Cord Blood Screening for Congenital Infectious Diseases and Haematological Change in Sulaimani Provence

Sirwan Muhammed Ameen¹, Chinar Hamed Sadiq¹, Samira Mohammad¹
¹Science and Education Sciences, School of Science, Department of Biology, University of Sulaimani, Iraq
²Corresponding author: Science and Education Sciences, School of Science, Department of Biology
Email: sirwan.muhammed@univsul.net

ABSTRACT

Toxoplasma gondii specific IgA, IgG, and Helicobacter pylori specific IgG antibodies were assessed by Chorus enzyme immune assay. Nineteen out of 70 (27.1%) cord blood serum samples were found positive for anti-T. gondii IgG antibody and there was only one (1.4%) positive for IgA. Regarding the detection of specific anti-H. pylori IgG, thirty cord blood samples were tested in which 26 (86.6 %) were found positive and 4 (13.4%) negative. The positive mean with H. pylori infection was significantly greater than H. pylori-negative mean (88.37 ± 53.69 and 5.8 ± 0.46, respectively, t value = 3.03, P = 0.005). The comparison of hematological profiles between positive and negative cord blood samples (IgG ≥ 8 and IgG < 4; IgA >1.2 and IgA < 0.8) showed no statistically significant variations in higher and lower value of IgG and IgA titration (P > 0.05). The Results revealed that all the cord blood serum samples were negative for IgA antibodies except that 1 of 70 (1.4%), indicate that all these newborn infants were rarely infected with congenital toxoplasmosis. Neonates born from H. pylori–infected mother, are provided with a great amount of specific IgG H. Pylori antibodies, which are transferred transplacentally. The CBC test shows the non-significantly effect of T. gonidii and H. pylori on most of the hematological parameters.

Keywords: Cord blood, Congenital Toxoplasmosis, H. pylori, Haematology

I. INTRODUCTION

Maternal infection during pregnancy still embodies an elusive zone in teratology. It is known that many infections can be transferred from mother to fetus across the placenta during fetal development, and umbilical cord of the newborn infant is a particularly common portal of entry for systemic infection. Certain pathogens including T. gondii that causes Toxoplasmosis can have adverse consequences for the fetus if they cross the placenta (e.g. miscarriage, stillbirth or severe complication for baby). (Wang and Yin 2014). It is among the most prevalent of human infections that affects an estimated to be 30–50% of the world population (Flegr et al. 2014). Congenital toxoplasmosis (CT) occurs only when a woman becomes infected with T. gondii during pregnancy and it can be asymptomatic or can have more serious maternal and neonatal consequences (Bollani et al. 2013). The risk of mother to child transmission of the parasite increases steeply with gestational age (GA) at maternal infection, with a probability of 10–25% in the first trimester of pregnancy, 30–45% in the second trimester, 60–65% in the third trimester, and up to 80% before childbirth (Capobianco et al. 2014). Infections in the pregnant women or infants born with congenital toxoplasma can be diagnosed by serological screening for toxoplasma antibodies (Aptouraman et al. 2012), if synthesis of specific anti-Toxoplasma antibodies (IgA, IgM, and/or IgG) was proven at birth or during the first year of life, and/ or if specific antibodies were still present after the age of 12 months (Faucher 2011). The results of previous studies suggest that the mothers play a crucial role in transmitting H. pylori infection to their children (Konno 2005; Escobar and Kawakami 2004). H. pylori recognized as the main etiological agent in a variety of gastroduodenal disorders, including chronic gastritis and peptic ulcer disease. The route of H. pylori transmission is not completely clarified. This microorganism commonly colonizes the stomach...
Previously intrafamilial clustering has been documented (Goodman and Correa 2000; Tindberg et al. 2001). In intrafamilial H. pylori infections, the infected parents, mainly the infected mothers, usually have been considered the possible source of transmission (Rothenbacher et al. 2002). Since then, several reasonable automated hematology analyzers have recently been developed for in-clinic usage. As complete blood count (CBC) is a typical hematological test that mostly used to inspect information from erythrocytes, leukocytes, and platelets indicating infections and disorders in the body. Several studies have demonstrated the H. pylori infection and Toxoplasmosis can alter haematological parameter (Saqib et al. 2015; Zaberi et al. 2007; Javadi et al. 2010).

The objective of the current study was to determine the seroprevalence of IgA and IgG anti-Toxoplasma, anti-H. pylori antibodies in cord blood serum and to access the usefulness of haematological parameters in diagnosing of toxoplasmosis and H. pylori infection.

II. METHODS AND MATERIAL

A. Ethics Statement

This study was approved by the ethics committee of the Maternity Hospital of Sulaimani. Informed consent was obtained from each women and personal data regarding demographic characteristics.

B. Collection of Samples

Between February and April 2015 cord blood samples of 70 women were collected at birth in the obstetric units of Maternity Hospital of Sulaimani. After delivery, blood samples were collected from cord vessels representing the existence of T. gondii and H. pylori antibodies in baby’s circulation. Serum samples were obtained by centrifugation at 2000 rpm for 10 min of clotted blood, stored under -20 °C until utilized.

C. T. Gondii and H. Pylori Antibodies in Serum

The anti-T. gondii IgG, IgA and anti-H. pylori IgG were detected by a Chorus enzyme immune assay (Chorus Elisa system, Disease Diag., Siena, Italy), which had been previously validated for Iranian, Turkey and Korean populations (Rocha et al. 1998; Oliveira et al. 1999; Lee et al. 2015). The assays were performed according to manufacturer’s instructions. The cut-off for both Toxoplasma and H. pylori-specific IgG test positive corresponds to ≥ 8 IU/ml, equivocal corresponds to ≥4 to <8 IU/ml and negative corresponds to <4 IU/ml. Anti-Toxoplasma-specific IgA were considered positive when the index was > 1.2 IU/ml, the result was equivocal when index was from 0.8-1.2 IU/ml and the test was regarded negative if the index was <0.8 IU/ml, according to the manufacturer’s instructions. Equivocal samples were retested.

D. Haematological Values

At birth 2 ml of Cord blood were collected in EDTA tubes from pregnant women in Maternity Hospital of Sulaimani City and complete blood count (CBC) tests performed by Orphée Mythic 18 hematology analyzer (SA, Switzerland) calibrated daily with standards provided by the manufacturer. It analyzes 18 parameters, leukocyte, and erythrocyte and thrombocyte parameters.

E. Statistical Analysis

Statistical significance for differences between categorical data calculated by pairwise two-tailed t-test and chi-square (χ²) using GraphPad Prism 6 (Software MacKiev, USA). Statistical significance was considered at \( P<0.05 \).

III. RESULTS AND DISCUSSION

To the best of our knowledge, this is the first study in Sulaimani to report the detections of anti-toxoplasma IgA and anti-H. pylori IgG in cord blood sera. Most congenitally infected newborn infants do not show any clinical symptoms of infection at birth. Diagnosis of congenital infections in newborns is, therefore, relying on biological investigations.

During delivery, 70 cord blood samples were collected from the participating women between February and April 2015 at birth in the obstetric units of Maternity Hospital of Sulaimani (Figure 1). The pregnant women’s mean age was 29±5.6 years old (range 16 to 40 years...
old). Distribution by age was as follows: 4.28% pregnant adolescents (16-18 years old), 80% pregnant adults (19-35 years old) and 15.71% older pregnant females (>35 years old).

**Figure 1:** Number of participants in each age group

A. Frequency of specific anti-*T. gondii* IgG and IgA antibodies in cord blood samples.

Nineteen out of 70 (27.1%) cord blood serum samples were found positive for anti-*T. gondii* IgG antibody and there was only one (1.4%) positive for IgA. There was a significant positive correlation between IgG and IgA amongst the cord blood samples (r= 0.63, P= 0.0001). No statistically significant difference was detected amongst different maternal age groups (P<0.05), the highest infection rate in observed in age groups 21-25 years old (Figure 2). IgG present in cord blood samples indicates maternal exposure to toxoplasmosis during her lifetime. Moreover, the presence of IgA in cord blood reflects CT. In comparison to previous studies, the prevalence IgG found in this study was lower than the prevalence found in 1998 in Bogotá (43%) and that reported in Bosa (45%) (Gomez-Marín et al. 1997). On the other hand, our results agree with previously reported in a study conducted in Bogota, Colombia (28.2%) (Angel-Mül ler et al. 2014). In 1999, Robert-Gangneux and his colleagues used immunofluorescence assay and ELISA to determine specific IgG in cord blood sera. The serological tests identified specific IgG in 54 of 57 uninfected newborns and 18 of 20 infected newborns with CT (Robert-Gangneux et al. 1999). An Iraqi study performed by Al-harís et al. showed that 105 neonates were positive for IgG (35%) and only one neonate with a positive IgM (0.33%) (Al-harís et al. 2015). Detection of specific anti-*Toxoplasma* IgG and IgM antibodies in cord blood is not quite satisfactory for early diagnosis of CT. Many studies have been published on this topic. Most of these studies confirmed the superiority of IgA testing compared to IgM testing (Bessières et al. 1992; Decoster et al. 1992; Decoster et al. 1991). In a similar study conducted in Lyon, France, on 41 congenital toxoplasmosis infants, 38 had IgA positive reaction at the 1: 20 dilution, 30 had IgA positive reaction at the 1:100 dilution. Among the 155 infants without congenital toxoplasmosis, nine had IgA at the 1:20 dilution and two had IgA at the 1: 100 dilutions (Gandilhon et al. 1994). Wallon et al. identified five infants with Toxoplasma-specific IgA but not IgM in a group of 89 Toxoplasma-infected infants (Wallon et al. 1999). Stepick-Biek and his collaborators observed that anti-Toxoplasma IgA antibodies were demonstrable in 8 of 9 infants/fetuses with CT. In certain, IgM antibodies could not be demonstrated (Stepick-Biek et al. 1990). In studies which have evaluated the two assays (IgM and IgA) in parallel, IgA assay has been considered to be the more sensitive (Patel et al. 1993; Decoster et al. 1992; Stepick-Biek et al. 1990).

**Figure 2:** Frequency of anti-*T. gondii* IgG and IgA antibodies in cord blood by maternal age group.

B. Frequency of specific anti-*H. pylori* antibodies in cord blood samples.

Thirty cord blood samples were tested. Maternal-specific anti-*H. pylori* IgG were detected in the cord-blood samples of 26 (86.6 %) newborns (Figure 3). The positive mean with *H. pylori* infection was significantly greater than *H. pylori*-negative mean (88.37 ± 53.69 and 5.8 ± 0.46, respectively, t value = 3.03, P = 0.005). Ashorn et al. (Ashorn et al. 1996) reported that anti-*H. pylori* IgG were detected in the cord-blood samples of
21 children (10-6%). Kuo et al. (2014) have reported similar results, who showed that only 89 (84.7%) out of 105 *H. pylori*-infected mothers had positive cord serum antibody detection from their babies (Kuo et al. 2014). It is well-known that specific anti-*H. pylori* IgG antibodies are transplacentally transported from mothers to fetuses (Blecker et al. 1994) and a close relationship between maternal and cord specific IgG levels were established (Kitagawa et al. 2001; Bunn et al. 2003).

C. Cord Blood Haematology

The comparison of hematological measurements between two groups of cord blood samples (IgG ≥ 8 and IgG < 4; IgA > 1.2 and IgA < 0.8) showed no statistically significant variations in higher and lower value of IgG and IgA titration (P > 0.05). The CBC test shows the non-significantly effect of *T. gonidii* on most of the hematological parameters. However, no significant up and down in both monocytes and PCT% parameters have found. These results agree with the tests made on cats by (Javadi et al. 2010). As they also found that most of the blood parameters have not significantly changed except the PCV, RBC and monocyte that were significantly high in cats with IgM ≥1/64. During the research, we found that there is no significant relation between CBC and *H. pylori* infection. As in (Saqib et al. 2010) results, they observed that *H. pylori* do not affect hemoglobin and MCV significantly. In contrast to our study Zuberi et al. (2007) noticed the significantly low level of hemoglobin in male and non-pregnant female patients with *H. pylori*. (Zuberi et al. 2007), also found significantly low levels of hemoglobin, ferritin and vitamin B12 in patients with *H. pylori* infection. Moreover, our result may be due to oral supplementation of Folic acid and Iron during pregnancy period.

![Figure 3: Percentage of cord blood samples tested positive for IgG. The number above each bar indicates the number of positive samples/number of tested samples.](image)

Table 1: Statistical data and serological results of the two groups of the 30 cord blood based on IgG titration (IgG ≥ 8, n=26; IgG<4, n=4)

|        | H. pylori IgG (IU/ml) | WBC $10^9$/ml | Lym $10^9$/ml | Mon $10^9$/ml | Gran $10^9$/ml | Lym % | Mon % | Gran % | RBC $10^9$/ml | HGB g/dl | HCT % | MCV $10^3$/μl | MCH pg | MCHC g/dl | RDW % | PLT $10^3$/μl | MPV μm | PCT % | PDW % |
|--------|-----------------------|----------------|----------------|---------------|---------------|-------|-------|-------|--------------|---------|-------|----------------|-------|------------|-------|-------------|-------|-------|-------|
| ≥ 8    | Mean                  | 88.37          | 11.24          | 4.05          | 1.38          | 5.83  | 36.5  | 12.3  | 51.0 9       | 4.24    | 14.1  | 44.9          | 4      | 106.06     | 33.41 | 31.52       | 15.2           | 31.8 | 7        | 93.78 | 0.5 | 6 | 12.0 7 |
|       | SD                    | 53.69          | 3.57           | 1.25          | 0.43          | 2.42  | 6.91  | 3.0   | 8.15          | 0.83    | 2.70  | 8.89          | 7.34   | 2.51       | 1.57  | 3.78        | 19.99          | 0.1 | 2 | 0.0 1 6 | 2.64 |
|       | SEM                   | 10.53          | 0.76           | 0.26          | 0.09          | 0.51  | 1.47  | 0.63  | 1.73          | 0.17    | 0.57  | 1.89          | 1.56   | 0.53       | 0.33  | 0.8         | 19.99          | 0.1 | 1 | 0.56 |
| <4     | Mean                  | 5.8            | 13.52          | 4.97          | 1.7           | 6.82  | 36.8  | 12.5  | 50.6          | 4.14    | 13.9  | 44.7          | 5      | 107.92     | 33.57 | 31.15       | 13.4 5         | 240.5 | 7.6 | 0.8 53 | 13.5 5 |
|       | SD                    | 0.46           | 2.06           | 3.57           | 2.24          | 0.37  | 2.33  | 11.7  | 2.4           | 13.67   | 0.08  | 0.29          | 2.41   | 5.07       | 0.34  | 1.22        | 2.2 8          | 0.8 | 53 | 0.0 32 | 1.44 |
|       | SEM                   | 0.09           | 1.03           | 0.76           | 1.12          | 0.18  | 1.16  | 5.85  | 1.2           | 6.83    | 0.04  | 0.14          | 1.2    | 2.53       | 0.17  | 0.61        | 1.1 4          | 0.4 | 2 | 0.0 1 72 | 0.72 |
Table 2: Statistical data and serological results of the two groups of the 70 cord blood based on IgG titration (IgG ≥ 8, n=19; IgG<4, n=51).

|          | IgG≥8 | IgG<4 |
|----------|-------|-------|
| T. gondii IgG (1IU/ml) | 69.75 | 49.46 |
| WBC 10³/µl | 12.26 | 4.02  |
| Lym 10³/µl | 4.39  | 0.97  |
| Mon 10³/µl | 1.45  | 0.34  |
| Grm 10³/µl | 6.5   | 3.06  |
| Lym % | 37.0 | 6.42 |
| Mon % | 12.1 | 4.37 |
| Grm % | 8.5  | 4.56 |
| RBC 10⁶/µl | 50.7 | 1.44 |
| HGB g/dl | 14.5 | 5.35 |
| HCT % | 45.6 | 27.96|
| MCV μm³ | 98.63| 33.48|
| MCH pg | 32.01 | 32.01 |
| ED W % | 15.4 | 4.28 |
| PL T 10⁵/µl | 6 | 239 |
| MP V μm² | 7.4 | 0.4 |
| PC T % | 0.1 | 1.3 |
| PDW % | 9.1 | 0.0 |

IV. CONCLUSION

In summary, this study revealed that the most of cord blood serum samples exhibited negative IgA level were indicated that is infrequent acute infection with T. gondii, while anti-T. gondii IgG were found in 27.1% serum samples of neonates born could be from maternal origin. We also conclude that neonates born from H. pylori–infected mother are provided with a great quantity of specific IgG H. Pylori antibodies, which are transported transplacentally. Regarding haematological parameters, all the values were within the normal limit and, therefore, we believed that unhelpful in this situation.

V. REFERENCES

[1] Flegr, J., Prandota, J., Sovičková, M., and Israili, Z.H. (2014). Toxoplasmosis a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. PLoS One. 9(3):e90203
[2] Escobar, M.L., and Kawakami, E. (2004). Evidence of mother-child transmission of Helicobacter Pylori infection. Arq Gastroenterol. 41(4):239-44.
[3] Konno, M., Fujii, N., Yokota, S., et al. (2005). Five-year follow up study of mother -to-child transmission of Helicobacter pylori infection detected by a randomamplified polymorphic DNA fingerprinting method. J Clin Microbiol. 43(5):2246-50.
[4] Wang, Y., and Yin, H. (2015). Reseach progress on surface antigen 1 (SAG1) of Toxoplasma gondii. Parasit Vector.7:180.
[5] Bollani, L., Strocchio, L., and Stronati, M. (2013). Congenital toxoplasmosis. Early Human Development. 89,S4: S70–S71.
[6] Faucher, B., Garcia-Meric, P., Franck, J., et al.(2012). Long-term ocular outcome in congenital toxoplasmosis: a prospective cohort of treated children. J Infect.64 (1):104-9.
[7] Capobiango, JD., Breganó, RM., Navarro, IT., et al.(2014).Congenital toxoplasmosis in a reference of Paraná, Southern Brazil. Braz J Infect Dis.18(4):364-71.
[8] Aptoarumani, M., Theodoridou,M., Syrogianopoulos, G., et al.(2012). A dedicated surveillance network for congenital toxoplasmosis in Greece, 2006-2009: assessment of the results. BMC Public Health.12:1019
[9] Goodman, K J., and Correa, P. (2000). Transmission of Helicobacter pyloril among siblings. Lancet. ;355:358-62.
[10] Tindberg, Y., Bengtsson, C., Bergström, M., and Granström, M. (2001). The accuracy of serologic diagnosis of Helicobacter pylori infection in school-aged children of mixed ethnicity. Helicobacter. 6(1):24-30.
[11] Gómez-Marín, J.E., Montoya-de-Londono, MT., and Castano-Osorio, J.C. (1997).A maternal screening program for congenital toxoplasmosis in Quindio, Colombia and application of mathematical models to estimate incidences using age-stratified data. Am J Trop Med Hyg.57:180-6
[12] Angel-Müller, E., Paula Houghton, M., Eslava, C., and Brenner, H. (2002). Role of infected parents in transmission of Helicobacter pylori to their children. Pediatr. Infect. Dis. J. 21:674-679.
[13] Al-haris, F.M., Saheb, H.S., and Abdul-Sada, KM. (2015). Investigation of Toxoplasmosis in Cord Blood of
Newborns at Al-Najaf Province, Iraq by Searching for IgG and IgM Antibodies. Int.J.Curr.Microbiol.App.Sci. 4(2): 314-321.

[15] Bessières, MH., Roques, C., Berrebi, A., et al. (1992). IgA antibody response during acquired and congenital toxoplasmosis. J clin Pathol. 45(7):605-8.

[16] Decoster, A., Darcy, F., Caron, A., et al. (1992). Anti-P30 IgA antibodies as prenatal markers of congenital toxoplasma infection. Clin Exp Immunol. 87(2):310-5.

[17] Decoster, A., Slizewicz, B., Simon, J., et al. (1991). Platelia-Toxo IgA, a new kit for early diagnosis of congenital toxoplasmosis by detection of anti-P30 immunoglobulin A antibodies. J Clin Microbiol.29(10):2291-5.

[18] Gandilhon, F., Peyle, L., Wallon, M., Peyron, F., and Mojon, M. (1994). Value of specific IgA assay on cord blood for the diagnosis of congenital toxoplasmosis. Serodiagn Immunoth Infect Dis. 6:17-20.

[19] Wallon, M., Dunn, D., Slimani, D., et al. (1999). Diagnosis of congenital toxoplasmosis at birth: what is the value of testing for IgM Aand IgA? Eur J Pediatr.156(8): 645-9.

[20] Stepick-Biek, P., Thulliez, P., Araujo, FG., and Remington, JS. (1990). IgA antibodies for diagnosis of acute congenital and acquired toxoplasmosis. J Infect Dis.162(1):270-3.

[21] Patel, B., Young, Y., Duffy, K., et al. (1993). Immunoglobulin-A detection and the investigation of clinical toxoplasmosis. J Med Microbiol. 38(4):286-92.

[22] Ashorn, M., Miettinen, A., Ruuska,T., Laippala, P., and Mäki, M. (1996). Seroepidemiological study of Helicobacter pylori infection in infancy. Arch Dis Child Fetal Neonatal Ed.74(2):F141-2.

[23] Kuo, F.C., Wu, CY., Kuo, CH., et al. (2014). The utilization of a new immunochromatographic test in detection of Helicobacter pylori antibody from maternal and umbilical cord serum. Biomed Res Int. doi: 10.1155/2014/568410.

[24] Blecker, U., Lanciers, S., Keppens, E., and Vandenplas, Y. (1994). Evolution of Helicobacter pylori positivity in infants born from positive mothers. J Pediatr Gastroenterol Nutr; 19(1): 87-90.

[25] Kitagawa, M., Natori, M., Katoh, M. et al. (2001). Maternal transmission of Helicobacter pylori in the perinatal period. J Obstet Gynaecol Res. 27(4): 225-230.

[26] Bunn, J.E., Thomas, J.E., Harding, M., Coward, WA., and Weaver, LT., (2003). Placental acquisition of maternal specific IgG and Helicobacter pylori colonization in infancy. Helicobacter. 8(5): 568-572.

[27] Javadi, S., Asri Rezaei, S., Tajik, H., Hadian, M., and Shokouhi, F. (2010) Haematological changes of cats with Toxoplasma gondii-specific antibodies'. Comp Clin Pathol. 19:307–310.

[28] Saqib, S., Hamid, J Q., and Sajid, N. (2010). Effect of Helicobacter Pylori Infection on Hemoglobin, MCV, and Vitamin B12’ Pakistan Journal of Medical and Health Sciences. 4(3):266-9.

[29] Zuberi, B.F., Afsar, S., Dadeer, R., et al. (2007).Hemoglobin, Ferritin , Vitamin B12 and Helicobacter pylori infection: a study in patients who underwent upper gi endoscopy at civil hospital Karachi’. J Coll Physicians Surg Pak. 17 (9):546-9.