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Phosphoglucomutase Types in Blood and Hair Roots Taken from Post-Transfusion Subjects

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ABSTRACT: Pre- and post-transfusion blood samples were collected from 22 subjects together with the corresponding plucked hair samples taken 2 days and 2 weeks after the transfusion. The phosphoglucomutase$_1$ (PGM_1) subphenotypes of blood and hair were determined by isoelectric focusing and the phenotypes confirmed by gel electrophoresis. Many of the post-transfusion blood samples showed an alteration in the PGM_1 bands when compared with the pre-transfusion samples. However, the PGM_1 types determined from the hair samples were identical to the corresponding pre-transfusion samples in all cases.

KEYWORDS: pathology and biology, phosphoglucomutase, blood, hair

Following crimes of violence control blood samples are required from the victims to establish their blood groups and relate them to bloodstains on clothing and at the scene of a crime. A problem sometimes occurs in cases where assault has led to considerable loss of blood requiring a blood transfusion. The patient's blood is cross-matched for ABO and Rhesus D factors but his red cell isoenzyme types are not identified. Therefore the isoenzyme types of the transfused blood need not correspond to the patient's own blood.

In such cases a pre-transfusion cross-match blood sample should be forwarded to the forensic science laboratory as a control sample for isoenzyme typing. However, if the hospital staff are unaware of the need to save the sample it is often destroyed soon after use. As red cells have an average life span of 120 days a considerable wait will then be required to ensure that all foreign blood has been cleared from the patient's blood stream. If the patient subsequently dies, then the original enzyme types can never be determined from his blood.

A logical solution to this problem is to investigate the possibility of obtaining the patient's true enzyme phenotype from another body tissue. In this study hair roots were examined as an alternative control sample because of their easy accessibility and the high levels of enzymes contained in the sheath cells surrounding them. Several isoenzymes are found in the sheath cells surrounding hair roots but phosphoglucomutase$_1$ (PGM_1) determined by isoelectric focusing (IEF) was chosen for this study as it has the highest discriminating power [*1*] of the commonly used enzyme systems.

PGM_1 has been extensively used in the typing of bloodstains since its polymorphic character was first demonstrated in 1964 by Spencer et al [*2*]. Subsequently the presence in semen

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[3], vaginal secretions [4,5], buccal cells [6], and latterly the sheath cells surrounding hair roots [7] of PGM_1 corresponding to the red cell phenotypes has been documented. The development of isoelectric focusing using polyacrylamide gels [8] or nonionic agarose [9] showed that ten distinct subphenotypes exist controlled by four alleles at the PGM_1 locus [10].

Materials and Method

Collection of Samples

Blood and hair samples were collected from 22 subjects. With one exception all the subjects were surgical patients mainly requiring orthopedic treatment. They received between 1 and 2.5 L of blood. The remaining subject was an assault victim who received approximately 5 L of blood.

The pre-transfusion blood samples were collected in plain tubes primarily for immunological cross-matching before transfusion. Corresponding post-transfusion blood samples were collected in ethylenediaminetetraacetic acid (EDTA), primarily for hemoglobin estimation, two days after transfusion.

Hair samples were plucked to obtain the hair root and surrounding sheath cells. They were collected two days and two weeks after transfusion. Because of the advanced age of many of the subjects sheath cell material was generally difficult to obtain. This led us to limit the study to the PGM_1 system only.

Isoelectric Focusing, Electrophoresis, and Isoenzyme Pattern Visualization

Isoelectric focusing was carried out according to the method of Randall et al [11]. The phenotype of each sample was checked by electrophoresis which was carried out according to the method of Spencer et al [2].

The blood samples were washed in saline and frozen to lyse the red cells before carrying out electrophoresis and IEF. The lysates were diluted $\times 8$ with water for IEF. The hair roots were untreated but were sectioned where necessary to provide ample samples for electrophoresis and IEF. The isoenzymes were visualized using a modification of the overlay proposed by Culliford [12].

Results and Discussion

The results for PGM_1 typing by IEF are given in Table 1. The PGM_1 phenotypes shown in the table were confirmed by starch gel electrophoresis in all cases.

There was no obvious difference between the pre- and post-transfusion blood samples from eight subjects. Either the transfused blood matched exactly the donors' blood or more likely the relatively small amounts of blood transfused to these subjects were insufficient to be detected. However in the casework situation it could not be assumed that a relatively small transfusion did not alter the isoenzyme pattern.

The remaining 14 paired blood samples did show an alteration in the post-transfusion PGM_1 bands. This was manifest as an extra isoenzyme band in each case although two extra bands would also be possible. Six of these subjects had only two bands present in the post-transfusion sample so this could be regarded as normal unless the pre-transfusion phenotype was known. Obviously if more than two bands are present then the analyst will be alerted to the presence of a mixture of blood. In this study the extra band was often weak because of the small amounts of transfused blood required as the result of an operation compared with an assault.

None of the hair samples collected either two days or two weeks after transfusion showed

TABLE 1—*PGM_1 bands from the blood and hair samples as determined by IEF.*

Subject	Pre-Transfusion Blood	Post-Transfusion Blood	Two-Day Hair	Two-Week Hair
1	2+1+	2+1+1−	...	...
2	2+1+	2+1+1−	2+1+	2+1+
3	1+	1+	1+	...
4	1+	2+1+	1+	1+
5	2+1−	2+1+1−	2+1−	2+1−
6	2+	2+	2+	2+
7	2+1+	2+2−1+	2+1+	2+1+
8	1+	2+1+	1+	1+
9	1+	2+1+	1+	1+
10	1+	1+	1+	1+
11	1+	2+1+	1+	1+
12	1+	1+	1+	...
13	2+	2+	2+	...
14	1+	2+1+	1+	1+
15	2+2−	2+2−1+	2+2−	2+2−
16	2−1−	2−1−	2−1−	2−1−
17	2+1+	2+2−1+	2+1+	2+1+
18	2−1−	2−1+1−	...	...
19	2+1+	2+1+	2+1+	2+1+
20	2+1+	2+1+	2+1+	2+1+
21	2+1−	2+1+1−	2+1−	2+1−
22	1+	2+1+	...	...

any alteration in the PGM_1 pattern. They all corresponded exactly to the appropriate pre-transfusion blood sample. This indicates that red cell PGM_1 contained in the vascular system supplying the hair root does not appear to influence the phenotype of PGM_1 synthesized in the epithelial sheath cells. Hence, a plucked hair sample is suitable as a control sample for PGM_1 typing following a blood transfusion.

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