Endocr (2010) 38:303–308
DOI 10.1007/s12020-010-9392-7

ORIGINAL ARTICLE

Haptoglobin phenotype in women with preeclampsia

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Received: 12 April 2010 / Accepted: 26 July 2010 / Published online: 23 October 2010

Abstract In pre-eclampsia, poor placentation causes both oxidative and endoplasmic reticulum stress of the placenta. The anti-oxidative protein Haptoglobin has three phenotypes: 1-1, 1-2, and 2-2. Haptoglobin 1-1 is a more potent antioxidant. Our objective was to determine whether haptoglobin 1-1 was less common in women with pre-eclampsia which is a disease with an oxidatives-stress component, compared to the healthy population. Haptoglobin phenotype was compared in 240 healthy and 120 preeclamptic gravida in a case–control study. Statistical analysis was performed using Chi square test. The prevalence of haptoglobin 1-1 was 13% among healthy women and 6% among preeclamptic women ($P = 0.049$). Secondary analysis was also performed. The prevalence of haptoglobin 1-1 is higher in healthy compared to pre-eclamptic subjects, a finding compatible with a protective role. Haptoglobin 1-1 might have a protective role in pre-eclampsia. Further work is needed with more Hp 1-1 subjects before we can conclude on the possible use of Haptoglobin phenotype to assess the risk of preeclampsia.

Keywords Haptoglobin · Phenotype · Preeclampsia · Oxidative stress

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Introduction

Preeclampsia is a leading cause of maternal morbidity and mortality, and one of the most common complications of pregnancy [1], characterized by hypertension and proteinuria, developing after 20 weeks of gestation. Many etiologies have been implicated in its pathogenesis [2–6], and it appears that the disease results from an abnormal invasion of the chorionic villi after implantation [3]. In the last few years, several studies have suggested that preeclampsia is in fact related to an imbalance of circulating angiogenic factors resulting in endothelial dysfunction [7] and increased oxidative stress. According to one hypothesis, an inflammatory process is involved, leading to oxidative stress [5]. This oxidative stress is the result of placental hypoxia and reperfusion, with production of reactive free oxygen species causing endothelial dysfunction [8]. Antioxidants, therefore, have been postulated to have a protective role against preeclampsia, even though inconsistent results have been obtained in studies that examined the relationship between various antioxidants like vitamin C and E and preeclampsia [6, 9, 10].

Haptoglobin is an alpha-2 sialoglycoprotein that is found in plasma. Its primary physiological role consists of binding free hemoglobin [11], thereby preventing the release of heme iron which can cause oxidative damage [12]. Therefore, Haptoglobin is considered to have antioxidative

properties [13]. Additional roles consist of inhibition of prostaglandin synthesis and induction of vascular endothelial proliferation [14].

The Haptoglobin gene is polymorphic with two classes of alleles denoted 1 and 2 and correspondingly there exists three Haptoglobin genotypes: Hp 1-1, Hp 2-1, and Hp 2-2 [15]. The Haptoglobin phenotype is defined as the polymer distribution of Haptoglobin molecules identified by electrophoresis in the serum of a given individual, with dimers found in Hp 1-1 individuals, linear polymers in Hp 2-1 individuals, and cyclic polymers found in Hp 2-2 individuals [11]. There is a 100% correspondence between the Haptoglobin genotype as determined by PCR and the Haptoglobin phenotype as determined by gel electrophoresis [16, 17]. Hp 1 and Hp 2 allelic protein products have different biological properties [11, 18]. Hp 1-1 binds and clears free hemoglobin with higher efficacy compared to the larger Hp 2-2 molecule. It is possible that the smaller size of Hp 1-1 improves the clearance of the Haptoglobin–Hemoglobin complex by the CD163 Haptoglobin–hemoglobin scavenger receptor compared to the larger Hp 2-2 [19]. Therefore, Hp 1-1 is considered to be a more potent antioxidant than the other two phenotypes. The relative frequency of the different Haptoglobin phenotypes varies among different ethnic groups and in different geographical areas [20].

The aim of our study was to determine the distribution of the various Haptoglobin phenotypes in preeclamptic and healthy gravida. Assuming a protective role for antioxidants in preeclampsia, we hypothesized that Hp 1-1 would be less prevalent in preeclamptic women compared to the control group, while Hp 2-2 would be more prevalent in preeclamptic women.

Results

Two hundred and forty healthy women and 120 women with preeclampsia were included in this study. Demographic and obstetrical data are presented in Table 1.

Table 1 Summary of demographic and obstetrical data in the two groups (Preeclampsia & Healthy)

	Preeclampsia ($n = 120$)	Healthy ($n = 240$)	P value
Age (SD)	29.9 (6.5)	30.9 (5.5)	0.05
Jewish patients (%)	75 (62.5)	150 (62.5)	0.05
Non-Jewish patients (%)	45 (37.5)	90 (37.5)	0.05
Gravidity (SD)	2 (1.7)	2.6 (1.8)	0.0001
Parity (SD)	1.5 (1.1)	2 (1.3)	0.0001
Gestational week at delivery (SD)	35.6 (4)	38.5 (2)	0.0001

SD standard deviation

Among women with preeclampsia, 7 had the 1-1 phenotype, 62 had the 2-1 phenotype, and 51 had the 2-2 phenotype. In the control group, 30 women had the 1-1 phenotype, 109 had the 2-1 phenotype, and 101 had the 2-2 phenotype. The results are shown in Fig. 1. The difference between the Hp 1-1 phenotype (vs. non 1-1 phenotype) prevalence in the two groups (e.g. preeclampsia and healthy) was statistically significant, with a $P > 0.05$.

Among women with the 1-1 phenotype, 7/37 (18.9%) had preeclampsia and 30/37 (81.1%) were healthy, in women with the 2-2 phenotype, 51/152 (33.6%) had preeclampsia and 101/152 (66.4%) were healthy, and in women with the 2-1 phenotype, 62/171 (36.3%) had preeclampsia and 109/171 (63.7%) were healthy ($P = 0.127$ refers to the comparison between all three phenotype groups of the percentage of preeclamptic women in each group).

Among women at first delivery ($n = 183$), 75% with the 1-1 phenotype were healthy ($n = 12$), while only 49.7% with the other two phenotypes ($n = 83$) were healthy ($P = 0.053$). The odds ratio for having preeclampsia among women at first delivery with the Hp 1-1 phenotype compared to the other two phenotypes was 0.329 (95% CI

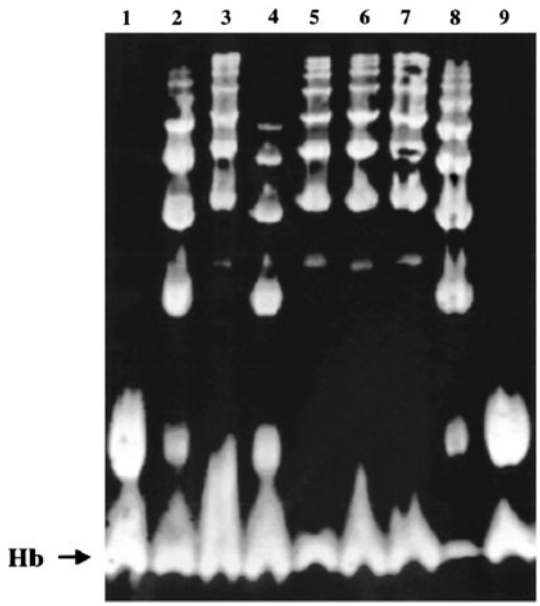

Fig. 1 Differentiation between Haptoglobin phenotypes by gel electrophoresis. Representative patterns of Hp phenotypes1-1, 2-1, and 2-2 after polyacrylamide gel electrophoresis hemoglobin-enriched serum. Bands correspond to Hp–hemoglobin complexes. A band visible in each lane corresponding to free unbound hemoglobin (Hb) is indicated with an *arrow*. Hp 1-1 shows a single rapidly migrating band. Hp 2-2 has a series of more slowly migrating bands. Hp 2-1 displays another series of slowly migrating bands and a weak band that migrates similar to the Hp 1-1 band. Sample preperation, electrophoresis, and staining with peroxidase were performed as described in Sect. 2. Hp 1-1 is present in *lanes 1 and 9*; Hp 2-1 is present in lanes *2, 4, and 8*; Hp 2-2 is present in *lanes 3, 5, 6, and 7*

Table 2 The frequency of the different phenotypes among groups according to severity of preeclampsia

	Preeclampsia		Healthy ($n = 240$)	P value
	Severe ($n = 66$)	Mild ($n = 54$)		
No. of 2-2 phenotype (%)	28 (42.4)	23 (42.6)	101 (42.1)	0.05
No. of 2-1 phenotype (%)	34 (51.5)	28 (51.8)	109 (45.4)	0.05
No. of 1-1 phenotype (%)	4 (6.1)	3 (5.6)	30 (12.5)	0.05

0.1–1.06) (P value = 0.063). In multiparous women, the difference in prevalence of the different phenotypes was not statistically significant ($P > 0.05$).

There were 66 women with severe preeclampsia and 54 women with mild preeclampsia. The distribution of the different phenotypes among them is shown in Table 2. The P value was 0.99 for all comparisons. The 2-2 phenotype was not found to be more prevalent in women with severe preeclampsia ($P > 0.05$). Additionally, no statistically significant difference was found when comparing women with severe preeclampsia to healthy women.

In women with preeclampsia, 38 gave birth at or before 34 full weeks, and 82 gave birth after 34 weeks. The distribution of the different phenotypes among them is shown in Table 2. The P value was 0.43 for the comparison in the 1-1 phenotype group, 0.126 for the 2-2 phenotype group, and 0.3 for the 2-1 phenotype group. Additionally, no statistically significant difference was found when comparing women with early preeclampsia to healthy women.

There were 33 women with the severe disease who gave birth at or before 34 weeks, and 87 women with other preeclampsia. The differences were not statistically significant (P value = 0.67, 0.22 and 0.4 for the 1-1, 2-2, and 2-1 phenotypes, respectively). Additionally, no statistically significant difference was found when comparing women with early and severe preeclampsia to healthy women.

Discussion

In this study, we have demonstrated that women with the Hp 1-1 phenotype had a significantly lower likelihood of developing preeclampsia suggesting that women with this type of Haptoglobin may be protected from developing preeclampsia. This appears to be consistent with several current models of the pathogenesis of preeclampsia and differences in the biological activity of the protein products of the two different Haptoglobin alleles. Specifically, hemoglobin-induced oxidative stress has recently been demonstrated to play an important role in the pathogenesis of preeclampsia [21]. The primary role of the Haptoglobin protein is to bind to hemoglobin and inhibit hemoglobin-induced oxidative stress [11]. Moreover, the Haptoglobin 1-1 protein is superior to the Haptoglobin 2-1 and Haptoglobin 2-2 proteins in blocking oxidative stress mediated by hemoglobin [19]. In a secondary analysis, we have examined the prevalence of the different haptoglobin phenotypes according to the severity of preeclampsia. Comparison according to these three definitions, in groups of severe disease, mild disease, and no disease, yielded no statistically significant differences in the distribution of the different phenotypes. This may possibly be related to the insufficient sample size of the groups. However, there was a clear trend of increasing prevalence of Hp 1-1 phenotype from early onset disease, to late onset disease to no disease, and a similar trend of increasing prevalence, from early severe disease to other disease to no disease. These results support the hypothesis that Hp 1-1 may offer protection against preeclampsia.

There were some differences in the obstetrical characteristics between healthy and preeclamptic women; the gravidity and parity were lower in the preeclampsia group, as was the mean gestational age at delivery. The lower gravidity/parity is explained by the well-known increased incidence of preeclampsia in nulliparous women. The lower gestational week at delivery is probably iatrogenic, as preeclampsia is known to be an indication for early induction of labor. However, we assume these differences would not act as confounding factors since they have no effect of the prevalence of the different haptoglobin phenotype, which is genetically determined. The ethnic origin which might actually affect the relative frequency of the different phenotypes was equal in the two groups, due to intentional stratification.

There have been three prior reports examining the relationship between the Haptoglobin type and preeclampsia. Chandra et al. [22] demonstrated that pregnancy-associated hypertension was significantly higher in Hp 2 individuals, consistent with our findings presented here. Raijmakers et al. [23] demonstrated no differences between healthy women and women with either severe preeclampsia or hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome in regard to the relative prevalence of the different haptoglobin phenotypes. Of note there were only 25 women in this study with preeclampsia with the majority of the study population having the HELLP syndrome so that the study population may have been too small to observe a protective effect of the Haptoglobin 1-1 type on preeclampsia. Finally, Depypere [24] demonstrated an increased prevalence of Hp 1-1 phenotype in women with preeclampsia, and even more so in women with the severe disease. One possible reason for the differences between our study and that of Depypere

may have been due to genetic differences between the two ethnically distinct populations.

In conclusion, our study is the fourth report in the medical literature to discuss the relationship between preeclampsia and haptoglobin phenotype. It is the largest, and its findings, that Haptoglobin 1-1 appears to provide protection against preeclampsia, are supported by pathophysiological mechanisms suggesting free hemoglobin plays an important role in the development of eclampsia and by functional differences between different Haptoglobin proteins.

Establishing the role of the haptoglobin phenotype in the future as a protective factor against preeclampsia might lead to its integration as a screening tool for predicting this disease. If prophylactic measures against preeclampsia are to be found in the future, determining the haptoglobin phenotype might establish a high risk group wherein prophylactic antioxidative treatment might be most useful.

Methods

Subjects

The study population consisted of 240 healthy and 120 preeclamptic women (matched by origin: Jewish/Arab), delivered at the Bnai-Zion Medical Center, which is a level 3 university hospital that mainly serves the urban population of the city of Haifa, Israel. All pregnant women giving birth after 24 weeks of gestation were eligible for the study. Women with chronic hypertensive disorder were excluded. Preeclampsia was defined as blood pressure exceeding 140/90 mmHg in repeat measurements, along with proteinuria of +1 or more measured by Mann–Whitney test (Mann–Whitney U test is the alternative test to the *t*-test. Mann–Whitney U test is a non-parametric test that is used to compare two population means that come from the same population. Mann–Whitney U test is also used to test whether two population means are equal or not) or 300 mg or more in 24 h urine collection. The group with severe preeclampsia ($n = 120$) was defined when one of the following existed: systolic blood pressure exceeding 160 mmHg, diastolic blood pressure exceeding 110 mmHg, proteinuria exceeding 5 g in a 24 h urine collection, laboratory findings consistent with the hemolysis, elevated liver enzymes and low platelets syndrome (HELLP), or the existence of intrauterine growth restriction. The control group ($n = 240$) consisted of healthy women with no known hypertension or a history of a hypertensive disorder of pregnancy in prior gestations, with normal blood pressure measurements throughout the entire hospital stay. For each woman, age, gestational age at delivery, number of prior gestations and deliveries, ethnic origin, severity of preeclampsia, and haptoglobin phenotype were documented. The study was approved by the Human Research Ethics Committee of the Bni-Zion Medical Center. Informed consent was obtained from all women.

Phenotyping

In women with preeclampsia, blood was collected upon arrival to the delivery room or maternity ward, and after the diagnosis of preeclampsia had been established. In healthy women, blood was collected 24 h after delivery in order to rule out cases of preeclampsia that developed intrapartum or postpartum. Five milliliters of blood were drawn from each woman through venipuncture. Samples were allowed to clot and stored at 4°C until analysis. A 10% hemoglobin solution in water was prepared from heparinized blood by first washing the blood cells five times in phosphate-buffered saline and then lysing the cells in 9 ml of sterile water per milliliter of pelleted cell volume. The cell lysate was centrifuged at $10{,}000 \times g$ for 40 min and the supernatant containing hemoglobin was aliquoted and stored at −70°C. Haptoglobin phenotyping was determined by gel electrophoresis and peroxidase staining using a modification of previously described methods [6, 25]. Briefly, serum (10 µl) was mixed with 2 µl of the 10% hemoglobin solution and the samples permitted to stand for 5 min at room temperature in order to allow the Haptoglobin–Hb complexes to form. An equal volume (12 µl) of sample buffer containing 125 mM TrisBase pH 6.8, 20% (w/v) glycerol and 0.001% (w/v) bromophenol blue was added to each sample prior to running on the gel. The Haptoglobin–Hb complex was resolved by polyacrylamide gel electrophoresis (PAGE) using a buffer containing 25 mM TrisBase and 192 mM glycine. The stacking gel was 4% polyacrylamide (29:1 acrylamide/bis-acrylamide) in 125 mM Tris-Base, pH 6.8 and the separating gel was 4.7% polyacrylamide (29:1 acrylamide/bis-acrylamide) in 360 mM Tris-Base, pH 8.8. Electrophoresis was performed at a constant voltage of 250 V for 3 h. After the electrophoresis was completed, the Haptoglobin–Hb complexes were visualized by soaking the gel in freshly prepared staining solution in a glass tray. The staining solution (prepared by adding the reagents in the order listed) contained 5 ml of 0.2% (w/v) 3,3′,5,5′-tetramethylbenzidine in methanol, 0.5 ml dimethylsulfoxide, 10 ml of 5% (v/v) glacial acetic acid, 1 ml of 1% (w/v) potassium ferricyanide and 150 µl of 30% (w/w) hydrogen peroxide. The bands corresponding to the Haptoglobin–Hb complex were readily visible within 15 min and were stable for over 48 h. All gels were documented with photographs. The staff performing the analysis was blinded to the presence/absence of preeclampsia in each individual.

Statistical analysis

SPSS 15 software was used [26]. The continuous and ordinal variables were presented by mean, median, and standard deviation. The categorical variables were presented in percentages. Statistical analysis was performed to check for differences between healthy and preeclamptic women in age, gravidity, parity, ethnic origin, and gestational age. Chi square test was used to check the difference between the categorical variables. Mann–Whitney test was used to check differences between the ordinal variables. Independent *t*-test was used to check differences between the continuous variables. Comparisons of the different phenotypes among women were performed using the chi square test. Logistic regression was used to check the relationship of multiple variables with preeclampsia and calculate the odds ratio with 95% confidence interval.

The distribution of the different phenotypes was compared in the following groups:

(1) Preeclamptic versus healthy, (2) Severe preeclampsia compared with mild preeclampsia and healthy women (separately), (3) Preeclampsia with delivery at or below 34 weeks compared with preeclampsia beyond 34 weeks and healthy women (separately), and (4) Severe preeclampsia at or below 34 weeks compared with other preeclampsia (mild preeclampsia at or below 34 weeks, mild preeclampsia beyond 34 weeks, and severe preeclampsia beyond 34 weeks) and healthy women. The study population was also grouped according to the haptoglobin phenotype, and the proportion of healthy and preeclamptic women was compared in each groups. In addition, in each of the three groups, stratification was performed according to parity (first delivery vs. repeat delivery).

Acknowledgment This work was supported in part by Abutbul Family in the memory of Abutbul Daniel

References

1. G. Pridjian, J.B. Puschett, Preeclampsia. Part 1: clinical and pathophysiologic considerations. Obstet. Gynecol. Surv. **57**, 598–618 (2002)
2. F.G. Cunningham, K.J. Leveno, S.L. Bloom, J.C. Hauth, L. Gilstrap, K.D. Wenstrom, *Williams Obsterics*, 22nd edn. (McGraw-Hill, New York, NY, 2005)
3. F. De Wolf, C. De Wolf-Peters, I. Brosens, The human placental bed: electron microscopic study of trophoblastic invasion of spiral arteries. Am. J. Obstet. Gynecol. **137**, 58 (1980)
4. A.D. Bardeguez, R. McNerney, M. Frieri, Cellular immunity in preeclampsia: alterations in T lymphocyte subpopulations during early pregnancy. Obstet. Gynecol. **77**, 859 (1991)
5. M.T. Gervasi, T. Chaiworapongsa, P. Pacora, Phenotypic and metabolic characteristics of monocytes and granulocytes in preeclampsia. Am. J. Obstet. Gynecol. **185**, 792 (2001)
6. C. Zhang, M.A. Williams, I.B. King, Vitamin C and the risk of preeclmapsia—results from dietary questionnaire and plasma assay. Epidemiology **13**, 382 (2002)
7. Walter.P. Mutter, S. Ananth Karumanchi, Molecular mechanisms of preeclampsia. Microvasc. Res. **75**(1), 1–8 (2008)
8. C.W.G. Redman, I.L. Sargent, Placental stress and pre-eclampsia: a revised view placenta 30, supplement A. Trophobl. Res. **23**, S38–S42 (2009)
9. J.M. Roberts, L. Myatt, C.Y. Spong, E.A. Thom, J.C. Hauth, K.J. Leveno, G.D. Pearson, R.J. Wapner, M.W. Narner, J.M. Thorp, G.B. Anderson, Vitamins C and E to prevent complications 13 of pregnancy-associated hypertension. N. Engl. J. Med. **362**(14), 1282–1291 (2010)
10. S.J. Mao, M.T. Yates, R.L. Jackson, Antioxidant activity and serum levels of probucol and probucal metabolites. Methods Enzymol. **234**, 505–513 (1994)
11. M.R. Langlois, J.R. Delanghe, Biological and clinical significance of haptoglobin polymorphism in humans. Clin. Chem. **42**(10), 1589–1600 (1996)
12. E. Tolosano, S. Fagoonee, E. Hirsch, F.G. Berger, Enhanced splenomegaly and severe liver inflammation in haptoglobin/hemopexin double-null mice after acute hemolysis. Blood **100**, 4201–4208 (2002)
13. C.F. Tseng, C.C. Lin, H.Y. Huang, H.C. Liu, S.J. Mao, Antioxidant role of human haptoglobin. Proteomics **4**(8), 2221–2228 (2004)
14. M.C. Cid, D.S. Grant, G.S. Hoffman, R. Auerbach, A.S. Fauci, H.K. Kleinman, Identification of haptoglobin as an angiogenic factor in sera from patients with systemic vasculitis. J. Clin. Investig. **91**(3), 977–985 (1993)
15. A.P. Levy, R. Asleh, S. Blum, N.S. Levy, R. Miller-Lotan, S. Kalet-Litman, Y. Anbinder, O. Lache, F.M. Nakhoul, R. Asaf, D. Farbstein, M. Pollak, Y.Z. Soloveichik, M. Strauss, J. Alshiek, A. Livshits, A. Schwartz, H. Awad, K. Jad, H. Goldenstein, Haptoglobin: basic and clinical aspects review. Antioxid. Redox Signal **12**(2), 293–304 (2010)
16. O. Smithies, Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. Biochem. J. **61**, 629–641 (1955)
17. R. Asleh, A.P. Levy, In vivo and in vitro studies establishing haptoglobin as a major susceptibility gene for diabetic vascular disease. Vasc. Health Risk Manag. **1**(1), 19–28 (2005)
18. W. Koch, W. Latz, M. Eichinger, A. Roguin, A.P. Levy, A. Schomig, A. Kastrati, Genotyping of the common haptoglobin Hp ½ polymorphism based on PCR. Clin. Chem. **48**, 1377–1382 (2002)
19. A.P. Levy, R. Asleh, S. Blum, N.S. Levy, R. Miller-Lotan, S. Kalet-Litman, Y. Anbinder, O. Lache, F.M. Nakhoul, R. Asaf, D. Farbstein, M. Pollak, Y.Z. Soloveichik, M. Strauss, J. Alshiek, A. Livshits, A. Schwartz, H. Awad, K. Jad, H. Goldenstein, Haptoglobin: basic and clinical aspects. Antioxid. Redox Signal. **12**(2), 293–304 (2010)
20. K. Carter, M. Worwood, Haptoglobin: a review of the major allele frequencies worldwide and their association with disease. Int. J. Lab. Hematol. **29**, 92–110 (2007)
21. M.G. Olsson, M. Centlow, S. Rutardottir, I. Stenfors, J. Larsson, Maaf B. Hosseini, M.L. Olsson, S.R. Hansson, B. Akerstrom, Increased levels of cell free hemoglobin, oxidative markers and the antioxidant heme scavenger alpha microglobulin in preeclampsia. Free Rad. Biol. Med. **48**, 284–291 (2010)
22. T. Chandra, T. Padma, S. Vishnupriya, R. Venkat Raman, Haptoglobin polymorphism in pregnancy induced hypertension. Am. J. Hum. Gen. **49**(suppl.), 130 (1991)
23. M.T.M. Raijmakers, E.M. Roes, R.H.M. te Morsche, E.A.P. Steegers, W.H.M. Peters, Haptoglobin and its association with the HELLP syndrome. J. Med. Genet. **40**, 214–216 (2003)

24. H.T. Depypere, M.R. Langloise, J.R. Delanghe, M. Temmerman, M. Dhont, Haptoglobin polymorphism in patients with pre-eclampsia. Clin. Chem. Lab. Med. **44**(8), 924–928 (2006)
25. A.P. Levy, I. Hochberg, K. Jablonski, H.E. Resnick, E.T. Lee, L. Best, B.V. Howard, Strong Heart Study. Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: the strong heart study. J. Am. Coll. Cardiol. **40**(11), 1984–1990 (2002)
26. B.P. O'Connor, Simple SPSS flexible SAS programs for analyzing lag-sequential categorical data. Behav. Res. Methods Instrum. Comput. **31**(4), 718–726 (1999)