye test, traditionally the reference test against which newer methods are compared, requires the use of live parasites. Most laboratories have abandoned the dye test in favor of simpler techniques that use killed antigens, such as the IFA, ELISA, agglutination, and indirect hemagglutination (IHA) tests (White *et al.*, 2014; Remington *et al.*, 2004).

Tests to detect IgM antibodies to T. gondii include the IgM IFA; double-sandwich enzyme-linked immunosorbent technique (DS-IgM ELISA); and IgM ISAGA (an agglutination test) (Remington *et al.,* 2004).

Because IgM antibodies usually appear within the first weeks of infection and disappear more rapidly than IgG antibodies, they are used to detect acute infection, it does not cross the placenta, so if it detected in fetal or neonatal blood samples represent synthesis by an infected fetus or infant (Halonen, 2013).

IgG titers peak at about 2 months, gradually drop thereafter, but remain detectable for years. A single high specific IgG titer is considered only suggestive of an acute infection (Munoz *et al.,* 2011).

IgM serological testing appears to be less sensitive for detecting infections acquired in the first trimester, but, early infection is frequently associated with clinically apparent disease and most cases are diagnosed on the basis of clinical findings (Yarovinsky, 2014). Further, an elevated anti-toxoplasma IgG antibody titre in the newborn may be of either maternal or fetal origin, i.e. it may reflect either an active congenital toxoplasmosis or simply the passively transferred antibodies of a maternal toxoplasmosis (Murat *et al.,* 2013).

Treating a child with congenital toxoplasmosis does not reverse CNS

damage but does markedly decrease late sequelae. A year of treatment is recommended for all congenitally affected infant; Pyrimethamine plus sulfadiazine act synergistically against *T. gondii*, these antimicrobial agents (plus leucovorin) currently constitute the standard treatment for toxoplasmosis (Helieh, 2014).

It has been reported that about one billion people worldwide are predicted to harbor Toxoplasma infection frequently with unknown lifelong health consequences (Halonen, 2013). The aim of the current study is estimation of the infection in newborns as the searching of this parasite in newborns is highly important in order to obtain an exact data about the occurrence of congenital toxoplasmosis besides to prediction of future spread of the parasite in our community.

Patients and Methods

Three-hundred newborns (one hundred sixty- five male and one hundred thirty- five females) selected in systematic random method to be included in our study. This study was performed on whom newborns delivered at Al-Zahraa teaching hospital in a period from the 1st of August 2010 to the 30th of April 2011. We describe the aim of our study and oral permission from mothers or other family members was obtained. Full maternal history was taken including residency, animal contact, history of (fever and/or lymphadenopathy) during pregnancy, history of toxoplasmosis, immune suppressive condition, mode of delivery. We also include history of abortion, intrauterine death or congenital anomalies in the previous pregnancies. Those mothers who show history of treatment against toxoplasmosis (were serologically positive for toxoplasmosis), with treatment given during pregnancy some for the 1st trimester only and others for whole pregnancy, but all with lack of serial follow up by their doctors (by history).

While regarding neonates, we took sex, gestational age, birth weight and presence of obvious clinical signs that are suggestive of congenital toxoplasmosis on physical examination, these are intrauterine growth retardation, hemorrhagic skin rash, hepatosplenomegaly, unexplained respiratory distress, generalized edema, neurologic abnormalities such as hydrocephaly.

Samples of cord blood were taken immediately after birth, were centrifuged (to obtain sera) and kept in deep freeze (at -20 C°) in the hospital laboratory till their use. A large number of samples were discarded as they were very small or hemolysed. We transport samples in freezed state to the outside laboratory (because of un availability of ELISA kits for toxoplasmosis). We use ELISA test for specific toxoplasma IgG and IgM by using commercial kits of Human Gesselschaft for Biochemical and Diagnostic mbH company - Germany. The human toxoplasma IgG and IgM ELISA is based on the classical technique in diagnosis. The absorbance of controls and specimens is determined by using automated ELISA system. The intensity of the color is directly proportional to the specific IgG and IgM concentration in the specimen.

Results of patients samples are obtained either by comparison with cut-off control in IU/ml or by quantitative estimation using a calibration curve. According to our procedure and kits used, cut-off values for toxoplasma IgM antibodies= 0.3 IU/ml, > 0.3 is considered to be positive. Cut-off value for toxoplasma IgG antibodies =5.5 IU/ml, value > 5.5 is considered to be positive.

Statistical analysis: We use SPSS version 11 with the use of Chi square (x^2) to determine the difference between categorical data in our cross sectional study and we set p-value < 0.05 to be statistically significant.

Result and Discussion

During the study period of nine months, total number of neonates included was 300 newborns, we found that 105 newborns (35%) were positive for IgG and only 1 neonate (0.33) was positive for IgM. Statistical analysis was performed to determine the significance of difference between our categorical data in presence of positive toxoplasma IgG antibody as following:

The neonate with a positive IgM was full term male of normal birth weight, who was a product of normal delivery of poorly educated mother that reside in rural area. The neonate had presented to us with hy a low APGAR score, admitted to the NCU with severe respiratory distress, receive assisted ventilation, unfortunately he died after few hours. When we deal with mothers and their families, we found that there is poor community education about the disease with its effect on neonates, risks of transmission, preventive measures and lack of serial follow up by their doctors to those with suspected infection during pregnancy.

Positive IgG are more reported among newborns that are male, term, of low birth weight, product of cesarean section delivery and in those with clinically suspicious signs of toxoplasmosis. There was also more reported history of abortions, intrauterine death, congenital anomalies, maternal toxoplasmosis (previous or recent pregnancy), diseases (fever and flu-like illness during pregnancy) and more with history of contact with domestic animals (especially cats).

Toxoplasmosis is found in every country and sero-positivity rates range from less than 10% to over 90% in adult population, congenital infection cause serious health effects and occur in the infants as a result of maternal transmission (Helieh, 2014).

In the present study, out of 300 newborns, we found 105 neonates (35%) was a positive for toxoplasma specific IgG antibodies, and one neonate (0.33%) was positive for IgM antibodies.

A Study in Egypt by El-Ghandoor *et al.* (2000), reveal the presence of toxoplasma-specific IgG antibodies in 19 (9.5%), and a positive IgM in 7 newborns (3.5%) in cord blood of 200 cases examined with ELISA.

In Brazil, Gesmar *et al.* (2006), used ELISA to detect IgG antibodies to *Toxoplasma gondii* and positive sera were re-tested to verify specific IgM and IgA antibodies in a capture ELISA. Seroprevalence of IgG antibodies against *T. gondii* was 51.6 per cent in the hospitals, while the frequency of congenital toxoplasmosis was 0.5 per cent, with specific IgM and/or IgA antibodies.

In the United States and Europe, the incidence of congenital toxoplasmosis is estimated to be 1–5 neonates per 10,000 live births (0.01-0.05%), with an estimated 400–4000 newborns yearly in the United States (Schmidt *et al.,* 2006). Data from IgM screening in Massachusetts revealed an incidence of congenital toxoplasmosis ≈1 newborn per 10,000 live births (Schmidt *et al.,* 2006). The regional New England program detects Toxoplasma-specific IgM in filter paper eluates at birth. Fifty of 635,000 neonates were diagnosed as being congenitally infected (1 of 10,000), although

they appeared healthy on routine pediatric examination after birth (Schmidt *et al.,* 2006).

In Southern America**,** in Quindio, Community Hospital, Colombia, screening test on 322 newborns with IgM ELISA at 2007 showed prevalence of 0.62% (Gomez-Marin *et al.,* 2007).

A retrospective study in Austalia from1998-2008 reveals that the incidence of congenital toxoplasmosis is 1/10000 (Prusa *et al.,* 2014). While in Marche region (Italy), an epidemiological study, show that the incidence of congenital toxoplasmosis is 0.01% (Ruffini *et al.,* 2014).

Statistical analysis of this study show that the seropositivity of toxoplasma IgG is 35%, which is higher than that reported in Egypt but lower than the study of Brazil.

While it is 0.33% for IgM antibodies, which is less than studies of Egypt, Brazil and South America but more than those of other studies of United states, Austalia, Italy and Europe. This show that the increasing seropositivity of toxoplasma IgG in societies are associated with lower incidence of congenital toxoplasmosis.

There is a significant correlation between positivity of Toxoplasma IgG antibodies and past history of abortion, congenital anomalies, history of animal contact, residency in rural area, fever or flu- like illness during pregnancy, history of toxoplasmosis (past or recent), normal birth weight and suggestive clinical presentation.

On the other hand, it shows no significant correlation with neonatal gender, immunological status of the mother, and maturity. Regarding toxoplasma specific IgM, there is significant correlation only with history of fever and flu-like illness in third trimester, but it is insignificant for all other parameters.

In comparison to other studies, Mostafavi *et al.* (2011) in Iran were found no difference between urban and rural places of residence in their study.

In Egypt, El-Ghandoor *et al.* (2000); study similarly revealed significant relation between (IgG and IgM antibodies) and parameters including contact of mothers with cats, their illness during pregnancy (fever, lymphadenopathy), previous abortions, and the occurrence of congenital anomalies in the newborns, but no significant association was found on studying other parameters, e.g, primigravida or multigravida, and rural or urban residence.

Results were also obtained by Lopez *et al.* (2014); in Portugal, where they reported that active toxoplasmosis was correlated with history of persistent lymphadenitis in pregnant mothers with elevated specific IgM, IgA and IgE antibodies in their newborn.

In the present study, 54.7% (40/73) of those with abnormalities in the forms of microcephaly, hydrocephaly, organomegaly, hypotonia, SGA, low Apgar score, hemorrhagic skin rash and generalized edema, were positive for toxoplasma specific IgG.

Nevertheless, detection of IgM antibodies was not a decisive mean for identifying infected newborns and for determination of the exact date of acquiring the infection (Abdul-Fattah *et al.,* 2010). Present serological methods differentiate poorly between acute and chronic toxoplasmosis in pregnant women (Borna *et al.,* 2013).

The following recommendations concluded for this study:

1. Congenital toxoplasmosis is not uncommon in our society.
2. There are poor preventive measures with lack of education and paucity of serial follow up (to high risk pregnancies).
3. Diagnosis of congenital toxoplasmosis by IgG and IgM by ELISA alone is difficult.
4. Poor compliance is another obstacle when follow up is required.

Table.1 The relation between positive IgG Antibody and neonatal clinical criteria

Clinical criteria			**+ve IgG**	**-ve IgG**	**p-value**
		NO.(%)	**NO. 105 (%)**	**NO.195 (%)**	
Sex	MALE	165 (55%)	62 (37.5%)	103 (62.4%)	0.3010
	FEMALE	135 (45%)	43 (31.8 %)	92 (68.14%)	
Presentation	Obvious suspicious clinical signs	73 (24.33%)	40 (54.79%)	33 (45.2 %)	0.0001*
	No obvious clinical signs	227 (75.66%)	65 (28.63%)	162 (71.3%)	
Gestational age	PRETERM	86 (28.6%)	29 (33.72%)	57 (66.27%)	0.2980
	TERM	214 (71.3%)	86 (40.18%)	128 (59.81%)	
Birth wt	NBW	242 (80.6%)	82 (33.88%)	160 (66.11%)	0.0460*
	LBW	40 (13.3%)	21 (52.5 %)	19 (47.5 %)	
	VLBW	15 (5%)	2 (13.3%)	1 13 (86.66 %)	
	ELBW	3 (1%)	0 (0%)	3 (100%)	

Table.2 The relation between maternal history and positive IgG result

Parameters No. (%)			IgG		p-value
			+Ve 105 (%)	-Ve 195 (%)	
Residence	Rural	84 (28 %)	21 (25%)	63 (75 %)	0.0240*
	Urban	216 (72 %)	84 (38.88%)	132 (61.11%)	
History of Abortion	Yes	88 (29.3%)	48 (54.54 %)	40 (45.45%)	0.0003*
	No	212 (70.6%)	57 (26.88 %)	155 (73.11%)	
History of IUD	Yes	21 (7%)	11 (52.38%)	10 (47.61 %)	0.0830
	No	279 (93%)	94 (33.6%)	185 (66.30 %)	
History of Congenital anomaly	Yes	45 (15%)	29 (64.44%)	16 (35.55 %)	0.0001*
	No	255 (85%)	76 (29.80%)	179 (70.19%)	
Contact with animals	Yes	166(55. 3%)	98 (59.03%)	68 (40.96 %)	0.0005*
	No	134(44. 6%)	7 (5.22%)	127 (94.77 %)	
Past History of toxoplasmosis (previous pregnancy)	Yes	24 (8 %)	15 (62.5 %)	9 (37.5 %)	0.0030*
	No	276 (92%)	90 (32.60 %)	186 (67.39 %)	
Recent history of toxoplasmosis (recent pregnancy)	Yes	5 (1. 66%)	5 (100 %)	0 (0%)	0.0022*
	No	295(98.3%)	100 (33.89%)	195 (66.10 %)	
Fever or LAP during 1st trimester of pregnancy	Yes	20 (6. 6 %)	16 (80 %)	4 (20%)	0.0002*
	No	280 (93. 3%)	89 (31.78%)	191 (68.21%)	
Fever or LAP during 3rd trimester of pregnancy	Yes	7 (2. 33%)	4 (57.14 %)	3 (42.85%)	0.0004*
	No	293 (97. 6%)	101 (34.47%)	192 (65.52 %)	
Mode of Delivery	NVD	197 (65. 6 %)	67 (34.01%)	130 (65.98%)	0.0420*
	CS	103 (34. 3%)	38 (36.89%)	65 (63.10%)	

* Significant at level of P □ 0.05

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