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The Sequential Determination of Glyoxalase-I Isoenzymes and ABO (H) Antigens From the Same Piece of Menstrual Blood Stain Thread

SWAROOP KAUR SAUND[a], RAKESH KUMAR GARG[b], SEEMA B.L.[c]

[a] M.Sc. Forensic Science, Post-graduate student, Punjabi University, Patiala, India
[b] Department of Forensic Science Lecturer, Punjabi University, Patiala - 147 002, India
[c] Scientific Officer, Punjabi State Forensic Science Laboratory, Sector 9, Chandigarh, India

Summary

Fifty paired stains of normal and menstrual blood have been analysed for the sequential determination of GLO-I isoenzymes and ABO(H) antigens from the same piece of cloth. It has been observed that ABO (H) antigens could be successfully determined after GLO-I isoenzyme typing, from the same fragment of the menstrual blood stains (98 per cent). The treatment of stains with reducing agent prior to insertion did not affect the results and the similar type of isoenzymes patterns were observed in the paired stains of the same individual. The GLO-I isoenzyme typing were detected for a longer period in stains stored at deep freezer as compared to room temperature. The GLO-I isoenzyme typing and ABO (H) antigens detection could be performed on the same thread particularly when the biological material submitted for forensic examination is limited.

Key words: *Glyoxalase-I - ABO (H) antigens - Menstrual blood stain.*

Blood in the form of stains are often encountered as a clue material in forensic cases like murder, suicide, assault, violence, sexual offences etc. The task of the forensic serologist often involved in the examination of stains is, if possible to determine their nature, species origin and carry out tests for as many genetic markers as are feasible for possible identifications of the victim or the suspect. But the determination of all these factors are greatly influenced by the external conditions and quantity of the material available through which the blood stain has passed before analysis.

A serological investigation of menstrual blood stains can sometimes become an important consideration in sexual assault cases and over the years several methods have been suggested for its identification (1-9). A common defence in cases of assaults on females is that the blood on women's garments is due to menstruation. Hence, if they are properly analysed, they can help in linking the criminal or the victim with the scene of crime and in the elimination of innocents not involved in the crime.

The purpose of this study is, therefore, that when the nature of the stain is established, the typability of various genetic markers on the material available could be performed. The simultaneous typing of a number of polymorphic systems from the same piece of thread have been attempted by a number of workers (10-18). As far as we are aware no information regarding the detectability of genetic markers simultaneously in menstrual blood stain is available. Therefore, in the present investigation, an

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extension of previously studied methods on normal blood stain has been made on menstrual blood stain for the typing of glyoxalase-I isoenzymes and ABO (H) antigens simultaneously from the same fragment of the stain.

MATERIAL and METHOD

Fifty stains of known nature of normal and menstrual blood were made from the students of Punjabi University, Patiala. Fresh blood samples (2-3 drops) were collected by finger prick method in normal saline and were analysed for ABO (H) blood grouping acoording to the technique (19). The menstrual blood stains on cloth pieces were dried and 15 stains were stored at room temperature range 20.3-40.5°C and remaining 35 samples in the deep freezer (-4°C) to study the stability of GLO-I isoenzymes and ABO (H) antigens. The stains were examined periodically under the two conditions for the typability of isoenzymes (GLO-I) and ABO (H) antigens from the same fragment of the stain.

Absorption-elution technique (20) was used for ABO (H) typing of same threads (1 cm) removed after electrophoresis of 30 minutes of menstrual blood stain.

Electrophoresis for GLO-I isoenzymes was performed in 1.2 mm thick mixed starch/agarose gel (21). Haemolysate was prepared by freezing thrice washed red blood cells of known type (GLO-I, 2-1 type). This was used for GLO-I isoenzymes typing.

Preparation of the buffers:

a) Tank Buffer (pH 7.6)
Tris 12.10 gm (0.1M)
Citric acid (Water) 6.05 gm (0.029M)
Distilled water to make one litre solution.

b) Gel Buffer (pH 7.6)
Tris 4.84 gm (0.2M)
Magnesium Chloride 0.8 gm (0.02M)
Distilled water to make 200 ml solution.

L-Histidine monohydrochloride approximately 7.0 gm (0.16M) was added to this solution while stirring to achieve final pH of 7.6.

The buffer is diluted 1:10 for use in the gel preparation.

c) Reaction Buffer (pH 6.8)
0.2 M Phosphate buffer
(A) Disodium hydrogen phosphate (anhydrous) 14.195 gm
Distilled Water 500 ml

(B) Sodium dihydrogen phosphate 15.60 gm
Distilled water 500 ml

Added (B) to (A) until the pH 6.8.

The haemolysates and three menstrual blood stain threads (1 cm each) were inserted in each of the gel slots after treating with 0.05 M dithiotheritol (DTT) for 10-15 minutes. The electrophoresis was conducted for 2.5 hours at 4°C at a constant voltage of 150 volts (approximately 7.5 volts/cm) and an initial current of 22 mega ampere. In the initial stages of experiment the voltage applied was low (100 volts) which was increased afterwards (5 minutes) to 150 volts. After half an hour of each run the inserted threads were removed from each slit and dried at room temperature for ABO (H) typing.

Location of bands:

The GLO-I activity was located by soaking Whatman 1 paper (12x14 cm) in the below given reaction mixture and placing this on the gel surface between the origin and anode. The gel plate was incubated at

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37°C for 30 minutes in humid chamber The overlay paper was removed carefully and was enclosed by the suitable templates.

Reaction mixture

Reduced glutathione 5.00 gm (0.04M)
Methyl glyoxal 0.1 ml (50% in water), (0.03 M)
Phosphate buffer 5 ml (0.2M) pH 6.8

Staining of GLO-I bands

A 1.5 per cent w/v agarose solution in distilled water was prepared and allowed to cool to approximately 60°C. One ml of iodine solution was added and mixed with the warm agarose solution. It was immediately poured over the gel surface and allowed to set. Blue colored GLO-I isoenzymes bands appeared against a colorless background immediately.

RESULTS and DISCUSSION

The results of menstrual blood stain for GLO-I isoenzymes typing are given in Table-I. It is evident from the table that the GLO-I isoenzyme types (2-2, 2-1 and 1-1) occured to the extent of 62.00, 30.00 and 8.00 per-cent. The GLO-I isoenzymes of phenotypes 2-2 has been observed to be the most common followed by 2-1 and 1-1. Similar type of frequencies of GLO-I has been reported in North Indian population by various researchers (9,22-24). The paired blood stains analysed (menstrual and normal blood stains) gave the same phenotypes in the given sample and these results are in confirmity with the findings of the other workers (25,26). All the three phenotypes observed in the present study are shown in Fig. 1.

The results of ABO (H) antigens typing after electrophoretic analysis of GLO-I isoenzymes are given in Table II. The blood group antigens ABO (H) has been correctly detected in all the stains tested (98 per cent) except one (2 per cent). One B type sample of menstrual blood stain gave negative results which may be due to the variations in the antigenic substances. In overall, the strength of the reaction in ABO (H) typing was observed to the maximum of double positive. Satisfactory results for ABO and MN grouping on blood stain could be achieved in approximately 65-75% (11,12). This study indicates that the ABO (H) antigens remains unaffected even after treatment with reducing agent (dithiotheritol) used prior to electrophoresis. Similar type of observations have been made (15,18) on blood stains.

Table I. GLO-I isoenzymes typing from menstrual blood stains.

Number of menstrual blood stain tested	GLO-I Phenotypes			Menstrual Blood Stains	
	2-2	2-1	1-1	Correctly typed	Incorrectly typed
50	31 *(62.00)*	15 *(30.00)*	4 *(8.00)*	50 *(100.00)*	

Figures in parenthesis indicate percentage.

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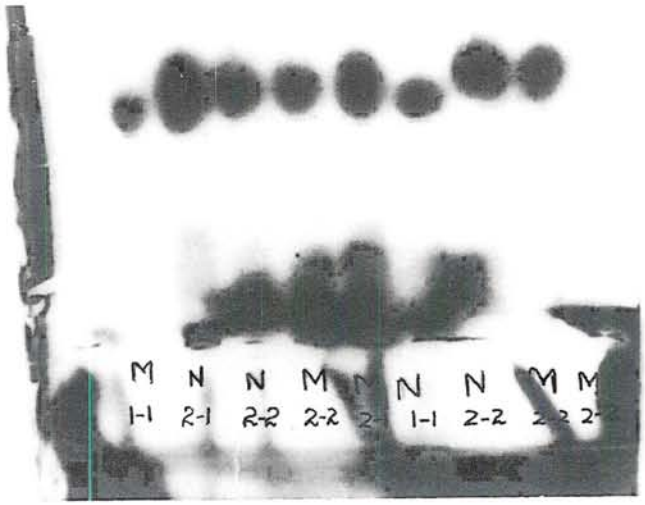

Figure I. Glyoxalase-I phenotype patterns.
N. Normal blood stain
M. Menstrual blood stain

Table II. ABO (H) Antigens detection from the same thread of menstrual blood stain after electrophoresis.

Blood group	Number of menstrual blood stains tested	ABO (H) antigen detection in menstrual blood stains	
		Positive	Negative
A	11	11 *(100.00)*	
B	20	19 *(95.00)*	1 *(5.0)*
O	13	13 *(100.00)*	
AB	6	6 *(100.00)*	
Total	**50**	**49** ***(98.00)***	**1** ***(2.00)***

Figures in parenthesis indicate percentage.

The results of stability of GLO-I isoenzymes under two different conditions (room temperature and deep freezer) are given in Table-III. It is evident from the table that the GLO-I isoenzymes could not be typed after twelve days of storage at room temperature in the month of June (temperature range 20.3-40.5°C) at the maximum humidity of 42 per cent while the samples stored at -4°C could be easily typed for the GLO-I activity

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Table III. Stability of GLO-I isoenzymes at room temperature and at -4 C.

Storage condition	Number of menstrual blood stains tested	GLO-I Isoenzymes Activity Days																						
		1-11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
20.3-40.5°C	15	+	+	DB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-4°C	35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	NT

DB: Diffused band not readable
NT: Not Tested

until the period of analysis (31 days). The intensity of the bands started decreasing after one week of storage at room temperature and after 12 days of storage the bands were diffused and uninterpretable. In the present study it has been observed that the ABO (H) antigens can be detected from the electrophoresed stains even when the isoenzymes typing is uninterpretable. Thus it is apparent from the present investigation that in menstrual blood stains GLO-I isoenzymes and ABO (H) antigens detection can be simultaneously performed from the same piece of thread.

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Reprints request to:

Dr. Rakesh Kumar Garg
Department of Forensic Science
Punjabi University
Patiala - 147002
India