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Haptoglobin Polymorphism and Morbidity in Children Born Prematurely

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Abstract: Haptoglobin (Hp) is an acute phase protein. There exists three different Hp phenotypes that differ in their pro-inflammatory and anti-inflammatory action. Inflammation and oxidative stress are critically involved in many pathophysiological processes in childhood. We previously reported on the relationship of in-hospital clinical events and the Hp phenotype in a cohort of consecutive premature infants. This study provides follow up of childhood morbidity data and its relationship to the Hp phenotype for the previously reported study premature infant cohort. A phone questionnaire was carried out among the parents and medical history records were evaluated. The age of participants varied from eight to eleven years. The questionnaire included information about diseases with an inflammatory component in pathogenesis, infections and need for hospitalization. Of the original 122 study participants, morbidity data at eight to eleven years was available for 101 participants, and 100 were enrolled in the study. A less anti-inflammatory Hp phenotype (Hp 2-2) was more prevalent in participants who suffered from diseases with an inflammatory component in their pathogenesis (RTI, pneumonia, UTI, OM and tonsillitis) when these entities were analyzed as a group (p=0.043). Subsequent comparison of the cumulative number of episodes of these entities between Hp phenotypes during all follow up period detected a trend in the same direction. Further research involving a larger population is required for better understanding of the Hp phenotype role in infantile and pediatric morbidity.

Keywords: Childhood Morbidity, Haptoglobin Phenotype, Infection, Inflammation

1. Introduction

Haptoglobin (Hp) is an acute phase protein with anti-inflammatory, anti-oxidant and bacteriostatic activities. The Hp gene has two alleles, 1 and 2, encoding for three phenotypes Hp1-1, Hp2-1 and Hp2-2. These phenotypes differ in their pro- and anti-inflammatory action [1]. It has been established that subjects with the Hp 1–1 phenotype were more likely to resist oxidative stress compared to those with Hp 2-2 phenotype. The Hp 1-1 protein prevents formation of active oxygen derivatives and hemoglobin (Hb)-induced oxidation. It also improves stability of Hp–Hb complexes. In contrast, the Hp 2-2 protein forms a less stable Hp–Hb complex. The Hp 2 allele is associated with higher oxidation stress and exaggerated inflammatory reaction. The Hp 2-2 phenotype has consistently been observed to be a risk factor in inflammatory diseases [1]. Compared to Hp 1-1, which enhances expression of anti-inflammatory cytokines, the Hp 2-2 phenotype promotes the production of pro-inflammatory cytokines. However, Hp 2–2 contributes to more efficient bacteriostatic action than Hp 1–1 [2]. The association between different Hp phenotypes with common

clinical conditions, such as coronary artery disease, hypertension, diabetes, infections and others has been studied [2-4].

In children, inflammation and oxidative stress are involved in multiple pathophysiological processes determining morbidity [5-7]. In our previous prospective clinical trial, Hp phenotype was evaluated in 122 preterm infants born in Carmel Medical Center, Haifa, Israel, and morbidities including sepsis, necrotizing enterocolitis, bronchopulmonary dysplasia (BPD), intraventricular hemorrhage and retinopathy of prematurity were recorded during hospitalization until discharge. A trend for increased risk of sepsis was demonstrated in Hp 2–2 infants with birth weight (BW) greater than 1,500 grams (p=0.078). The lack of differences in the morbidity parameters in very low birth weight infants was explained by the low level of Hp protein in their serum [8].

This follow up study was designed as an attempt to investigate if there is an association between Hp phenotypes and long term morbidity in children that were born prematurely and had been enrolled in our previous study. In this study, infectious and inflammatory conditions that were associated with the different Hp phenotypes were assessed.

2. Methods

After the approval of hospital Ethics Committee, long term morbidity data of previous study participants who had been investigated for Hp phenotype during the neonatal period were collected. Absence of an informed consent and the presence of congenital severe chronic diseases were considered as exclusion criteria.

Out of 122 participants of the reported study, the parents of 103 children (84.4%) were accessed by phone from May 2017 to June 2018. The parents of two children (1.64% of total) refused to give an informed consent. Disappointingly, the phones of 7 families (5.7%) were not connected and it was impossible to identify an alternative accessible phone numbers. 12 families (9.8%) were unavailable for multiple phone calls at any time. As a result their children became ineligible as potential participants for this study. Informed consent was obtained from parents of 101 children (82.8% of the previous study participants). The parents answered a phone questionnaire. In addition, source verification by means of medical history records review was performed. In the medical records of nine study participants, no visits to any medical facilities appeared and no additional data were found, so in these participants, morbidity information was based on the phone questionnaires only. The questionnaire included information of diseases diagnosed after the participants' discharge from the neonatal department, with an emphasis on the diseases with an inflammatory component in pathogenesis, infections, and need for hospitalization. The age of children followed during the study was eight to eleven years.

One participant was excluded from the study because of primary hyperoxaluria, renal and liver transplantation and dependency on hemodialysis. Therefore, one hundred participants (82% of the original cohort) were enrolled in this study. Statistical analysis was performed using IBM statistics (SPSS) version 24 software. Continuous variables are presented by mean, SD or median. Categorical variables are presented in percentages. Patients were segregated by Hp phenotype into two categories: combined 1-1 and 2-1 versus 2-2. Demographic and clinical characteristics were compared between the two categories using Chi Square test for the categorical variables and independent t- test or Mann-Whitney test, as appropriate, for the continuous variables. P-value <0.05 was defined as statistically significant.

3. Results

In the study group, six children (6%) had Hp1-1 phenotype, 43 (43%) children had Hp 1-2 phenotype and Hp 2-2 phenotype appeared in 51 children (51%). As the number with the Hp 1–1 phenotype was insufficient for statistical analysis by itself, this subgroup of participants was combined with the Hp 2-1 patients' subgroup. Such combination of Hp phenotypes to non Hp 2-2 subgroup has been used previously to demonstrate the anti-inflammatory influence of Hp-1 allele [4]. Demographic characteristics of the participants stratified by Hp type (n=100), compared to the initial participants group (n=122) [8] are presented in Table 1. This comparison was performed to determine if there had been a selection bias in the recruitment of the previous study participants for this study. No statistically significant differences were revealed between the present and previous study groups with respect to their Hp phenotype distribution in regards to gender, BW, gestational age and ethnicity.

Table 1. *Demographic characteristics of the study group participants, compared to initial participants group.*

	Hp phenotypes			**Hp phenotypes**		
	1–1/2–1 (n=63) [total of n=122]	**1–1/2–1 (n=48) [total of n=100]**	**p value**	**2–2 (n=59) [total of n=122]**	**2–2 (n=52) [total of n=100]**	**p value**
Male gender N (%)	27 (42.9%)	21 (43.8%)	0.925	30 (50.8%)	26 (50.0%)	0.929
Gestational age (GA) weeks mean (SD)	32.4 ± 2.89 (26–35.7)	31.7±2.9 (26-35)	0.209	32.5 ± 2.5 (24–37)	32.0±2.7 (24-35)	0.313
BW in grams mean (SD)	609–3,008 (1,666 ± 552.11)	1621.2±572.3 (609-3008)	0.677	734–2,584 (1,738 ±491.3)	1708.6±503.2 -7342584	0.756
Ethnicity			0.281			0.424
Ashkenazi Jews	28 (44.4)	27 (56.3)		32 (52.5)	22 (42.3)	
Non Ashkenazi Jews	22 (34.9)	16 (33.3)		18 (30.5)	22 (42.3)	
Arabs + Druse	13 (20.6)	5 (10.4)		10 (16.9)	8 (15.4)	

Morbidity parameters are presented in Table 2. No statistically significant differences were found between Hp phenotypes in the incidence of respiratory tract infection (RTI),

pneumonia, urinary tract infection (UTI), otitis media (OM), and tonsillitis. Grouping disorders with an "inflammation" component in the pathophysiological mechanism was then performed. This consolidated group of disorders involving inflammation included RTI, pneumonia, UTI, OM and tonsillitis. Mean (SD) of morbidity data in the participants from the non Hp 2-2 subgroup was 1.58 (±2.31), and mean (SD) in Hp 2-2 subgroup was 2.2692 (±2.33570), and this difference was statistically significant (p=0.043). After the number of episodes of RTI, pneumonia, UTI, OM and tonsillitis during the entire follow up period was taken in account, and comparison between the Hp phenotype subgroups was performed, the same trend was established. Mean (SD) of this morbidity data in the participants from non Hp 2-2 subgroup was 1.90 (±2.79), and mean (SD) in Hp 2-2 subgroup was 2.65 (±2.90), (p=0.061).

Table 2. *Morbidity characteristics.*

Data	Hp phenotypes		P Value
	1–1/2–1 (n=48)	2–2 (n=52)	
BPD	8 (16.7%)	9 (17.3%)	p>0.99
Respiratory Tract Infection	18 (37.5%)	20 (38.5%)	p=0.921
Pneumonia	5 (10.4%)	7 (13.5%)	p=0.640
Urinary Tract Infection	2 (4.2%)	8 (15.4%)	p=0.094
Otitis Media	15 (31.3%)	19 (36.5%)	P=0.577
Tonsillitis	14 (29.2%)	21 (40.4%)	p=0.240
Scarlet fever	0	1 (1.9%)	p>0.99
Dysentery and acute gastroenteritis	6 (12.5%)	4 (7.7%)	p=0.514
Hyper-reactive airways	12 (25.0%)	11 (21.2%)	p=0.648
Asthma	3 (6.3%)	9 (17.3%)	p=0.089
Celiac disease	1 (2.1%)	1 (1.9%)	p>0.99
Atopic dermatitis	5 (10.2%)	9 (17.3%)	p=0.321
Urticaria	2 (4.2%)	0	p=0.228
Anaphylaxis	0	1 (1.9%)	p>0.99
Adenoid and/or tonsills hyperthrophy	4 (8.3%)	5 (9.6%)	p>0.99
Adenoidectomy and/or tonsillectomy	2 (4.2%)	3 (5.8%)	p>0.99
Atrial septum defect/ventricular septum defect	2 (4.2%)	0	p=0.228
Syncope	1 (2.1%)	0	p=0.480
Abdominal Pain	1 (2.1%)	4 (7.7%)	p=0.364
Gastro-Esophageal Reflux	0	4 (7.7%)	p=0.119
Congenital uretherocele, partial uretherectomy	1 (2.1%)	0	p=0.480
Vesiculo-uretheral reflux	1 (2.1%)	1 (1.9%)	p>0.99
Multicystic kidney	0	1 (1.9%)	p>0.99
Hypospadias	0	1 (1.9%)	p>0.99
Hearing Impairment	5 (10.4%)	3 (5.8%)	p=0.475
Serous otitis and ventilation tubes insertion	2 (4.2%)	3 (5.8%)	p>0.99
Thyroglossal duct cyst	1 (2.1%)	0	p=0.480
Brachial cleft sinus/cyst	0	1 (1.9%)	p>0.99
Dacryostenosis/dacryostomy	0	1 (1.9%)	p>0.99
Strabismus	1 (2.1%)	0	p=0.480
Cryptorchidism and orchiopexy	0	2 (3.8%)	p=0.496
Inguinal hernia repair	5 (10.4%)	3 (5.8%)	p=0.475
Transient sinovitis	3 (6.3%)	5 (9.6%)	p=0.717
Fracture/trauma	8 (16.7%)	9 (17.3%)	p=0.932
Failure to thrive	1 (2.1%)	1 (2.0%)	p>0.99
Short stature	2 (4.2%)	2 (3.8%)	p>0.99
Tic disorder	1 (2.1%)	1 (1.9%)	p>0.99
Epilepsy	1 (2.1%)	0	p=0.480
Enuresis/encopresis	1 (2.1%)	1 (1.9%)	p>0.99
External hydrocephalus	0	2 (3.8%)	p=0.496
Attention deficit hyperactivity disorder	11 (22.9%)	8 (15.4%)	p=0.445
Developmental delay global	0	1 (1.9%)	p>0.99
Skin lesions (pigmented nevus, hemangioma)	0	3 (5.8%)	p=0.244
Vitiligo	1 (2.1%)	0	p=0.480
Hospitalization rate	24 (49.0%)	22 (43.1%)	p=0.558

It is known that wheezing episodes and asthma are common in children born prematurely and suffering from BPD [9]. In this study, the incidence of wheezing episodes and asthma in participants suffering from BPD was compared between the Hp groups. Asthma diagnosis was considered after 5 years of age [10]. BPD was diagnosed in 17 participants. Eight of them belonged to the non Hp 2-2 subgroup, and nine had Hp 2-2 phenotype. Asthma was

determined in one non Hp2-2 participant (12.5% of those defined as BPD in this subgroup), and in four Hp2-2 participants (44.4% of BPD patients from Hp2-2 subgroup) (p=0.294).

Bacteriological data was collected from medical records. A total of 12 blood cultures taken from participants were sterile. Four participants were ascribed to non Hp 2-2 subgroup and eight to Hp 2-2 subgroup. Ten participants were suspected for UTI. Two of them (4.2%) belonged to non Hp 2-2 subgroup and eight (15.4%) were ascribed to Hp 2-2 group (p=0.094). Eight patients had positive urine cultures with different Gram-negative bacteria growth. Due to small number of positive urine cultures it was not possible to compare the Hp subgroups regarding this parameter.

24 (49.0%) children with non Hp 2–2 phenotype and 22 children (43.1%) with Hp 2–2 phenotype need hospitalization, but the difference between the groups was not statistically significant (p=0.558).

4. Discussion

This study followed late morbidity in children who were evaluated for Hp phenotype in the neonatal period. One hundred participants were enrolled in the study.

The pathophysiological mechanisms of many pediatric diseases have an inflammatory component. Oxidative stress is an integral part of pneumonia and OM pathogenesis [5, 10-13]. Infections also have a prominent role in this study group [6, 7, 14-16].

RTIs are characterised by a mucosal neutrophilic inflammatory reaction and pro-inflammatory cytokines release [17]. Elevated concentrations of pro-inflammatory cytokines, as well as chemokines for cellular infiltration and factors for cellular proliferation were found in viral rhinitis [18]. Disturbed cytokine production in OM contributed to altered inflammatory response and otitis-prone condition [19]. Scharer et al suggested a systemic inflammatory response development during acute OM, based on detection of elevated cytokines concentrations, especially interleukin 6 (IL-6) and IL-8 [14]. It appeared that IL-8 had been up-regulated in acute tonsillitis [20].

Neutrophil infiltration is a part of RTIs pathogenesis and in asthma exacerbations [21]. Increased cytokine concentrations were detected in asthmatic patients with pneumonia and bronchitis [15]. Anti- and pro-inflammatory cytokines appear to play a role in acute immune response to respiratory syncytial infection (RSV), subsequent hyper-reactive airways and asthma development [7]. Urine IL-6 and IL-8 were increased in children with febrile UTI, compared to children with fever of other origin [21].

In this follow up study, statistical difference between the non Hp 2-2 and Hp 2-2 phenotypes was not found in any single morbidity. However, when disorders with an inflammation component in their pathogenesis were grouped together (RTI, pneumonia, UTI, OM and tonsillitis), there was a significantly higher prevalence of this inflammatory phenotype in the Hp2-2 group (p=0.043). Subsequent comparison of the cumulative number of episodes of these diseases between the Hp phenotypes during entire observation period marked a trend in the same direction (p=0.061).

Hp phenotypes are involved in Epstein-Barr virus (EBV) infection and patients with non Hp 2-2 phenotype are less prone to positive EBV serology [16]. F Yang et al found that expression of the human Hp transgene in alveolar macrophages was upregulated when the transgenic mice carrying a human Hp 2 gene were treated with endotoxin. They also detected human Hp messenger RNA in circulating eosinophils in humans and in Hp transgenic mice. They suggested that Hp is involved in a variety of lung inflammatory disease, including respiratory allergy and asthma [22]. In this study, no statistically significant differences between the incidence of asthma among the non Hp 2-2 and the Hp 2-2 phenotypes were detected.

Papp et al demonstrated that in the celiac population phenotype Hp2-2 had a predisposition to a more severe clinical course [23]. In this study none of the participants had celiac disease.

BPD is a common complication of extreme prematurity. High levels of pro-inflammatory cytokines like IL-1β, IL-6, IL-8 and TNF-α, in the tracheal aspirate of the mechanically ventilated preterm infant were associated with BPD development [24]. Children with BPD have higher risk for hospitalization over the first few years of life due to respiratory infections, and they are more prone to suffer from asthma later in childhood [9, 25]. The Hp 2-2 phenotype was noted to be a risk factor in diseases which have pathogenesis of inflammation. [4]. This study compared the incidence of asthma among participants with BPD between the Hp subgroups, however the differences were not found to be statistically significant.

The Hp 2–2 phenotype has been reported to have higher bacteriostatic activity compared to non Hp 2-2 phenotypes [2]. In this study, all blood cultures taken from participants were sterile. Due to the small number of positive urine cultures it was not possible to compare the Hp subgroups. No statistically significant differences were found between the Hp subgroups regarding this parameter. Our data does not reflect the possible bacteriostatic effect of the Hp 2-2 phenotype on morbidity rates.

The study has some limitations. The study is retrospective. The sample size of the participants was relatively small. In part of the cases medical history records review didn't reveal additional data, and most morbidity information was based on phone questionnaires only.

5. Conclusion

This study followed late morbidity in children who were evaluated for the Hp phenotype in the neonatal period. The comparison of the consolidated group of diseases with a clear involvement of inflammation in their pathogenesis, which included RTI, pneumonia, UTI, OM and tonsillitis, demonstrated preponderance for the phenotype with less anti-

inflammatory activity, Hp2-2 (p=0.043). The difference between the anti-inflammatory of Hp 1 and Hp 2 alleles is not well underestimated. Further research involving a larger population is required for better understanding the possible influence of different Hp alleles on pediatric morbidity.

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