Haptoglobin Revisited

K. J. Joshi*, B. T. Shah and Dr. (Mrs.) V. R. Rathod****

Directorate of Forensic Science Laboratory, Kalina, Mumbai.

DOI: 10.29322/IJSRP.8.9.2018.p8170
http://dx.doi.org/10.29322/IJSRP.8.9.2018.p8170

Abstract- Haptoglobin (abbreviated as Hp) is a protein in the blood plasma that binds free hemoglobin released from erythrocytes with high affinity and thereby inhibits its oxidative activity. Three phenotypes of Hp found in humans: Hp 1-1, Hp 2-1, and Hp 2-2. The Haptoglobin groups can be used in the cases of disputed paternity in forensic science. As we know in science and also in forensic science the collection of reliable data is very important. If large data is available, any conclusion based on this data is reliable and undisputable. Different population studies had carried out all over the world to study the gene frequency of these three haptoglobin phenotypes.This study is an effort in this direction as to study the changes in the percentage of different Haptoglobin types and to compare it with the previously available data.

Index Terms- Electrophoresis, Genotype, Gene frequency, Haptoglobin, PAGE, Phenotype, Population Study

I. INTRODUCTION

Haptoglobin is produced mostly by hepatocytes but also by other tissues: e.g. skin, lung, and kidney. The Hp protein is composed of two pairs of ‘a’ chain and ‘b’ chain, and the polymorphism of the protein reflects inherited variations in the Hp a-chain polypeptides. The a chain of the Hp2 protein is composed of 142 amino acid residues and is a product of a partial gene duplication of the Hp1 a chain, which has 83 amino acid residues. The molecular weight of Hp varies depending on the type. It is between 90,000 and 100,000 daltons in case of type Hp1-1 and has a sedimentation coefficient of 4.4s. Hp2-2 has a molecular weight of around 400,000 daltons and has a sedimentation coefficient of 7.5 s. Hp2-1 gives two picks of 4.4 and 6.5s with molecular weight of 200,000 Daltons (2).

Hp exists in two allelic forms in the human population, so called *Hp1* and *Hp2*; the latter one having arisen due to the partial duplication of *Hp1* gene. Three phenotypes of Hp, therefore are found in humans: Hp1-1, Hp2-1, and Hp2-2 (3). An inert allele, *Hp0*, at the Hp locus has been postulated by studies on the anomalous inheritance of Hp phenotypes, and the homozygosity of *Hp0* has also been suggested as a cause for so-called ahaptoglobinemia.

An allelic deletion from the Haptoglobin gene (Hp) of individual; results into ahaptoglobinemia. The Hp gene cluster consists of coding regions of the a chain and b chain of the haptoglobin gene (Hp) and of the a chain and b chain of the haptoglobin-related gene (Hpr), in tandem from the 50 side. Southern blot and PCR analyses have indicated that the individual with ahaptoglobinemia was homozygous for the gene deletion and that the gene deletion was included at least from the promoter region of Hp to Hpr a but not to Hpr b (Hpdel). Although certain pathological states, such as severe hemolysis and liver dysfunction, are known to lead to secondary ahaptoglobinemia, some reports have claimed that the hypo- or ahaptoglobinemia (Hp0 phenotype) observed in tropical countries has a genetic origin (Allison et al. 1958; Giblett and Steinberg 1960) (4).

The Haptoglobin groups were used in forensic science to solve paternity dispute cases as the inheritance of Haptoglobin is determined by two allelic genes viz. M and N which have co dominant mode of inheritance (5). Also, collection of reliable data is very important in forensic science. If large data is available, any conclusion based on this data is reliable and undisputable (6), (7).

II. MATERIALS AND METHODS

The separation of haptoglobin was carried out using poly acrylamide gel electrophoresis .

With tris glycine buffer system (pH 8.3). This method was developed by Budowle and Chow in 1985 (8) and was improved by Stolorow (9).

I] Apparatus:

Horizontal electrophoresis apparatus (Laurell and Nilehn)

Shandon power pack (constant voltage 500v)

II] Reagents:

All following required chemicals were obtained from Sigma (USA).

Acryl amide (Polymer for gel preparation)

Bis acryl amide (Monomer for gel preparation)

TEMED (Free radical stabilizer)

APS (Ammonium per Sulphate)

Tris (For buffer preparation)

Glycine (For buffer preparation)

Phenolphthalein and Hydrogen peroxide (Indicator) are laboratory reagents.

III] Blood samples:

The blood samples were collected from the population at random such that the selected people will represent the cross section of the population. The blood was collected in sterile eppendorff tubes. These tubes were centrifuged to separate serum from the blood. The serum was stored at -20° C in eppendorff tubes for further use.

IV] Preparation of hemolysate:

The hemolysate was prepared from blood obtained from blood bank. Blood was centrifuged and cells were washed with physiological saline. Heme was released by lysing RBC using equal volume of distilled water.

V] Test sample preparation:

One drop of serum separated by centrifugation from the blood under investigation was mixed with one drop of hemolysate. The tube was incubated at 37° C for 20 mins before electrophoresis to make sure the bond between Heme from blood occurs with haptoglobin from serum.

VI] Method:

Electrophoresis: The plates used for PAGE were washed thoroughly with distilled water. 8% polyacryl amide gel was prepared using Acryl amide, Bis acryl amide, Tris buffer, APS and TEMED. Horizontal plates were prepared with sandwich gel technology using plastic spacers. These plastic spacers create wells for the sample application. 10µl samples were loaded in each well using micropipette. Plate was placed in electrophoresis apparatus. The electrophoresis was carried out at 4° C for 5 hrs at 500Volts

Staining: The peroxidase like activity of hemoglobin is used to identify hemoglobin haptoglobin complex. Hence phenolphthalein and hydrogen peroxide are the reagents used as indicators. After electrophoresis, the gel was layered with

phenolphthalein for one min. The excess of phenolphthalein was decanted and then plate was layered with hydrogen peroxide to obtained pink coloured bands.

III. OBSERVATIONS

The complete study in this research paper is carried out using Tris –Glycine buffer system. Previously Haptoglobin studies were carried out using barbiturate buffer (11). Results obtained with suggested new buffer i.e. with Tris Glycine buffer system are eqully good and give highly resolved pattern oh Haptoglobin. The results obtained are reliable and reproducible. Hence; laboratories without specialized equipments and experts can still obtain highly resolved Hp bands from serum. Also, Tris Glycine buffer is non hazardous and non poisonous buffer system hence; continuous contact with Tris Glycine will not be harmful for people working continuously with it.

Stains like amido black, benzidine also can be used but staining with phenolphthalein is easy and convenient as the reagents are harmless and readily available.

The haptoglobin typing for 500 samples was carried out. Mainly three different Hp types were obtained from the studied population. The results are as follows.

Table 1: Different Hp phenotypes observed in the study

Hp Type	No of people	% obtained
0-0	70	14
2-1	80	16
2-2	350	70
1-1	-	0

As it was observed in previous studies (15); in this study also Hp 2-2 phenotype dominates the population. Hp 2-1 type is much lesser than Hp 2-2 type. Surprisingly changes in phenotype Hp 0-0 and Hp 1-1 are observed. Earlier no Hp 0-0 was observed but our study shows this increase up to 14%.

IV. RESULTS AND DISCUSSION

As we know in science and also in forensic science the collection of reliable data is very important. If large data is available, any conclusion based on this data is reliable and undisputable. Therefore there is a need to generate more systematic data bank. This paper is an effort in the same direction. The additional information obtained through this experiment will either help to strengthen the previous findings or if there are any different findings then it will open up new doors for the scientists to work in so far explored directions.

Tris Glycine buffer is non hazardous and non poisonous buffer system hence; continuous contact with Tris Glycine will not be harmful for people working continuously with it.

Population study for Maharashtra was carried out by Malvankar et. al where 395 samples were studied (11).

Table 2: Comparison of the DATA obtained to determine the gene frequency of Hp from the same geographical areas.

Hp Type	% obtained in recent studies	% obtained in previous studies (11)
0-0	14	0
2-1	16	24.87
2-2	70	71.99

1-1	0	3.14

In that study no sample was observed with Hp 0-0. Where as our population study shows 14% of the population is of the type Hp 0-0. This change in trend need to take in the consideration as far as the population study is concern from forensic point of view. The results obtained from earlier studies (Malvankar et al.) from population of Maharashtra showed that 71.99% of population was of Hp 2-2 type, 24.87% was of Hp 2-1 type and 3.14% population was of Hp 1-1 type. (11)

Comparing the obtained results which are also from the population of Maharashtra, we can say that; the % of Hp 2-2 type are almost similar ie. 70%. Surprisingly changes in phenotype Hp 0-0 are observed. Earlier no Hp 0-0 was observed but our study shows this has increased up to 14%. This significant increase in Hp 0-0 phenotype is certainly due to the changes in the genotype. Hence the gene frequency is changing for haptoglobin in the population of Maharashtra. During our recent studies no Hp 1-1 is been observed where as it was 3.14% of the total population in earlier population study of Maharashtra. Occurrence of Hp genotype can be found out by finding the gene frequency as shown in the following table.

Table 3: Hp gene frequency

Genotype	**Gene frequency**
Hp 0	0.21
Hp 1	0.08
Hp 2	0.71

V. CONCLUSION

- Haptoglobin typing for the population of 500:

  Hp 2-2 = 70%

  Hp 2-1 = 16%

  Hp 0-0 = 14%

- The gene frequency for Haptoglobin:

  Hp 2 = 0.71

  Hp 1 = 0.08

  Hp 0 = 0.21

- Changes in genotype of Haptoglobin are observed by looking at the gene frequency and comparing them with previous studies. Further more study with more number of population needs to be carried out regarding the same subject to get better idea of gene expression. Also study can be carried out on the basis of sex, caste and religion to observe the trends in the genotype accordingly.
- The numbers of observations examined are too low to arrive at any conclusion. But the significant differences in the observations indicate more deep study.

VI. ACKNOWLEDGEMENT

We are thankful to the former director of The Directorate of Forensic Science Laboratory Dr. (Mrs.) Rukmini Krishnamurthy for making the laboratory available for our practical work. Also we are thankful to Dr. K. V. Kulkarni; the present Director of the Directorate of Forensic Science Laboratory for his kind support during this study.

REFERENCES

[1]Dae-Hyun Ko, Ho Eun Chang, Taek Soo Kim, Eun Young Song, Kyoung Un Park, Junghan Song, and Kyou Sup Han; 'A Review of Haptoglobin Typing Methods for Disease Association Study and Preventing Anaphylactic Transfusion Reaction'; BioMed Research International,Volume 2013, Article ID 390630, 6 pages

[2] Beir M, 'Electrophoresis, Theory, Methods and Applications', Vol 2, Academic press, New york and London, 1967

[3] Wassell J., 'Haptoglobin: function and polymorphism' Clin Lab. 2000;46(11-12):547-52.

[4] Giblett E.R.,' Genetic markers in human blood', F.A. Davis Co. publishers, Philadelphia, 1969

[5] Galatius, Gensen, 'The use of haptoglobin patterns in the cases of disputed paternity', Methods of Forensic Science, Vol.1, Inter science publications, New York, 479, 1962

[6] Bhasin M.K, Walter H., ' The distribution of genetical morphological behavioral traits among the people of India'; 1992

[7] Nandi A.D., Mehta A.K., Singh M.M.; 'Studies of Haptoglobin blood stains in Gujrat population'; Journal of Indian Academy of Forensic Sciences, Vol 30, July 1991

[8] Budowle B., Chow G.H., ' Techniqual note: Discontinuous poly acryl amide gel electrophoresis for typing haptoglobin ib blood stains', Journal of Forensic Sciences, Vol. 30, July 1985.

[9] Stolorow M.D., Wraxall B.G.D., ' An efficient method to eliminate streaking in the electrophretic analysis of haptoglobin in blood stains', Journal of Forensic Sciences, Vol 24, 856, Oct 1979

[10] Harris H., 'The principles of human Biochemical genetics', American Elsevier publishing Co. Inc. New York, 1975

[11] Kamble D.S., Malvankar A.G., Mokashi R.H., 'Studies of haptoglobin phenotype', Forensic science laboratory, Mumbai.

[12] Polticelli F, Bocedi A, Minervini G, Ascenzi P.; 'Human haptoglobin structure and function--a molecular modelling study'. FEBS J. 2008 Nov;275(22)

[13] Sadrzadeh SM, Bozorgmehr J., 'Haptoglobin phenotypes in health and disorders', Am J Clin Pathol. 2004 Jun;121 Suppl:S97-104

[14] Grick F.H., 'General nature of genetic code of protein', Nature, Vol. 192, 1227, 1961

[15] Smithies O., 'Improved procedure for starch gel electrophoresis', Journal Of Biochemistry, Vol. 71, 585, 1959

[16] Harris H., Robson E.B., 'A typical segregation of haptoglobin types in man', Nature, Vol. 182, 1324, 1958

[17] Smithies O., 'Group variation in occurrence of new protein components in normal human serum', Nature, Vol. 175, 307, 1955

[18] Biology method manual, Metropolitan police, Forensic Science Laboratory, Mumbai.

[19] Culliford B.J., 'Biology methods maual', Metropolitan police forensic science laboratory, London.

First Author- K. J. Joshi, M.Sc. Biochemistry, Directorate of Forensic science laboratory, Mumbai, kjbioc@yahoo.com
Second Author- B. T. Shah, M.Sc. Biochemistry, Directorate of Forensic science laboratory, Mumbai, bhavesh.dude@hmail.com
Third Author- Dr. (Mrs.) V.R. Rathod, Ph.D.,Directorate of Forensic science laboratory, Mumbai, varsha_rathod@hotmail.com

Correspondence Author-- K. J. Joshi, kjbioc@yahoo.com, krupalijjoshi@gmail.com, +91-8329028730.