

# Polymorphism of Haptoglobin in Patients with Premature Rupture of Membrane

Jin-Kyung Cho,<sup>1,\*</sup> Yeun-Hee Kim,<sup>2,\*</sup> In-Yang Park,<sup>2</sup> Jong-Chul Shin,<sup>2</sup> Mi-Kyung Oh,<sup>1</sup> Seon-Joo Park,<sup>1</sup> Nam-Hoon Kim,<sup>1</sup> and In-Sook Kim<sup>1</sup>

Departments of <sup>1</sup>Natural Sciences and <sup>2</sup>Obstetrics and Gynecology, College of Medicine, The Catholic University of Korea, Seoul, Korea.

**Purpose:** To investigate whether allelic polymorphism of haptoglobin (Hp) is associated with premature rupture of membrane (PROM), the Hp phenotypes of pregnant women with PROM were analyzed. **Patients and Methods:** The Hp phenotypes of 221 pregnant Korean women (187 control and 34 PROM patients) were determined by benzidine/hydrogen peroxide staining, following native polyacrylamide gel electrophoresis of hemoglobin-mixed sera. The Hp allele frequencies were calculated from the data of Hp phenotypes, and overall association with PROM was evaluated using Pearson Chi-Square test. **Results:** The polymorphic distribution of the patients cohort who underwent a normal delivery (control group) was similar to that of healthy Koreans. In contrast, however, patients with PROM showed significantly higher occurrence of the Hp 1-1 phenotype than control group (23.5% vs 8.0%). Hp 2-2 phenotype was lower in PROM cohort (38.2%) than in the control group (48.7%). The *Hp*<sup>1</sup> allele frequency in PROM group was significantly higher than that in the control group (0.426 vs 0.297,  $p = 0.034$ ) with odds ratio of 1.762 (95% CI: 1.038 - 2.991). **Conclusion:** These findings suggest that pregnant Korean women who possess *Hp*<sup>1</sup> allele (expressed as Hp 1-1 phenotype) have higher incidence of PROM than women with *Hp*<sup>2</sup> allele (expressed as Hp 2-2 phenotype). This is the first study that evaluated the significance of Hp polymorphism with respect to the development of PROM.

**Key Words:** Haptoglobin, genetic polymorphism, phenotype, premature rupture of membrane (pregnancy)

Received August 20, 2008  
Accepted November 7, 2008

This study was supported by a grant of the Korea Health 21 R&D project, Ministry of Health & Welfare, Republic of Korea (A050209).

\*These authors contributed equally to this study.

Reprint address: requests to Dr. In-Sook Kim, Department of Natural Sciences, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, Korea. Tel: 82-2-590-1269, Fax: 82-2-535-4526, E-mail: [ikim@catholic.ac.kr](mailto:ikim@catholic.ac.kr)

## INTRODUCTION

Premature rupture of membrane (PROM) is characterized by membrane rupture prior to the onset of full-term labor. PROM complicates approximately 8% of pregnancies at term.<sup>1</sup> The main clinical concern for PROM is a high risk of infectious morbidity and mortality in newborns.<sup>2</sup> To reduce the risk of fetal and maternal complications, it is required to find specific biomolecules associated with development of PROM.

The membrane rupture is related to collagen destruction and programmed cell death in fetal membranes. The risk factors for PROM involve reproductive tract infection, cigarette smoking, and uterine overdistention, and various mediators of PROM, including matrix-degrading enzymes, prostaglandins and inflammatory cytokines, can be produced in the fetal membranes and deciduas.<sup>1-3</sup> Recent studies suggest that reactive oxygen species (ROS) might damage connective tissues of chorioamnion and induce PROM.<sup>3-5</sup> However, the exact mechanism of PROM is still unclear.

Haptoglobin (Hp) is an acute phase protein which plays roles in the systemic host defense in response to inflammation.<sup>6</sup> The protein binds hemoglobin (Hb), thereby protecting cells from oxidative damage that occurs during hemolytic injury.<sup>7</sup> In addition, Hp exerts an inhibitory effect on prostaglandin synthesis<sup>8</sup> and a stimulatory effect on differentiation of endothelial cells.<sup>9</sup> Therefore, Hp is considered to have functions as an antioxidant, immunomodulator and angiogenic factor.

Although it has been well-known that the main site of Hp biosynthesis is liver, several recent

studies demonstrated that the Hp protein is locally expressed in female reproductive organs such as the ovary, endometrium, and decidua.<sup>10,11</sup> The Hp can also be detected in amniotic fluid and vaginal fluid.<sup>12</sup> In addition, the plasma Hp level is elevated during pregnancy in a biphasic pattern with peaks in the first and third trimester.<sup>13</sup> These findings suggest that Hp may play a role in human reproduction and its activity may be involved in obstetric and gynecologic diseases.

The human Hp gene has 2 major alleles,  $Hp^1$  and  $Hp^2$ , which are expressed as 3 major phenotypes (Hp 1-1, Hp 2-2 and Hp 2-1). The Hp 1-1 and Hp 2-2 correspond to homozygote of the  $Hp^1$  and  $Hp^2$  alleles, respectively, while the Hp 2-1 is heterozygous for the two alleles ( $Hp^1/Hp^2$ ).<sup>14</sup> It has been reported that the allelic polymorphism of Hp is associated with susceptibility and severity of specific diseases such as atherosclerosis, Parkinson's disease, gestational diabetes and preeclampsia.<sup>15-18</sup>

To date, the association of Hp with PROM has not been reported. To elucidate whether the Hp polymorphism is related to a high risk of PROM, we investigated the Hp phenotypes/genotypes of pregnant Korean women without or with PROM in the present study.

## PATIENTS AND METHODS

### Subjects

A total of 221 Korean term pregnant women were recruited into our study between 1 September 2006 and 31 June 2007 at Kangnam St. Mary's hospital in Seoul, Korea. The patients were divided into 2 groups: Group 1 (PROM group) comprised 34 pregnant women who delivered with PROM after 37 completed weeks of gestation, while Group 2 (Control group) was composed of 187 normal term pregnant women without PROM. All women were non smokers with singleton pregnancy. There were neither signs of pregnancy-related diseases, except PROM, nor any history of medical treatments. Patients were diagnosed with membrane rupture when they demonstrated at least 2 of the following indices on speculum examination: vaginal pooling of fluid with or without

Valsalva maneuver, a positive nitrazine paper test, or microscopic evidence of ferning on a dried vaginal smear. All patients signed a written consent form to donate blood for research purpose, which was approved by the Institutional Review Board of The Catholic University of Korea in Seoul.

Blood was collected from each patient at the time of delivery. Serum fraction was obtained from the blood by centrifugation and stored at  $-80^{\circ}\text{C}$  until analysis.

### Hp phenotyping

Hp phenotypes of individuals were determined by benzidine/hydrogen peroxide staining after native polyacrylamide gel electrophoresis of Hp-hemoglobin (Hb) complexes.<sup>19</sup> Briefly, the Hp in serum was converted into Hp-Hb complex by mixing 15  $\mu\text{L}$  of serum with excess Hb, and electrophoresed on 8% native polyacrylamide gel. After electrophoresis, the gel was soaked in 1% benzidine /20% acetic acid solution, and a small amount of hydrogen peroxide was immediately added to it while shaking. The bands representing Hp-Hb complexes in the gel could be visualized by the peroxidase activity of Hb. In the resultant electrophoretic pattern, Hp 1-1 type shows only a single band, whereas Hp 2-2 type shows slow-migrating polymer bands. Hp 2-1 type represents both 1-1 type and polymer forms.<sup>19</sup>

### Statistics

Data were analyzed by 2-tailed Student's test and expressed as mean  $\pm$  standard deviation (SD). The Hp allele frequencies were calculated from the data of Hp phenotypes, and overall association with PROM was evaluated using Pearson Chi-Square test. All statistical calculations were performed by the SPSS 12.0 program for Windows.  $P < 0.05$  was considered statistically significant.

## RESULTS

To analyze Hp phenotypes, 34 pregnant women who suffered from PROM and 187 pregnant women who underwent normal delivery were recruited. There were no significant differences between the

**Table 1.** Clinical Characteristics of Patients

	Group 1 PROM (n = 34)	Group 2 Control (n = 187)
Nullipara, n (%)	23 (67.6%)	114 (61%)
Age (yrs)	31.5 ± 3.3	31.8 ± 3.5
Gestational age at delivery (wks)	39.3 ± 1.1	39.3 ± 1.6
Neonatal birth weight (kg)	3.3 ± 0.4	3.2 ± 0.4
5 min Apgar score	9.4 ± 0.6	9.4 ± 0.9

PROM, premature rupture of membrane; Group 1 (PROM), patients who delivered with PROM; Group 2 (Control), patients who delivered without PROM.

Values are given as mean ± SD or percentages.

**Table 2.** Haptoglobin Polymorphism in Pregnant Women

	Group 1 PROM, n (%)	Group 2 Control, n (%)	<i>p</i> value*
Hp 1-1	8 (23.5)	15 (8.0)	0.006
Hp 2-1	13 (38.2)	81 (43.3)	
Hp 2-2	13 (38.2)	91 (48.7)	
Total, n (%)	34 (100.0)	187 (100.0)	

PROM, premature rupture of membrane; Group 1 (PROM), patients who delivered with PROM; Group 2 (Control), patients who delivered without PROM; Hp, haptoglobin.

\*Comparison between Hp 1-1 type and the other types by Pearson Chi-Square test.

PROM and the normal delivery (control) groups in the clinical characteristics such as nullipara, maternal age, gestational age at delivery, neonatal birth weight and Apgar score at 5 minutes (Table 1).

As for Hp polymorphism, the pregnant women with normal delivery (control group) showed Hp 1-1, 2-1, and 2-2 phenotypes at 8.0%, 43.3%, and 48.7%, respectively (Table 2). The Hp phenotypic distribution in these women was found to be statistically similar to ratios in healthy Koreans.<sup>20</sup> The distribution rate of Hp phenotype for total population enrolled in this study (n = 221) was also similar; 10.4%, 42.5%, and 47.1% for Hp 1-1, 2-1 and 2-2, respectively. In contrast, however, patients with PROM demonstrated the Hp 1-1 type at 23.5%, which is a significantly higher rate than 8.0% of control group. The percentage to express the Hp 2-2 type was lower in PROM cohort (38.2%) than in the control group (48.7%). By Pearson Chi-Square test, Hp 1-1 type was

significantly prevalent in PROM group as compared with control (23.5% vs 8.0%, *p* = 0.006) with odds ratio of 3.528 (95% CI: 1.362 - 9.141) (Table 2).

The Hp allele frequencies were calculated from the distribution data for Hp phenotypes (Table 2) and evaluated using Pearson Chi-Square test. As shown in Table 3, the frequency of *Hp*<sup>1</sup> allele in the PROM group was significantly higher than that of the control group (0.426 vs 0.297, *p* = 0.034) with odds ratio of 1.762 (95% CI: 1.038 - 2.991). These findings suggest that term pregnant women with the *Hp*<sup>1</sup> allele have higher incidence of PROM than women with the *Hp*<sup>2</sup> allele.

## DISCUSSION

Several studies have demonstrated the relation of Hp polymorphism with susceptibility and severity of clinical symptoms in specific disorders,

**Table 3.** The Frequency of Haptoglobin Allele in PROM and Control Groups

Hp allele	Frequency		<i>p</i> value*
	Group 1 PROM	Group 2 Control	
<i>Hp</i> <sup>1</sup>	0.426	0.297	0.034
<i>Hp</i> <sup>2</sup>	0.574	0.703	

PROM, premature rupture of membrane; Group 1 (PROM), patients who delivered with PROM; Group 2 (Control), patients who delivered without PROM; Hp, haptoglobin.

\*Pearson Chi-Square test was performed between Group 1 and Group 2.

including angiopathies, diabetes, Parkinson's disease and preeclampsia.<sup>15-18</sup> However, to the best of our knowledge, the correlation between PROM and Hp has not yet been studied. In the present study, we found that the frequency of individuals afflicted with PROM was significantly higher in women expressing the Hp 1-1 than in women expressing the Hp 2-2, suggesting that individuals with the *Hp*<sup>1</sup> allele are more susceptible to PROM as compared to those with the *Hp*<sup>2</sup> allele (Tables 2 and 3).

Hp proteins with the 3 phenotypes show phenotype-dependent functional differences in anti-oxidant, anti-inflammatory and angiogenic activities;<sup>7,9,21</sup> Hp 1-1 has a higher anti-inflammatory activity than Hp 2-2. On the contrary, Hp 2-2 possesses more potent angiogenic activity compared to Hp 1-1 and plays a role more efficiently during angiogenic process, while Hp 2-1 shows an intermediate activity. Although the exact mechanism involved in higher incidence of PROM in individuals with Hp 1-1 is not known yet, it is quite plausible to suggest that the lower angiogenic potential in carriers of Hp 1-1 may contribute less effectively to the repair of damaged connective tissues of chorioamnion and may develop PROM more easily.

Hp forms a stable Hp-Hb complex by irreversible binding with Hb. The Hp-Hb ligand binds to CD163, which is known as a Hb scavenger receptor, and is endocytosed. Recent studies demonstrated that the binding of Hp-Hb complex to CD163 receptor increases the expression of anti-inflammatory modulators such as interleukin-10 and heme oxygenase-1.<sup>22</sup> It was also reported that the CD163 surface ligation induces tyrosine kinase-

dependent calcium mobilization as well as casein kinase II- and protein kinase C-dependent secretion of proinflammatory cytokines (interleukin-6 and interleukin-1 $\beta$ ).<sup>23</sup> Kristiansen et al. (2001) reported that affinity of the Hp-Hb complex for CD163 was Hp phenotype-dependent;<sup>24</sup> the complex of Hp 2-2 and Hb exhibits higher functional affinity for CD163 than the complex of Hp 1-1 and Hb, although both Hp 1-1 and Hp 2-2 can efficiently remove Hb through CD163 receptor. In addition, the role of Hp in inflammatory response is phenotype-dependent, showing that Hp 1-1 and Hp 2-2 make dominant Th2 response and Th1 response, respectively.<sup>21</sup> Therefore, to understand higher incidence of PROM in the individuals with Hp 1-1, because of the reason that PROM is an inflammation-associated gestational disease, further studies are required, focusing mainly on the expression of inflammatory regulators via CD 163 signaling in amniotic tissues or decidua.

Preterm PROM is the cause of nearly half of all preterm delivery and a high risk factor of perinatal morbidity and mortality.<sup>2</sup> Because the pathophysiologic processes involved in subclinical infection are considered to be similar in both term and preterm PROM, the evidences for term PROM in this study might be applied similarly to preterm PROM. Nevertheless, it is necessary to confirm exact relationship of Hp to preterm PROM.

In conclusion, pregnant women with Hp 1-1 type have higher incidence of PROM than those with other Hp types. To our best knowledge, this is the first study that evaluated the significance of Hp phenotype with respect to the development of PROM. The findings suggested that the Hp polymorphism may be potentially applied to the

prevention of PROM during pregnancy.

## REFERENCES

- Hannah ME, Ohlsson A, Farine D, Hewson SA, Hodnett ED, Myhr TL, et al. Induction of labor compared with expectant management for prelabor rupture of the membranes at term. TERMPROM Study Group. *N Engl J Med* 1996;334:1005-10.
- Mercer BM, Goldenberg RL, Meis PJ, Moawad AH, Shellhaas C, Das A, et al. The Preterm Prediction Study: prediction of preterm premature rupture of membranes through clinical findings and ancillary testing. The National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. *Am J Obstet Gynecol* 2000;183:738-45.
- Woods JR Jr. Reactive oxygen species and preterm premature rupture of membranes - a review. *Placenta* 2001;22 Suppl A:S38-44.
- Woods JR Jr, Plessinger MA, Miller RK. Vitamins C and E: missing links in preventing preterm premature rupture of membranes? *Am J Obstet Gynecol* 2001;185:5-10.
- Borna S, Borna H, Daneshbodie B. Vitamins C and E in the latency period in women with preterm premature rupture of membranes. *Int J Gynaecol Obstet* 2005;90:16-20.
- Quaye IK. Haptoglobin, inflammation and disease. *Trans R Soc Trop Med Hyg* 2008;102:735-42.
- Melamed-Frank M, Lache O, Enav BI, Szafrank T, Levy NS, Ricklis RM, et al. Structure-function analysis of the antioxidant properties of haptoglobin. *Blood* 2001;98:3693-8.
- Jue DM, Shim BS, Kang YS. Inhibition of prostaglandin synthase activity of sheep seminal vesicular gland by human serum haptoglobin. *Mol Cell Biochem* 1983;51:141-7.
- Cid MC, Grant DS, Hoffman GS, Auerbach R, Fauci AS, Kleinman HK. Identification of haptoglobin as an angiogenic factor in sera from patients with systemic vasculitis. *J Clin Invest* 1993;91:977-85.
- Sharpe-Timms KL, Ricke EA, Piva M, Horowitz GM. Differential expression and localization of de-novo synthesized endometriotic haptoglobin in endometrium and endometriotic lesions. *Hum Reprod* 2000;15:2180-5.
- Berkova N, Lemay A, Dresser DW, Fontaine JY, Kerizit J, Goupil S. Haptoglobin is present in human endometrium and shows elevated levels in the decidua during pregnancy. *Mol Hum Reprod* 2001;7:747-54.
- Katnik I, Dobryszczyka W. Enzyme immunoassay to measure low levels of haptoglobin in biological fluids. *J Immunoassay* 1990;11:503-17.
- Haram K, Augensen K, Elsayed S. Serum protein pattern in normal pregnancy with special reference to acute-phase reactants. *Br J Obstet Gynaecol* 1983;90:139-45.
- Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. *Clin Chem* 1996;42:1589-600.
- Asleh R, Miller-Lotan R, Aviram M, Hayek T, Yulish M, Levy JE, et al. Haptoglobin genotype is a regulator of reverse cholesterol transport in diabetes *in vitro* and *in vivo*. *Circ Res* 2006;99:1419-25.
- Costa-Mallen P, Checkoway H, Zabeti A, Edenfield MJ, Swanson PD, Longstreth WT Jr, et al. The functional polymorphism of the hemoglobin-binding protein haptoglobin influences susceptibility to idiopathic Parkinson's disease. *Am J Med Genet B Neuropsychiatr Genet* 2008;147B:216-22.
- Mustafa S, Vukovich T, Prikoszovich T, Winzer C, Schneider B, Esterbauer H, et al. Haptoglobin phenotype and gestational diabetes. *Diabetes Care* 2004;27:2103-7.
- Depypere HT, Langlois MR, Delanghe JR, Temmerman M, Dhont M. Haptoglobin polymorphism in patients with preeclampsia. *Clin Chem Lab Med* 2006;44:924-8.
- Lai IH, Lin KY, Larsson M, Yang MC, Shiau CH, Liao MH, et al. A unique tetrameric structure of deer plasma haptoglobin - an evolutionary advantage in the Hp 2-2 phenotype with homogeneous structure. *FEBS J* 2008;275:981-93.
- Yang SE, Min WK, Park H, Chun S, Nah J, Kim JQ. Distribution of haptoglobin phenotypes in a Korean population, using the semi-automated PhastSystem. *Ann Clin Biochem* 2000;37(Pt2):205-9.
- Guetta J, Strauss M, Levy NS, Fahoum L, Levy AP. Haptoglobin genotype modulates the balance of Th1/Th2 cytokines produced by macrophages exposed to free hemoglobin. *Atherosclerosis* 2007;191:48-53.
- Philippidis P, Mason JC, Evans BJ, Nadra I, Taylor KM, Haskard DO, et al. Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses *in vitro*, in resolving skin blisters *in vivo*, and after cardiopulmonary bypass surgery. *Circ Res* 2004;94:119-26.
- Ritter M, Buechler C, Kapinsky M, Schmitz G. Interaction of CD163 with the regulatory subunit of casein kinase II (CKII) and dependence of CD163 signaling on CKII and protein kinase C. *Eur J Immunol* 2001;31:999-1009.
- Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, et al. Identification of the haemoglobin scavenger receptor. *Nature* 2001;409:198-201.