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Haptoglobin A Pleiotropic Marker in Polycystic Ovary Syndrome-A Study from South India

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders affecting 4-12% of women worldwide; contributing to long term complications associated with high oxidative stress and chronic low grade inflammation, demanding the understanding of diverse contributing factors for better management of this multifactorial condition. Haptoglobin polymorphic forms, an acute phase protein vary with respect to antioxidant activity, angiogenesis and immunomodulatory function. In order to analyze the distribution of various phenotypes of Hp in relation to PCOS, the present study was carried out. A total of 193 patients and 187 healthy age matched, ultrasound scanned controls were included in the present study. Blood samples were obtained for Hp phenotyping by PAGE. The percentage of Hp1-1, Hp1-2 and Hp2-2 phenotypes were 2.07, 33.67, 64.24 and 0.53, 49.19 and 50.26 in patients and controls, respectively. Sizeable increase in the frequency of Hp2-2 (15.5%) and decrease in the frequency of Hp 1-2 in the patients compared to controls suggests heterozygous condition to be significantly protective over the homozygotes in PCOS. The present investigation may be the first one from South Indian population, dealing with distribution of haptoglobin phenotypes in PCOS patients and its relevance with respect to anthropometric measures and serum malondialdehyde levels.

Key words: Haptoglobin, oxidative stress, polycystic ovary syndrome, phenotype, malondialdehyde

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders affecting 4 to 12% of women of reproductive age worldwide (Knochenhauer *et al*., 1998; Farah *et al*., 1999; Hahn *et al*., 2005), however, the estimated frequency may raise to 4-25% depending on the diagnostic criteria used (Jones *et al*., 2010). It is a complex disorder with clinical features changing throughout the life span (Balen and Dunger, 1995; Elting *et al*., 2000). A combination of genetic and environmental factors is implicated for this syndrome. It is characterized by hyperandrogenism, polycystic ovaries and chronic anovulation along with insulin resistance, abdominal obesity and dyslipidemia as frequent metabolic traits (Lee *et al*., 2005). Other key pathophysiologic feature of PCOS is altered gonadotropin-releasing hormone dynamics, causing increased LH and decreased FSH coupled with anovulation (Farzadi and Zadeh, 2006). Such multiple factors predisposes the individual to serious long term consequences such as obesity, type 2 diabetes, endometrial hyperplasia, thyroid dysfunction and cardiovascular disease (Ovalle and Azziz, 2002; Legro, 2003; Hardiman *et al*., 2003).

Till date, a number of studies revealed the association of Haptoglobin (Hp) phenotypes with a variety of complex disorders like type II diabetes, Cardiovascular Disorders (CVD), cancers and reproductive anomalies associated with high Oxidative Stress (OS). OS refers to an imbalance between Reactive Oxygen Species (ROS) and the antioxidant defense system which buffers the oxidative damage. The resultant OS causes increased tissue/cellular damage manifested by lipid peroxidation, protein oxidation, DNA damage (Pasaoglu *et al*., 2004; Marjani, 2010; Sadrzadeh and Bozorgmehr, 2004) and hemoglobin induced oxidative injury. Hemoglobin (Hb) released from erythrocytes is highly toxic and mediates iron driven oxidative stress and inflammation (Langlois and Delanghe, 1996; Conn *et al*., 2000). To surpass this activity, Hp an acute phase protein synthesized by the liver under the influence of several cytokines, binds to free Hb released from destructed RBCs stoichiometrically and irreversibly (Saeed *et al*., 2003); forms soluble complexes (Hp-Hb), thereby reduces the generation of ROS, ameliorates OS and hence plays an important role as an antioxidant. The antioxidant capacity of Hp 2-2 in circulation is lower than that of Hp1-1 b e cause Hp 2-2 binds to hemoglobin with lower affinity than Hp 1-1 (Ghadam *et al*., 2008; Lee *et al*., 2005; Marjani, 2010). It also inhibits the synthesis of prostaglandins and vasodilatation mediated by nitric oxide and exerts immunomodulatory actions (Langlois and Delanghe, 1996).

The genetic polymorphism of serum Hp is controlled by two co-dominant alleles (Hp1 and Hp2) and results in three phenotypes, designated as Hp1-1, Hp2-1 and Hp2-2. Hp1-1 is a homodimer, Hp1-2 a linear polymer and Hp2-2 is a large circular polymer (Conn *et al*., 2000). Different functional properties of these polymorphism (s) are associated with a variety of human disorders (Sadrzadeh and Bozorgmehr, 2004); however, there are not many studies with respect to PCOS. The present investigation may be the first one from South Indian population, dealing with distribution of haptoglobin phenotypes in PCOS patients and its relevance with respect to anthropometric measures and serum malondialdehyde levels. To our knowledge this is also the first report from the Asian population with respect to PCOS and Hp.

MATERIALS AND METHODS

The current study was carried out in 380 subjects comprising of 193 patients and 187 normal ultrasound scanned women as controls. None of controls had signs or symptoms of hyperandrogenism, menstrual dysfunction or history of infertility. Patients were selected based on Rotterdam criteria proposed by ESHRE in 2003 (ESHRE Workshop, 2003) and were obtained from Government Maternity Hospital, Hyderabad, India. Informed consent from the study group and ethical clearance was obtained from institutional ethical committee (Department of Genetics, Osmania University). The anthropometric details required for Body Mass Index (BMI) and Waist Hip (W/H) ratio were recorded.

Five milliliter of blood through veinipuncture from all the participants was obtained; serum was separated and frozen at -20°C until assayed. Samples were prepared, PAGE was performed and the gels were stained with benzedine for Hp phenotyping by Davis method (Davis, 1964). Hp phenotyping was performed essentially as described previously. Briefly, samples were pretreated at 37°C by mixing 10 μL serum and 10 μL of freshly prepared hemolysate and electrophoresed using a 10% polyacrylamide gradient gel at 150 V for 3 h and the bands were visualized with benzedine (Sigma) developing solution. Electrophorograms (Fig. 1) were analyzed for Hp phenotypes. Serum was also subjected for estimation of malonaldialdehyde (MDA) levels, a marker of lipid peroxidation according to Nadiger *et al*. (1987).

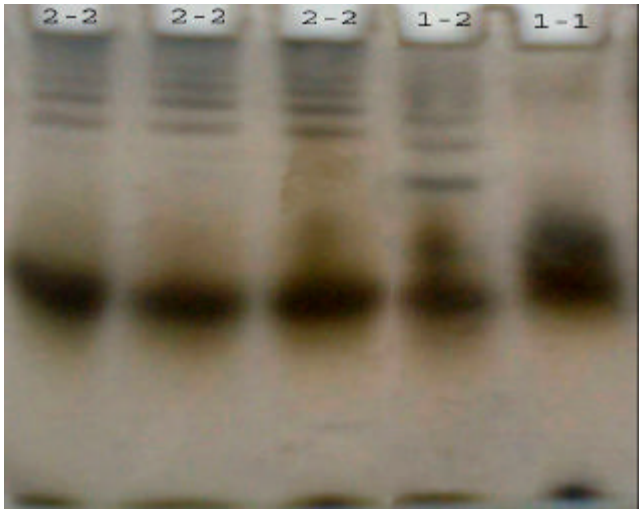

Fig. 1: Gel picture showing different phenotypes of haptoglobins

STATISTICAL ANALYSIS

All the values were expressed as Mean±Standard Deviation (SD) unless otherwise stated. For statistical comparisons between the patients and control group, t-test for independent samples were used. The association of Hp phenotypes with PCOS was explored using the chi square. Odds Ratio (OR) with 95% Confidence Interval (CI) in the two study groups were calculated to assess the phenotype (s) associated with high risk. A two-tailed p value of less than 0.05 was considered to be significant. The SPSS package (16th version) was used to perform statistical analysis.

RESULTS AND DISCUSSION

The data analysis of 380 individuals (193 patients and 187 controls) revealed 2.07% Hp1-1, 33.67% Hp1-2 and 64.24% Hp2-2 in patients and 0.53%, 49.19% and 50.26% of Hp 1-1, Hp 1-2 and Hp 2-2 phenotypes in controls respectively. Sizeable increase in the frequency of Hp2-2 (15.5%) and decrease in the frequency of Hp 1-2 (16%) in the patients compared to controls, suggestive of heterozygous condition being protective over the homozygotes in PCOS. Hp 2-2 vs others and Hp 1-2 vs others revealed an OR value of 1.98 and 0.46 correspondingly, demonstrating a twofold increased frequency of Hp 2-2 phenotype in the patient group compared to the other phenotypes (Table 1, 2).

There is a remarkable difference with respect to Hp1 allele in different populations (Carter and Worwood, 2007) all over the world. Four out of 193 patients and one out of 187 in controls were with Hp1-1 phenotype in the present study. As the number of individuals belonging to this category was limited, this section of women were not considered for further analysis in relation to quantitative measures such as anthropometric and biochemical analysis.

A perusal of Table 3 revealed that indicators of obesity (BMI), abdominal adiposity (W/H) and oxidative stress (MDA) all were significantly elevated in the patient group compared to the controls ($p<0.05$).

Further categorization of the patients and controls with respect to different Hp phenotypes in relation to BMI (25.38±4.76 vs 25.41±5.42) and W/H (0.78±0.05 vs 0.78±0.05) revealed no

significant difference between Hp 1-2 and Hp 2-2. This lack of difference observed in present study could not be explained on the basis of graded circulating hp levels as shown by Alvarez-Blasco *et al.* (2009) (Table 3). The mean MDA levels were appreciably elevated in individuals with Hp 2-2 phenotypes compared to Hp 1-2 in patients, however, did not reach the significant level within the patient group (400.64±142.13 vs 363.33±144.56, p = 0.119).

Haptoglobin polymorphism causes a gene dosage effect resulting in a graded decrease in the haptoglobin ability to bind to hemoglobin (Hp 1-1>Hp 1-2>Hp 2-2) there by the antioxidant capacity (Langlois and Delanghe, 1996). These polymorphic forms also results in a graded increase in the angiogenic and inflammatory effects of haptoglobin, with the Hp2-2 phenotype expressing the strongest properties (Hp2-2>Hp1-2>Hp1-1) (Quaye, 2008). Elevated MDA levels in PCOS women compared to the controls indicates higher oxidative stress in the former group compared to the controls similar to the observations of Sabuncu *et al.* (2001). The borderline increased mean MDA levels in the Hp 2-2 PCOS women compared to women with Hp 1-2 is suggestive of the association of Hp 2 allele with higher oxidative stress which could be due to the free Hb remains in the circulation longer and causes greater oxidative stress (Melamed-Frank *et al.*, 2001; Mishra *et al.*, 2010).

Another significant observation is the elevated frequency of Hp1-2 individuals in controls compared to the patients. However, the mean MDA levels did not vary in these two groups, this may suggest that the antioxidant property of this molecule alone may not be the primary contributory factor for PCOS. The haptoglobin molecule being involved in a number of protein interactions it is difficult to assign a single explanation for the protective role of Hp 1-2 phenotypes in the present study. Investigation (s) pertaining to the physiological role of these molecules may help in translating present results for better understanding of the behavior of this pleiotropic molecule at the molecular level.

Table 1: Showing the distribution of haptoglobin gene polymorphism in controls and patients

Groups	HP 1-1 N (%)	HP 1-2 N (%)	HP 2-2 N (%)
Controls (187)	1 (0.53)	92 (49.19)	94 (50.26)
Patients (193)	4 (2.07)	65 (33.67)	124 (64.24)
Allelic frequency	Hp1		Hp2
Controls	0.24		0.76
Patients	0.18		0.82

Table 2: Showing the odds ratio of patient and control group

Phenotypes	OR	Lower limit	Upper limit	p-value
Hp 1-1	4.54*	0.50	41.06	0.05
Hp 1-2	0.46 *	0.29	0.72	0.0008
Hp 2-2	1.98 *	1.28	3.06	0.0029

OR: Odds ratio. OR was calculated by taking particular phenotype VS others *- p value<0.05

Table 3: Anthropometric measures and serum mean MDA levels in the study group

Characteristics	Total Ct (187)	Total Pt (193)	Hp1-2 Pt (65)	Hp1-2 Ct (92)	Hp2-2 Pt (124)	Hp2-2 Ct (94)
BMI (kg mt^{-2})	22.45±3.91	25.48±5.24*	25.38±4.76*	22.14±3.82	25.41±5.42*	22.76±4.51
W/H	0.77±0.04	0.78±0.05*	0.78±0.05*	0.77±0.04	0.78±0.05*	0.76±0.04
MDA (μg dL^{-1})	258.35±74.27	363.92±128.81*	363.33±144.56*	283.58±91.26	400.64±142.13*	258.353±74.27

Quantitative data are presented as Mean±SD, Ct: Controls, Pt: Patients, MDA: Malondialdehyde BMI: Body mass index, W/H: Waist to hip ratio. *: p value<0.05

CONCLUSION

In conclusion, individuals with Hp1-1 and Hp 2-2 phenotypes exhibited significantly increased risk (5 folds and two folds over the heterozygote) for PCOS, while Hp 1-2 phenotype demonstrated a significantly protective role over both the homozygotes (OR = 0.46) in South Indian women. To our knowledge this is the first report regarding the distribution of haptoglobin phenotypes in PCOS patients and also in connection with various anthropometric measures and lipid peroxidation marker (MDA) in relation to South Indian population.

Further studies with multiple markers reflecting various function of Hp molecule may help in understanding the role of Hp a pleiotropic molecule in the pathophysiology of such multifactorial disease like PCOS for better management of this condition which is associated with long term morbidity and mortality.

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