# Genetic Polymorphism of Red Cell Phosphoglucomutase (PGM) in Population of Maharashtra and its Applications in Forensic Science

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**Abstract:** Any polymorphism in human blood has a potential use for the forensic scientist in his attempt to typify blood in dried stain form or to separate and distinguish between two or more possible sources for that stain. $PGM_1$ typing is an important system used to solve the paternity disputes as well as other medicolegal cases. The present study was undertaken to determine the gene frequency of Phosphoglucomutase ($PGM_1$) subtype in the population of Maharashtra. Isoelectric focusing was used for the separation of the $PGM_1$ types from dried blood stains. The study could help in finding any characteristic signs of any phenotype in a population. The Gene frequencies were estimated as follows:
$1^+ = 0.61$, $1^- = 0.19$, $2^+ = 0.12$, $2^- = 0.07$.

**Index Terms:** Isoenzymes, IEF (Isoelectric Focusing), Gene frequency, polymorphism, PGM (Phosphoglucomutase), alleles, phenotype**.**

## I.INTRODUCTION

The multiple forms of an enzyme catalyzing the same reaction are isoenzymes or isozymes. Isoenzymes are common in sera and tissues of all vertebrates, insects, plants and unicellular organisms. Different tissues may contain different isoenzymes and these isoenzymes may differ in their affinity for substrates. Electrophoresis, Ion exchange chromatography, Chemical inhibition, Heat inactivation techniques can be used for the separation of Isoenzymes. Phosphoglucomutase (PGM) is widely distributed in mammalian tissues. It is present in the red blood cell, semen and sometimes in vaginal secretion.

Genetics of Phosphoglucomutase (PGM):
The polymorphism of the phosphoglucomutase (PGM) system was first demonstrated by starch gel electrophoresis studies conducted by Spencer et al. (1) on the enzyme from the human red cells. Pedigree studies (2) have shown that two common autosomal alleles $PGM_1^1$ and $PGM_1^2$ are situated on the same locus of chromosome 1(3). They are responsible for determining the three common phenotypes $PGM_1^1$, $PGM_1^2$ and $PGM_1^{2\text{-}1}$. These are observed by starch gel electrophoresis (1). The electrophoresis pattern of the $PGM_1^1$ is characterized by two isoenzymes ‘a’ and ‘c’ while isoenzymes ‘b’ and‘d’ are associated with the $PGM_1^2$ phenotype; the heterozygote $PGM_1^{2\text{-}1}$ has all four isoenzymes (1).Subsequent investigation have shown that a further two loci $PGM_2$ and $PGM_3$ located on different chromosomes viz 17 and 18 exist, each of which determines a separate set of isoenzymes which are inherited independently in a simple Mendelian fashion (4-6).

Principles of Isoelectric Focusing:
The isoelectric point (pI) of a protein is defined as the pH where the net charge of the protein is zero. A protein in an environment with pH below its pI will be positively charged, and when the pH is above the proteins pI, the charge will be negative. Since the charge of the protein determines the direction of migration in an electric field at a pH above or below its pI, a protein will migrate toward the anode or cathode, respectively. When the pH is equal to its pI, the protein possesses a net charge of zero and cannot migrate in an electric field (7). IEF exploits this behavior for protein separation allowing for sensitivity and resolution not possible with conventional electrophoresis.

The difference between the resolving capabities of IEF and conventional electrophoresis lies in the pH with in the resolving gel. Protein separation is accomplished in a pH gradient using IEF while the pH is essentially constant throughout the resolving gel with

conventional electrophoresis. Thus, conventional electrophoresis separates proteins based on mobility (charge to mass ratio differences) and IEF separates proteins based on pI values. The result is narrower, more resolved bands on IEF gels.

## II. MATERIALS AND METHODS

Selection of the subjects**:**
The blood samples were collected from 490 individuals of both sexes within the age range from 18 to 60 years selected at random to represent the cross section of population. This included families belonging to different religions.

Preparation of Bloodstains:
The blood stains were prepared from healthy individuals. Bloodstains were made from finger pricks on starch free cotton cloth. The stains were then air-dried, labeled and kept in small bottles. The bottles were also labeled and stored in deep freeze.

Isoelectric Focusing :

A. Reagents:
All reagents used for electrophoresis and subsequent staining were of Sigma or an Equivalent grade. All the reagents were prepared in Distilled water.
For one plate of 0.3mm thickness
Sucrose (37.5%)-4ml
Acrylamide (6%)-4ml
Acrylogel (9%)-4ml
Riboflavin (0.01%)-0.1ml
Ampholine (pH5-7)-0.5ml
The mixture was mixed and degassed.
A convenient way to remove the ampholine was by using a disposable luer syringe with a 21g hypodermic needle. It was ensured that the ampholine and acrylamide solutions were uniformly mixed by gently swirling of the flask and then degassing under vacuum.

B. Procedure:

1. Preparation of ultra-thin plates:
   1. Both the plates – thin plate (2mm thickness) and thick plate (5mm thickness) were cleaned thoroughly with methanol so that the plates were grease free.
   2. Thin plate was kept on leveling platform and then clean spacer of 0.3 mm thickness was placed on it.
   3. The degassed mixture was poured on the thin plate and the thick plate was slided to cover it. Care was taken to avoid trapping of air bubbles between the two plates.
   4. The plates were clamped and immediately kept on fluorescent tube for 2.5 hours to allow photopolymerisation to occur.
   5. Then the plates were kept in fridge ($4^0$C) for about half an hour for easy separation of plate. The top plate was removed immediately prior to sample application.
   6. The plates were detached by inserting scalpel blade in between thick plate and spacer from one corner.
   7. The thin plate with gel was kept on a cooling plate in the IEF instrument. The temperature of cooling plate was $5^0$C.

All the IEF work was conducted on the LKB 2217 Ultraphor electrophoresis cells and the LKB2303 MultiDrive XL power supply, supplied by LKB Pharmacia Instruments Sweden.

2. Prefocussing:
The conditions for the Prefocussing are as follows:

| Volts | Current | Wattage | Volt hours | Time |
|---|---|---|---|---|
| 1500 volts | 15 amp | 15 watts | 0 | 30 minutes |

3. Preparation of sample for loading on gel:
0.5 x 0.5 piece of the bloodstain was taken & soaked in distilled water. The fibres were teased to get proper extract. After prefocussing was over, the samples were loaded by using 0.75 x 0.5 cm 1mm Whatman paper, soaked in the above extract. Also known standards of $PGM1^+1^-$, $2^+2^-$ and $1^+2^-$ were loaded in same way.

4. Condition for run: The conditions for the electrophoresis are as follows:

| Volts | Current | Wattage | Volt hours | Time |
|---|---|---|---|---|
| 1500 volts | 15 amp | 15 watts | 3500 | 2.20 hours |

After 30 mins the electrophoresis was stopped. The filter papers were removed and then the electrophoresis was again started. When volt hours reached 3500/3500 the electrophoresis was stopped which is approx. 2.20 to 2.30 hrs. The plate was removed from the cooling plate and kept on leveling stand for staining (8).

C. Staining Procedure:

1. Stock solution
a. Glucose -1-phosphate -300mg/ml
b. Magnesium chioride-200mg/ml.
c. MTT Tetrazolium-250mg/ml.
d. NADP-15mg/ml.
e. PMS-25mg/ml.
f. Glucose-6-phosphate dehydrogenase -20ul
g. PGM staining buffer – (0.06M tris HCL) – pH 8.0.

2. Staining of gel:
300mg Agarose was taken in 20ml Buffer and allowed to melt. The glass strips were kept on the border of the gel and were blocked by agarose.15ml of PGM reaction buffer was taken in conical flask and 0.2ml G-1-P, 0.2 ml NADP, 0.2 ml MgCl2, 0.2 MTT, 0.2ml PMS, 20ul G-6pd was added to it. Also remaining melted agarose was added in conical flask, poured on the plate and the solution was scraped with rod while pouring. The solution was cooled in dark. The plate was kept in incubator at $37^0$C for about 20 mins and the results were observed.

## III. RESULTS & DISCUSSION

PGM is a phosphotransferase (E. C. NO 2.7.5.1) which catalyses the conversion of Glucose -1-phosphate to Glucose-6-phosphate in the presence of small amounts of Glucose -1, 6-diphosphate in an early stage of the glycolytic pathway. Glucose 1, 6- diphosphate is required as a cofactor together with magnesium ions. The Mg ++ ion greatly enhances the activity of PGM (1, 9). Glucose -6-phosphate is used as a substrate for Glucose -6- phosphate dehydrogenase, which forms 6-phosphogluconate. This enzyme requires NADP as coenzyme, which itself is reduced to NADPH. Phenazine methosulphate (PMS) is used as a transfer agent. MTT tetrazolium is reduced to a blue colored formazan as shown in Figure 1 and the NADPH is oxidized back to NADP. The bands of PGM activity are hence seen as blue bands on a yellow background on the starch gel (1, 9).

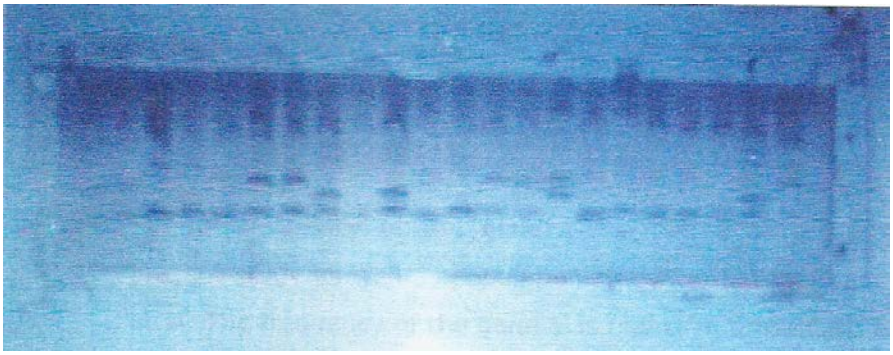

Figure 1: Blue bands of PGM subtypes by IEF

Ishimoto and Kawata (10) first investigated the separation of PGM by isoelectric focusing at low voltage. They confirmed the existence of 3 $PGM_1$ phenotypes. They obtained more bands than are normally seen by starch gel electrophoresis.
Later work by Burdett and Whitehead (11) confirmed these observations. Bark et al. (12) examined 150 blood samples and proposed that the more complex band patterns, which gave ten distinct phenotypes instead of the three normally seen by starch gel electrophoresis, were due to the presence of four isoenzymes which they called 1+, 1-, 2+, 2-. They suggested that the 1+1-and 2+2-isoenzymes were equivalent to the “a” and “b” isoenzymes, respectively, formerly proposed by Spencer et al. (1).

In a study conducted by Kuhnl et al. (13) using a wide spectrum ampholine gradient (pH 3.5-9.5) demonstrated the existence of 10 phenotypes by isoelectric focusing which they believed were also due to the presence of two additional common alleles at the $PGM_1$ locus (2). Sutton and burgess (14) have shown that these alleles are inherited in a Mendelian fashion.
In the present study all ten phenotypes expected in a system with four co dominant alleles have been observed. In Table 1 the subtype distributions in examined population are shown. The Gene frequencies were estimated as follows:
$1^+ = 0.61$, $1^- = 0.19$, $2^+ = 0.12$, $2^- = 0.07$. Also, the observed gene frequency is compared with the gene frequencies given by Spencer et al (1)

TABLE 1: $PGM_1$ Phenotype and Gene Frequency distribution in population of Maharashtra

| Phenotype | Number observed | Gene frequencies in present study | Gene frequencies by Spencer et al |
|---|---|---|---|
| 1+1+ | 150 | | |
| 1+1- | 150 | $PGM^{1+}1 = 0.61$ | $PGM^{1+}1 = 0.618$ |
| 1-1- | 8 | | |
| 1+2+ | 93 | $PGM^{1-}1 = 0.19$ | $PGM^{1-}1 = 0.122$ |
| 1+2- | 50 | | |
| 1-2+ | 10 | $PGM^{2+}1 = 0.12$ | $PGM^{2+}1 = 0.142$ |
| 1-2- | 14 | | |
| 2+2+ | 6 | $PGM^{2-}1 = 0.07$ | $PGM^{2-}1 = 0.118$ |
| 2+2- | 6 | | |
| 2-2- | 3 | | |
| Total | 490 | | |

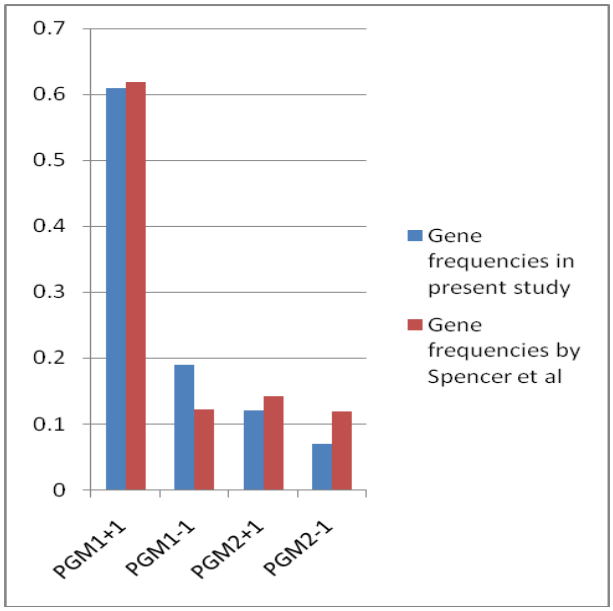


Figure 2: Graph showing gene frequencies in present study and study conducted by Spencer et al (1).

The frequency of gene $1^+$ is almost same in the present study as well as population study carried by Spencer et al (1). The frequency of gene $1^-$ is higher in the present study. The frequency of gene $2^+$ is slightly lower in present study. There is a significant difference in the frequency of $2^-$ in both the population studies. It is very less in present study.

TABLE 2: Gene frequency comparison between the genders

| Gene | Frequency | |
|---|---|---|
| | Males | Females |
| $1^+$ | 0.625 | 0.6 |
| $1^-$ | 0.2125 | 0.17 |
| $2^+$ | 0.1125 | 0.12 |
| $2^-$ | 0.05 | 0.11 |

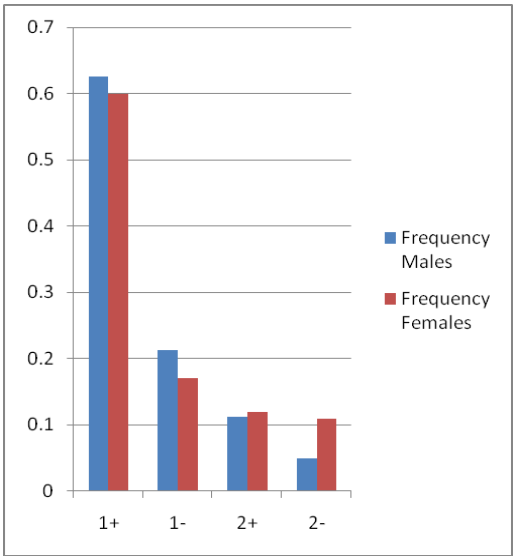


Figure 3: Graph of gene frequency comparison between the genders

The frequency of the gene $1^+$ is marginally higher in males than in females. The occurrence of gene $1^-$ is slightly higher in males than in females. The frequency of gene $2^+$ is slightly higher in females than in males. The frequency of gene $2^-$ is very high in females compared to that in males.

Thus, though the trend of the gene frequency is same in the present population study i.e. $1^+$ followed by $1^-$ , $2^+$, $2^-$ , a significant difference in the individual gene frequencies was observed from study carried by Spencer et al (1). So, the gene frequency i.e. a particular phenotype may be a characteristic of that population and could be used for distinguishing the person.

The value of any polymorphic system in discriminating between different individuals is most conveniently expressed in terms of its discriminating power (DP). The discriminating power (DP), which is 0.58 for the three phenotypes by starch gel electrophoresis, increases to 0.79 for the ten phenotypes observed by IEF, which is of great forensic value.
In some criminal cases the fresh blood sample is not available. In that case PGM can be done from a deep-seated muscle, bone marrow and hair roots. Also, the material required is very less. Thus, PGM can reduce the burden on DNA profiling.
The PGM typing of the blood can be compared with that of the semen in cases of sexual offences, since the PGM of blood and semen have identical mobility in electric field. This would help in the conviction of the criminal with more convincing evidence. The same can be applied in case of a paternity dispute where the PGM typing of blood can be compared with that of hair roots.

## IV. CONCLUSION

The PGM polymorphism could be used to solve the paternity cases in a shorter time and economically. It takes a long time to get the DNA profiling reports of the accused. Also, the cost of DNA profiling is very high and requires sophisticated Genetic Analyzer. Instead the PGM subtyping can be routinely used by the forensic scientist to identify the accused person by elimination test. This would reduce the burden on DNA profiling and will help to solve the paternity dispute.

Similarly, PGM can also be done for other routine cases in the forensic lab. Presence of a particular phenotype of the Phosphoglucomutase ($PGM_1$) characteristic of a particular population of the area, if any, will be helpful in narrowing down the criminal and would make easy identification of the accused in criminal cases.

Thus, in this study PGM $_1$ polymorphism by using IEF technique was conducted on the population of Maharashtra. The IEF technique used in this study gives well separated, sharp and more concentrated bands. It would require very less stained material as compared to any prevailing methods. Besides this technique is less time consuming

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## REFRENCES

[1.] N. Spencer, D. A. Hopkinson and Harry Harris, “Phosphoglumutase Polymorphism in Man”, Nature, 1964, 204, 742-745.
[2.] H. Harris, D. A. Hopkinson, J. E. Luffuman and S. Rapley, “Electrophorectic Variation in red cell enzymes in Hereditary Disorders of Erythrocyte Metabolism”, E. Beutlaer, Ed; Grune& Stratton, New York, 1968, 7-13.
[3.] N. Vang Cong, C. Billardon, J. Y. Picard, J. Frezal, “Gnetique- Liaison Probable (Linkage) Entre les Locus PGM1 et peptidase c. Chez L. Homme”, Comptes Rendus Hebdomadaries des Seances de l Academie des Sciences. Serie (D): Sciences, Nature, 1971, 485-487.
[4.] D. A Hopkinson and H. Harris, “Evidence for a second structural locus determining human phosphoglucomutase”, Nature, 1965, 208, 410-412.
[5.] D. A. Hopkinson and H. Harris, “Rare phosphoglucomutase phenotypes”, Ann. Hum. Genetics, 1966, 30, 167-178.
[6.] D. A. Hopkinson and H. Harris, “A third phosphoglumutase locus in man”, Ann. Hum. Genetics, 1963, 31, 359-367.
[7.] Keith Wilson and John walker, “Practical Biochemistry: Principles and techniques”, Fifth Ed; Cambridge Unipress, 2000.
[8.] A. G. Malwankar and R. H. Mokashi, “Isoelectric Focusing of phosphoglucomutase (PGM) UNDER Indian ambient conditions”, Forensic Science Laboratory, Vidyanagari, Mumbai.
[9.] Otto Prokop & Gerhard Unlen Bruck “Human blood & serum groups”, Maclaren & sons, London 1st edition.
[10] G. Ishimoto and M. Kawata, “The typing of red cell enzymes by isoelectric focusing in gels”, Rep. Nat. Res. Inst. Police Sci., Japan 1972, 25, 13-16.
[11.] P. E. Burdett and P. H. Whitehead, “The separation of the phenotypes of phophoglumutase, erythrocyte acid phosphatase and some haemoglobin variants by isoelectric focusing”, Analyt Biochem ,1977,77, 419-428.
[12.] J. E. Bark, M. J. Harris and M. Firth, “Typing of the common phosphoglucomutase variants using isoelectric focusing –A new interpretation of the phophoglucomutase system”, J. Forensic Sci. Soc, 1976, 16, 115-120.
[13.] P. Kulni, U. Schmidtmann and W. Spielmann, “Evidence for two additional alleles at the PGM 1 Locus (phosphoglucomutase – E.C. 2.7.5.1)”, Human Genetics, 1977, 35, 219-223.
[14.] J. G. Sutton and R. Burgess, “Genetic Evidence for four common Alleles at the phosphoglucmutase-1 locus detectable by Isoelectric Focusing”, Vax Sanguinis, 1978, 34(2), 97-103.

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