Objective: Highly sensitive haptoglobin measurement should be used in neonates because the haptoglobin concentration in neonates is lower than that of adults. The aim of this study was to establish the reference values of haptoglobin levels in the cord blood of uninfected neonates.

Methods: The cord blood of 29 preterm and 51 term babies was collected, and data from the mother and the newborn were recorded. The haptoglobin concentrations of 80 cord blood samples were simultaneously measured by enzyme-linked immunosorbent assay (ELISA; Assaypro, St Charles, MO, USA) and immunoturbidimetry assay (Roche Diagnostics, Basel, Switzerland). C-reactive protein (CRP) was also measured by immunoturbidimetry assay (Roche Diagnostics, Switzerland).

Results: Mean values of CRP and ELISA haptoglobin were not significantly different between preterm and term babies. The 2.5 percentile and 97.5 percentile values of ELISA haptoglobin concentration were as follows: 80 neonates, 0.01 mg/dL and 0.59 mg/dL; 29 preterm babies, 0.08 mg/dL and 0.18 mg/dL; and 51 term babies, 0.07 mg/dL and 0.23 mg/dL. There were no differences in ELISA haptoglobin concentration according to maternal underlying diseases, delivery method, usage of antibiotics or steroids before delivery, gestational age, gender of baby, or twin gestation.

Conclusion: A highly sensitive haptoglobin method should be used to determine the haptoglobin concentration in Korean newborns because the reference values of cord blood haptoglobin concentration in Korean newborns are less than the lower detection limit for commonly used immunoturbidimetric haptoglobin measurement methods.

Key Words: Cord blood, Enzyme-linked immunosorbent assay, Haptoglobins, Immunoturbidimetry, Newborn

Introduction

Haptoglobin is a plasma α₂-glycoprotein synthesized primarily by hepatocytes. Haptoglobin plays important roles in innate host defense responses and hemoglobin metabolism. Haptoglobin is an acute phase protein, and its synthesis is rapidly and dramatically increased in response to numerous inflammatory stimuli. This increase in haptoglobin production during inflammation is due to transcriptional activation of the haptoglobin gene.¹ Haptoglobin binds to oxygenated, free hemoglobin with high affinity. Free hemoglobin can be harmful to the body, promoting the accumulation of hydroxyl radicals, resulting in oxidative tissue damage.² Once irreversibly bound to haptoglobin, free hemoglobin loses its oxidizing ability. The haptoglobin–free hemoglobin complex is rapidly removed from the circulation by a specific receptor found on the surfaces of monocytes and macrophages.³ When haptoglobin is completely saturated with free hemoglobin, levels can require 5–7 days to recover because haptoglobin synthesis is not increased by low haptoglobin levels.² The reference value of plasma haptoglobin varies according to age¹ and haptoglobin phenotype frequency by racial origin;²,⁵ this range is 30 to 300 mg/dL in adults.⁶ Therefore, in adults, serum haptoglobin levels can be used as a screening tool for inflammation, infec-
tion, and in vivo hemolysis.

Most methods used previously have failed to consistently measure haptoglobin in neonates, particularly at birth and in preterm newborns.\textsuperscript{4,7,8} Accurate determination of haptoglobin in newborns is essential because normal newborns can be misinterpreted as having ahaptoglobinemia, and conversely, infected newborns can be misinterpreted as normal if haptoglobin is measured with an insufficiently sensitive assay. However, there are no data on reference ranges of haptoglobin in Korean neonates. The aim of this study was to establish the reference values of haptoglobin levels of the cord blood in uninfected neonates.

Methods

The Institutional Review Board of Yonsei University Wonju Severance Christian Hospital (approval no. CR317056) approved this study. We explained the study to all mothers and obtained written informed consent.

Twenty-nine preterm (22 to 36 week’s gestation) and 51 term (37 to 42 week’s gestation) neonates born at Wonju Severance Christian Hospital between August 2017 and October 2017 were enrolled prospectively. Umbilical venous cord blood at delivery time was collected in serum separator tubes (Becton Dickinson, Franklin Lakes, NJ, USA), centrifuged, and stored at −80°C until testing. Clinical data included delivery mode, maternal underlying diseases (diabetes mellitus, hypertensive disease, etc.), type of rupture of the membranes and duration of the time to delivery, usage of maternal antibiotics, usage of maternal corticosteroids, gestational weeks, birth weight, and multiple pregnancy. C-reactive protein (CRP) and immunoturbidimetric haptoglobin (i-haptoglobin) were tested with COBAS 8000 (Roche Diagnostics, Basel, Switzerland). Highly sensitive enzyme-linked immunosorbent assay (ELISA) haptoglobin (e-haptoglobin) concentrations were measured in triplicate with a lower limit of detection of 0.002 mg/dL (Assaypro, St Charles, MO, USA).

Data were analyzed using IBM SPSS Statistics version 23 (IBM Corporation, Armonk, NY, USA). Linear regression analysis was performed to define the correlation coefficients between e-haptoglobin concentration and gestational days. A Kolmogorov–Smirnov test was performed to verify the assumption of normality of e-haptoglobin concentrations according to neonates’ demographic characteristics. Distribution of a variable was determined with normality when the \( P \) value of Kolmogorov–Smirnov test was larger than 0.05. Statistical significance of the difference in the mean values of e-haptoglobin according to neonatal categorical demographic characteristics were determined by the Mann–Whitney test and \( \chi^2 \) test. The \( P \) value was determined at the 0.05 level of a two-tailed test.

Results

Demographic characteristics of the 80 newborns are shown in Table 1. All of the 80 neonates had CRP concentrations less

| Table 1. Demographic Characteristics of 80 Newborns |
|-----------------------------------------------|
| Demographic characteristics | No. (%) of neonates |
|-------------------------------|
| Cases                        | Preterm  | Term  | Total |
| Preterm                       | 29 (36.3)| 51 (63.8)| 80 (100) |
| Term                          |          |        |       |
| Gender                       |          |        |       |
| Male                         | 13 (44.8)| 29 (56.9)| 42 (52.5) |
| Female                       | 16 (55.2)| 22 (43.1)| 38 (47.5) |
| Delivery mode                |          |        |       |
| Cesarean section             | 21 (72.4)| 35 (68.6)| 56 (70.0) |
| Vaginal delivery             | 8 (27.6)| 16 (31.4)| 24 (30.0) |
| Rupture of amniotic membrane |          |        |       |
| Spontaneous                  | 14 (48.3)| 15 (29.4)| 29 (36.3) |
| No spontaneous               | 15 (51.7)| 36 (70.6)| 51 (63.7) |
| Twins                        |          |        |       |
| Yes                          | 6 (20.7)| 6 (11.8)| 12 (15.0) |
| No                           | 23 (79.3)| 45 (88.2)| 68 (85.0) |
| Maternal underlying diseases |          |        |       |
| Yes                          | 8 (27.6)| 15 (29.4)| 23 (28.8) |
| No                           | 21 (72.4)| 36 (70.6)| 57 (71.2) |
| Maternal usage of antibiotics|          |        |       |
| Yes                          | 21 (72.4)| 18 (35.3)| 39 (48.8) |
| No                           | 8 (27.6)| 33 (64.7)| 41 (51.2) |
| Maternal usage of steroids   |          |        |       |
| Yes                          | 20 (69.0)| 0 (0)  | 20 (25.0) |
| No                           | 9 (31.0)| 51 (100)| 60 (75.0) |
| Gestational age (week)       | 34±2.4   | 38±1.1 | 37±2.7 |
| Newborn weight (g)           | 2,192±562| 3,146±456| 2,801±676 |

Values are presented as mean±standard deviation or number (%).
than 0.02 mg/dL, except 3 neonates (one term baby, 0.04 mg/dL, and two preterm babies, 0.03 mg/dL). Blood culture was carried out in 26 neonates, all the results were negative. All of the 80 neonates had i–haptoglobin concentrations of less than 10 mg/dL (Table 2).

There was no correlation between e–haptoglobin concentration and gestational days ($r=0.061$). Based on the neonates’ demographic characteristics data, there was no normality of e–haptoglobin distribution, with the exception of twins. Mean values of e–haptoglobin did not show a significant difference according to the neonates’ demographic characteristics (Table 2). There were no differences in e–haptoglobin concentration according to maternal underlying diseases, delivery method, usage of antibiotics or steroids before delivery, gestational age, gender of baby, and twin gestation (Table 2).

The 2.5 percentile and 97.5 percentile values of e–haptoglobin were as follows: 80 neonates, 0.01 mg/dL and 0.59 mg/dL; 29 preterm babies, 0.08 mg/dL and 0.18 mg/dL; and 51 term babies, 0.07 mg/dL and 0.23 mg/dL (Fig. 1).

**Discussion**

Haptoglobin can be quantitated in terms of its hemoglobin–
binding capacity or by immunologic means such as nephelometry and ELISA. Published reference values of haptoglobin using hemoglobin-binding capacity for children at birth are zero, whereas mean concentrations in 1- to 7-day-old newborns are 10 mg/dL, with 95% range of 0 to 41 mg/dL. Kanakoudi et al. reported that haptoglobin was undetectable by nephelometry in 80% of the preterm and 60% of the term infants at birth. Gitlin and Biasucci reported that small amounts of haptoglobin were found in 9 of the 21 embryonic and fetal sera of 7.5-39 weeks’ gestation and that the postnatal serum haptoglobin concentrations ranged from a low of 1% of the adult serum pool to as much as 230% of the adult pool. Blood levels of haptoglobin increase by 200-500% during the 7 days after the onset of inflammation and only slowly return to normal during the 3-4 weeks after the removal of the pathologic insult. However, the blood haptoglobin concentration in case of neonatal infection is less than the lower detection limit of 10 mg/dL measured by immunoturbidimetry method, because haptoglobin concentration in cord blood of uninfected newborns in this study ranged from 0.01 mg/dL to 0.59 mg/dL. As a result, clinical studies based on low detection sensitivity have raised serious doubts about the reliability of haptoglobin concentrations for the prediction of neonatal sepsis. Recent studies using highly sensitive immunoassays have focused on haptoglobin as the potential diagnostic tool for risk stratification and identification of infants with early onset neonatal sepsis. Haptoglobin was elevated in confirmed early onset neonatal sepsis group but not in presumed sepsis group and had 95% area under the receiver operating characteristic curve in predicting confirmed early onset neonatal sepsis. However, procalcitonin, fibrinogen, α-2-macroglobulin and tissue plasminogen activator were not significantly different between confirmed early onset neonatal sepsis and other groups. Since synthesis of haptoglobin is not stimulated by hemolysis or clearance of the hemoglobin–haptoglobin complexes, reduced levels of serum haptoglobin are reasonable markers of intravascular hemolysis. In neonates, however, haptoglobin levels are low, and normal children or adult values may not be achieved until several months of age. Thus, some reports commented that the serum concentration of haptoglobin is not a reliable parameter of hemolysis in newborns. This outcome is due to the low detection sensitivity of haptoglobin. It is noteworthy that hemolysis in newborns can easily lead to depletion of haptoglobin because the haptoglobin concentration in the blood of newborns is very low, and the free hemoglobin accumulated in the kidney is likely to cause oxidative renal damage. Therefore, haptoglobin should be measured by highly sensitive enzyme immunoassay if cord blood is used for screening newborns.

There was a report measuring haptoglobin in cord blood of healthy preterm and term babies by ELISA in which median values of preterm and term babies were 5.90 mg/dL and 4.62 mg/dL, respectively. However, our results showed that the median values of preterm and term babies were 0.08 mg/dL and 0.06 mg/dL, respectively. Haptoglobin is made up of two α and β polypeptide subunits, interconnected by a disulfide bridge between the two α chains. While the β polypeptide chain is constant in all populations, molecular polymorphism of haptoglobin occurs as a result of genetic alterations in the α-chain. Two alleles of α-chain, Hp 1 and Hp 2, give rise to three major Hp phenotypes: Hp 1-1, Hp 2-1, and Hp 2-2. The frequency of the three major phenotypes varies according to geographic region. Hp 1-1 individuals have the highest plasma concentration of haptoglobin, and the highest hemoglobin binding capacity, whereas Hp 2-1 heterozygotes have intermediate and Hp 2-2 subjects have the lowest values.

![Fig. 1. Box and whisker plot displaying the distribution of haptoglobin concentrations measured by enzyme-linked immunosorbent assay (e-haptoglobin). In the plot diagram, the central rectangle is the interquartile range, the whiskers above and below the box show the locations of the minimum and maximum excluding outliers, the line within the box marks the median, and + symbol means the mean of e-haptoglobin.](image-url)
heterozygous individuals have a series of multimers (e.g., dimers, trimers) by virtue of intermolecular disulfide linkages through the duplicated light chain. Owing to steric hindrance between molecular sites on the multimers, the different phenotypes of haptoglobin yield measurements of antigen- or hemoglobin-binding capacity that may be discrepant with the absolute amount of haptoglobin present in a sample. Accordingly, the reference range for haptoglobin is broader for an entire population of different phenotypes than within individual phenotypes. For this reason, interpretation of haptoglobin concentrations is soundest for serial measurements in the same individual. The frequencies of haptoglobin phenotypes in healthy Korean adults, in decreasing order, were Hp 2–2 45.9%, Hp 2–1 44.3%, and Hp 1–1 9.8. The low frequency of haptoglobin phenotype Hp 1–1 in Korean population accounts for the low haptoglobin concentration in cord blood of Korean newborns. Additional studies are needed to further elucidate the relationship between haptoglobin phenotype and serum haptoglobin concentration in newborn.

**Conflict of Interest**

No potential conflict of interest relevant to this article was reported.

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