**Introduction**

Since the successful transplantation of umbilical cord blood from a newborn girl to her 5-year-old brother with Fanconi’s anemia at the St. Louis Hospital in Paris in 1988, fetal cord blood stem cell research and transplantation have taken an increasing share of the limelight [1]. Compared with adults’ hematopoietic stem cells, those in fetal cord blood have distinct advantages, including the capacity of forming more colonies in culture, a high cell-cycle rate, autocrine production of growth factors, and long telomeres. Cord blood CD34^+^38^-^ cell telomere length is around 12 kb, whereas bone marrow stem cell telomere length is around 8 kb [2]. However, the drawback of stem cells from fetal cord blood is their limited availability and quantity (average, 70–100 mL). Bone marrow and umbilical cord blood contain 1–3% stem cells, while only 0.1% of adult peripheral blood contains stem cells.

Pathogens detected in fetal cord blood represent fetal intrauterine infection. The infections might have originated from ascending maternal infection or indirect infection from the maternal sexual partner with subsequent fetal swallowing or absorption from infected amniotic fluid. Although the placenta serves as a filter for the fetus to prevent pathogens of large particulate matter, pathogens with a molecular weight less than 1,000 Da can pass to the fetus.

During pregnancy, cell-mediated immunity undergoes selective inhibition. On the one hand, this ensures fetal survival within the maternal body; on the other hand, this decreases maternal immunity from pathogenic infections [3].

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**Pathogens in Maternal Blood and Fetal Cord Blood Using Q-PCR Assay**

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**SUMMARY**

**Objective:** To evaluate the rates of infection of asymptomatic fetuses and mothers through fetal cord blood and maternal blood examination results.

**Materials and Methods:** Quantitative PCR (Q-PCR) was used to detect pathogens in maternal peripheral blood and fetal cord blood after delivery of term pregnancy at Buddhist Tzu Chi Medical Center, Hualien, between July 2002 and June 2003.

**Results:** We used Q-PCR to detect pathogens in 29 samples of maternal blood. The maternal hepatitis B virus (HBV) DNA detection rate was 51.72% (15/29); for human cytomegalovirus DNA, the detection rate was 10.34% (3/29) and for *Chlamydia trachomatis* DNA the detection rate was 3.45% (1/29). No *Neisseria gonorrhoeae* DNA was detected. Whereas, in 29 samples of paired fetal cord blood, the detection rates were 27.59% (8/29), 10.34% (3/29), and 3.45% (1/29) for HBV DNA, *C. trachomatis* DNA, and *N. gonorrhoeae* DNA, respectively. No human cytomegalovirus DNA was detected in fetal cord blood.

**Conclusion:** Our results revealed an unexpectedly high incidence of pathogens in fetal cord blood. Screening for the above pathogens in donor cord blood in cord blood banks using Q-PCR is strongly urged to decrease morbidity and mortality rates in fetal cord blood stem cell transplant recipients. [Taiwanese J Obstet Gynecol 2006;45(2):114–119]

**Key Words:** cord blood, pathogens, Q-PCR, term pregnancy

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Recipients of hematopoietic stem cell transplants might contract infections, fevers, and other nonimmune rejection-related symptoms. These infections are important factors affecting transplant success. Further, the recipients are usually immunocompromised, which increases susceptibility to infections [4].

In our study, we used quantitative PCR (Q-PCR) to detect pathogens in pregnant women and fetal cord blood. The pathogens studied were hepatitis B virus (HBV), human cytomegalovirus, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae*. The goals of this study were to understand the rates of infection of sexually transmitted pathogens in pregnant women and the umbilical cord blood, to prevent fetal cord blood transplants from infecting their recipients, and to decrease morbidity and mortality rates in fetal cord blood transplant recipients.

**Materials and Methods**

**Sample studied**

With informed consent, 2 mL of peripheral blood samples from 29 healthy mothers after delivery of their term singleton infants and paired 5 mL umbilical cord blood were collected during delivery at Hualien Buddhist Tzu-Chi General Hospital between January 2002 and June 2003. All were stored in 1.5-mL sodium heparin-containing vacutainers (BD, Franklin Lakes, NJ, USA), and kept in a refrigerator maintained at 4°C. All samples were sent to the laboratory within 24 hours of collection, where 2 mL of blood was placed in 2 mL tubes and centrifuged at 3,000 rpm for 15 minutes. The serum on top was then separated into clean test tubes kept at –20°C for reserve purposes. The study protocol was approved by the Hospital Research Ethics Committee.

**Purification of pathogen and cellular nucleic acid from the plasma of fetal cord blood and maternal peripheral blood**

Above-stored plasmas were thawed to room temperature. The precipitated protein was cleared by centrifugation at 1,500g for 5 minutes. Pathogens and cells in the cleared plasma layer (0.5 mL) were precipitated by mixing with 100 µL PEG/NaCl solution (20% PEG 8000, 2.5 M NaCl) at room temperature for 30 minutes. The precipitate was collected by centrifugation at 12,000 rpm for 10 minutes. Pathogens and cellular nucleic acid were purified by 200 µL phenol extraction and finally dissolved in 250 µL TE buffer.

**Q-PCR assay using Taqman probe**

The assay was performed in a PE 7700 Q-PCR thermal cycler following the manufacturer’s instructions (Applied Biosystems, Foster City, CA, USA). Q-PCR assay was modified in our laboratory. Reaction was in a volume of 20 µL containing 1× PCR buffer [50 mM Tris–HCl, 10 mM KCl, 5 mM (NH4)2SO4, 2 mM MgCl2 (pH 8.3)], 0.2 mM of each dNTP, 0.5 unit of FastStart Taq (Roche Applied Science, Indianapolis, IN, USA), 0.375 µM of each primer, 0.25 µM of Taqman probe (5'-labeled with FAM and 3' labeled with BHQ, purified by PAGE or HPLC), and various amounts of template DNA (2 µL). The assay was performed using a two- or three-step method, that is: initial steps included hold at 50°C for 2 minutes and 95°C for 4 minutes, then 35–60 cycles of 95°C for 15 seconds and 60°C for 1 minute or 95°C for 15 seconds and 55°C for 30 seconds and 72°C for 30 seconds. After the reaction, the linear plot (cycle number vs. fluorescence value), standard curve, and copy number report table were collected for analysis. The presence of genomic DNA as the successful preparation of template DNA was monitored using primers specific to the human β-globin gene. The primer sequences used to generate the positive control plasmid for Q-PCR are listed in Table 1. The primer and probe sequences for the Q-PCR are available on request. The concentration, expressed in copies per milliliter, was calculated using the following equation:

\[
C = \hat{c} \times \frac{V_{DNA}}{V_{PCR}} \left( \frac{1}{V_{EXT}} \right)
\]

**Table 1. PCR primers used to generate the positive control plasmid for Q-PCR**

<table>
<thead>
<tr>
<th>Name</th>
<th>F Primer</th>
<th>R primer</th>
<th>Name of plasmid clone</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>CTTCCAGGAAACATCACTACC</td>
<td>AAAGCCCTGCGAACCACCTGA</td>
<td>pHBV</td>
<td>231</td>
</tr>
<tr>
<td>HCMV</td>
<td>CCTAGTGTGGATGACCTACGGCCA</td>
<td>CAGACACAGTGCCTCCGGCTCCTC</td>
<td>pCMV</td>
<td>75</td>
</tr>
<tr>
<td>CT</td>
<td>GAAGACGGTTAATACCGGGCT</td>
<td>GATGGGTTAGACCATCACC</td>
<td>pCT</td>
<td>398</td>
</tr>
<tr>
<td>NG</td>
<td>GTCTAGGGTCGGCTGCCTGTTT</td>
<td>AGCCGGGCAATAGAGACAC</td>
<td>pNG</td>
<td>280</td>
</tr>
<tr>
<td>β-globin</td>
<td>ACACACAGTGTGGTCTACTACC</td>
<td>GTCTCCACATGCGCATT</td>
<td>—</td>
<td>189</td>
</tr>
</tbody>
</table>

*HBV = hepatitis B virus; HCMV = human cytomegalovirus; CT = Chlamydia trachomatis; NG = Neisseria gonorrhoeae.*
where $C$ is the target concentration in serum (copies/mL); $O$, the target quantity (copies) determined by sequence detector in PCR; $V_{DNA}$, the total volume of DNA obtained after extraction; typically 250 μL per extraction; $V_{PCR}$, the volume of DNA solution used for PCR, typically 2 μL; and $V_{EXT}$ is the volume of serum extracted, typically 200 μL.

**Statistical analysis**
Data obtained from the laboratory were analyzed using Microsoft Excel’s descriptive statistics. Deductive statistics using SPSS 11.0 paired-samples t test. A $p$ value of less than 0.05 was statistically significant.

**Results**
We used quantitative PCR to analyze all 29 samples of maternal blood and paired fetal umbilical cord blood. The maternal HBV DNA detection rate was 51.72% with an average value of 646,804.18 copies/mL, while the median was 76.58 copies/mL and the range was 1.42–4,598,712 copies/mL. The HCMV DNA positivity rate was 10.34% with an average value of 6,805.2 copies/mL, while the median was 2,938.12 copies/mL and the range was 10.26–17,467.22 copies/mL. C. trachomatis DNA positivity rate was 3.45%, and the number of DNA copies was 672.93 copies/mL.

N. gonorrhoeae DNA was not detected in the maternal blood. The internal control β-globin gene DNA average value was 1,431.66 copies/mL, while the median was 150.28 copies/mL and the range was 1.08–7,567.85 copies/mL. In the paired samples of fetal umbilical cord blood, the HBV DNA detection rate was 27.59%, while the average value was 36,789.54 copies/mL, the median was 3.21 copies/mL and the range was 1.77–294,286.24 copies/mL. C. trachomatis DNA positivity rate was 10.34%, while the average value was 10.21 copies/mL, the median was 10.02 copies/mL, and the range was 4.57–16.02 copies/mL; N. gonorrhoeae DNA positivity rate was 3.45%, and the number of copies was 1,135.54 copies/mL. HCMV DNA was not detected in the cord blood. The internal control β-globin gene DNA average value was 101,925.3 copies/mL, while the median was 102 copies/mL, and the range was 11.67–1,321,264.1 copies/mL (Figure; Table 2).

**Table 2. Quantitative analysis of pathogen DNA in 29 serum samples of maternal and cord blood**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Positive number</th>
<th>Mean (copies/mL)</th>
<th>Median (copies/mL)</th>
<th>Range (copies/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-globin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>29</td>
<td>1,431.66</td>
<td>150.28</td>
<td>1.08–7,567.85</td>
</tr>
<tr>
<td>CB</td>
<td>29</td>
<td>101,925.30</td>
<td>101.99</td>
<td>11.67–1,321,264.08</td>
</tr>
<tr>
<td>HBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>15</td>
<td>646,804.18</td>
<td>76.58</td>
<td>1.42–4,598,712.00</td>
</tr>
<tr>
<td>CB</td>
<td>8</td>
<td>36,789.54</td>
<td>3.21</td>
<td>1.77–294,286.31</td>
</tr>
<tr>
<td>HCMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>3</td>
<td>6,805.20</td>
<td>2,938.12</td>
<td>10.26–17,467.22</td>
</tr>
<tr>
<td>CB</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>1</td>
<td>672.95</td>
<td>—</td>
<td>672.95</td>
</tr>
<tr>
<td>CB</td>
<td>3</td>
<td>10.21</td>
<td>10.02</td>
<td>4.57–16.62</td>
</tr>
<tr>
<td>NG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CB</td>
<td>1</td>
<td>1,135.54</td>
<td>—</td>
<td>1,135.54</td>
</tr>
</tbody>
</table>

*HBV = hepatitis B virus; HCMV = human cytomegalovirus; CT = Chlamydia trachomatis; NG = Neisseria gonorrhoeae; MB = maternal blood, CB = cord blood.*
HBV DNA was detected in 51.72% of maternal blood and 27.59% of cord blood, while the HBV DNA positivity rate was significantly higher in maternal blood than in cord blood ($t = 0.022$). The average value of maternal blood HBV DNA and cord blood HBV DNA was 646,804.18 and 36,789.54 copies/mL, respectively. Maternal blood HBV DNA concentration was higher than cord blood HBV DNA concentration, but did not reach statistical significance ($t = 0.114$). We also found that in the 12 cases of HBsAg(−) mothers, the HBV DNA average value was 488,305.20 copies/mL; while for the three cases of HBsAg(+) mothers, the HBV DNA average value was 959,359.38 copies/mL, with no significant statistical differences ($p = 0.598$).

**Discussion**

HBV infection is caused by a small, partial double-stranded DNA virus. The HBV genome measures approximately 3.2 kbp. The major modes of HBV transmission are contact with blood and bodily fluids and vertical transmission from mothers to newborns. In this study, the high rate of HBV infection found in fetal cord blood was the result of vertical transmission from mother to infant during the perinatal period [5,6].

Nagata et al [7] reported the use of Q-PCR to test the responses of chronic HBV patients to interferon therapy and found that the HBV DNA values of peripheral blood in HBsAg(−) and anti-HBs(+) patients were still positive, but that the HBV DNA concentration was usually less than 400 copies/mL. High values of HBV DNA signified that the virus was presently replicating or that the liver was undergoing progressive damage [8]. Chen et al [9] analyzed 1,762 pregnant HBV carriers and perinatal mothers to infant transmission rate and found that the HBsAg positivity rate during pregnancy was 8.3% (147/1,762), among which 33.3% were HBeAg positive (49/147), 71.4% of the newborns were HBsAg positive in the gastric fluid (50/70), 55.7% were HBsAg positive in the maternal breast milk (34/61), and 20.6% were HBsAg positive in the newborn blood (13/63). At the same time, Chen et al [9] found that if maternal HBsAg and HBeAg were both positive, then the newborn’s blood HBsAg positivity rate was 81.8%. If only the mother was HBsAg positive, the risk of vertical transmission decreases to 18.6%. Vaginal delivery and cesarean section revealed no differences in vertical transmission rates. HBV infection is a widespread disease in those of Chinese descent; its prevalence, determined using general antigen screening, is 10–20%, and when we used Q-PCR to test the 29 samples of maternal and fetal cord blood, the HBV DNA detection rates were 51.72% and 27.59%, respectively. The HBV DNA positivity rate of the maternal blood was significantly higher than that of the fetal cord blood. For those who were HBsAg(+), the HBV DNA positivity rate was significantly higher than in those who were HBsAg(−). However, we analyzed 29 samples of maternal blood HBsAg(−) (12/29) and HBsAg(+) (3/29) for HBV DNA concentration and found that serum HBsAg(−) samples had HBV DNA average values of 488,305.20 copies/mL; and HBsAg(+) samples had HBV DNA average values of 959,359.38 copies/mL ($p = 0.598$), which showed no statistically significant difference. In our study using the Q-PCR technique, the HBV detection rate was higher than those reported in the literature.

The sensitivity of using PCR technology to diagnose congenital HCMV infection from the amniotic fluid is 80%, and the specificity is 100% [10]. The sensitivity can reach 75% when ELISA is used to search for cord blood anti-HCMV IgM antibodies, whereas viral culture sensitivity reaches only 30%. In the US, female serum anti-HCMV antibody positivity rate was 55–85% during the reproductive age, with the rate of primary HCMV infection during pregnancy being 0.7–4%, while the rate of persistent infections was 13.5% [11]. Pregnant women from mainland China had a serum anti-HCMV IgG positivity rate as high as 94.6%, anti-HCMV IgM positivity rate of around 3.1–13.44%; the newborn blood or cord blood anti-HCMV IgM positivity rate was around 0.6–8.5% and urine HCMV-DNA positivity rate, 6.5–27.3%. There was no gender predilection for HCMV infection. In Singapore, Wong et al [12] used the ELISA method to screen for serum HCMV IgG in 120 pregnant women and found the prevalence to be 87%. In Egypt, Nawawy et al [13] used the ELISA method to test 150 pregnant women and their newborns for serum HCMV IgG and IgM, HBsAg, HBeAg, and Toxoplasma-IgG. In women, they found a prevalence of IgM with HCMV IgG of 96%, a prevalence of HBsAg of 8%, a prevalence of Toxoplasma-IgG of 43%, and vertical transmission rate of 33%. The newborn HCMV IgG rate was 96% and HBsAg rate was 17%, while HCMV IgM and HBeAg were not detected.

We used real-time Q-PCR to test 29 samples of maternal blood and fetal cord blood. The HCMV DNA positivity rate was 10.34% (3/29), and no HCMV DNA was found in the fetal cord blood. This shows that the cord blood is relatively more pure with respect to HCMV. In this study, the HCMV DNA positivity rate was lower than those reported in the literature.

Zhang et al [14] used the PCR technique to analyze 772 samples from pregnant women nearing labor and found that the rate of positive results for C. trachomatis in the cervix was 11.3% (87/772), with a
vertical transmission rate of 37% (amniotic fluid, newborn conjunctivae, and nasopharyngeal swab sample positivity). However, *C. trachomatis* was not detected in the 81 samples of cord blood, leading to the conclusion that ascending sexual infection is an important route of intrauterine fetal infections. Similarly, Dong et al [15] used the PCR technique to screen 120 samples of cervical discharge during early pregnancy, and found a positivity rate of 8.3% (10/120) for *C. trachomatis* DNA in the cervix, while the *C. trachomatis* DNA positivity rate was 5.1% (3/59) in chorion obtained from early abortions. In Hungary, *C. trachomatis* infection rate during pregnancy was around 5.74% [16]. In the US, *C. trachomatis* infection comprised 25–50% of pelvic inflammatory disease, and 50–80% of patients with *C. trachomatis* infection were without symptoms. In Bissau, one researcher found that the *N. gonorrhoeae* infection rate in the female reproductive tract was 17% (34/200) [17]. According to the socioeconomic status level in the US, *N. gonorrhoeae* infection rates in pregnant women ranged from 0.5% to 10%, and if the maternal reproductive tract was infected with *N. gonorrhoeae*, there was a vertical transmission rate of 30–40% which caused ophthalmia neonatorum. For asymptomatic women, the *C. trachomatis* infection rate in their reproductive tracts ranged from 6.1% to 10.1%, and the *N. gonorrhoeae* infection rate was 2.3% [18,19]. Honohan et al [20] reported a microbial contamination rate of fetal cord blood of 13%, with low microbial load and low pathogenicity. We used Q-PCR to test 29 samples of maternal blood and fetal cord blood from Hualien’s Tzu-Chi Medical Center and found *C. trachomatis* DNA detection rates of 3.45% (1/29) and 10.34% (3/29), respectively; *N. gonorrhoeae* DNA within the cord blood was 3.45% (1/29), and no *N. gonorrhoeae* DNA was detected in the maternal blood. These results were similar to those reported in the literature.

We did not detect pathogen DNA in the maternal blood but found pathogen DNA in fetal cord blood, like that of *N. gonorrhoeae*. Possible reasons include sub-detectable threshold levels of bacteria in the maternal blood. The cord blood infection may have come from an ascending sexual infection that may have risen from maternal sexual activities with an infected partner. In addition, the sample could have been contaminated.

During our data analyses, we unexpectedly found a high incidence of pathogen DNA in both maternal and cord blood samples, and hence repetitive assays were performed to ensure no false-positive results in our laboratory. It would be desirable to conduct a prospective study of these pregnant women for asymptomatic infection as well as for the newborns.

We feel that using PCR technology to detect cord blood pathogenic infections is rapid, sensitive, and specific. Our research disclosed significant pathogen infection in newborns. In addition, we would favor pathogen screening in cord blood banks using Q-PCR to decrease infection in transplant recipients.

References


