CHARACTERISATION OF VAGINAL SECRETION STAINS IN FORENSIC CASES - A CRITICAL REVIEW

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INTRODUCTION

The usual contribution of a forensic scientist to the investigation of a sexual assault is to identify the offender by grouping any semen found contaminated with vaginal secretions because in these cases victim is known to us. The typing of genetic markers like cell surface antigens serum proteins and enzymes can be very valuable in the investigation of crime by establishing the the identity of the individual. However, the knowledge about the presence or absence of various genetic parameters, their level of detection, stability and the distribution of their phenotypes in population concerned will help in deciding which parameters should be studied from body fluids or even mixtures of the two body fluids.

The vaginal secretions have been identified using various tests for epithelial cells, Doderlein's bacilli, lactic acid and mucus by various workers. Graves et al (1978), Martin (1981), Martin and Cheshire (1982) and Joshi et al (1986) reported the presence of ABO(H) substances in vaginal secretion stains.

Schwerd and Stock (1982) attempted typing of PGM and AK on starch gel, EsD on cellulose acetate sheets and GLO-I on agarose gel from vaginal secretions. Sato et al (1983) typed PGM, EsD and 6-PGD from vaginal secretions by starch gel electrophoresis. Garlo (1985) reported PGM & EsD activity in post coital vaginal swabs by electrophoresis. Ablett (1983) identified SAP and VAP by isoelectric focussing. Endogenous and post coital components of vaginal swabs were studied by Bryson et al (1988).

In the Present Study, in continuation with the earlier work, the detection and persistence of epithelial cells, lactic acid, mucus, Doderlein's bacillus, ABO(H), Rh(D), G-6-PD and GLO-I from vaginal secretion stains have been reported.

MATERIALS AND METHODS

400 semen free vaginal discharge samples were collected on sterilised cotton cloth pieces, dried at room temperature and kept in serially marked envelops. Blood samples of the same subjects were also collected for typing of ABO(H) and Rh(D) blood groups and for preparing haemolysates. The information regarding name, age, time of sexual abstinence, pregnancy or non-pregnancy and date of collection of the sample was also recorded.

Epithelial cells and Doderlein's bacillus were examined microscopically after staining with Papanicolaou staining procedure and Gram's staining procedure respectively. Mucus and lactic acid were detected by chemical tests (Examination of Biological Stains, 1980). The technique of Kind (1960) was used with slight modifications for ABO(H) typing, whereas Rh(D) typing was done according to Bargagna et al (1982) technique. In case of Rh(D) typing, low ionic strength solution was used and prepared according to the method of Low and Masseter (1974).

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The electrophoresis for GLO-I was carried out in 1.2 mm thick mixed starch agarose gel (1:2) as described by Sehajpal et al (1986). After the electrophoresis, the gel was overlayed with Whatman filter paper no. 1 soaked with methyl glyoxal and reduced glutathione dissolved in reaction buffer and incubated at 37°C for 30 minutes. The charecteristic blue bands of GLO-I were visualised by pouring iodine solution on the surface of the gel after blotting excessive reaction mixture. The electrophoresis for G-6-PD was conducted in 1.2 mm thick mixed starch - agarose gel (1:2). The haemolysates and extracts from vaginal secretion stainswere treated with 0.05M Cleland's reagent for 20-30 minutes before loading into the gel. Using the method as described in WHO Technical Report no. 3 366 (1967) ,the gel was developed by agar overlay method. Blue bands against yellow background showed G-6-PD activity.

RESULTS AND DISCUSSION

The epithelial cells could be detected upto 4½ months whereas Doderlein's bacilli could not be detected beyond one month. Number of epithelial cells decreased with increase in the age of the stain. Rothwell and Harvey (1978) have questioned the validity of vaginal epithelial cells being identified on penile swabs. Their study indicated that false positive findings can be obtained and that this approach to determine whether a male has recently had intercourse may not be reliable one. Lactic acid was detected upto 10 months and the intensity of reaction increased with the passage of time. Mucus was not detected in dried stains but could be detected in liquid form only.

Using absorption-elution technique, 86.00% subjects were found to be secretors. The intensity of the reaction for h was more in all the groups as compared to A and B. Even in AB group, readable activity of H substance was detected. The ABO(H) substances from vaginal secretion stains showed that with the increase in the time of the stain, the number of positive results decreases. Some discrepancy in the time till which all the stains could be correctly typed was observed for different groups e.g. the samples of O group could be typed upto 40 days. The samples of B group could be typed for 46 days whereas samples of A and AB blood groups could be typed upto 42 days. The samples which showed weak reactions (3-4 cells clump) were considered as negative. After 30 days, some samples of A and O groups showed B activity. This activity increased with the passage of time. Graves et al (1978) also observed a similar phenomenon. This antigenic activity was reported to be due to some bacteria or bacterial enzyme like E.coli which might have converted the terminal sugar of blood group specific substances to change its specificity (Jenkins et al 1972).

Rh(D) antigens could not be detected in any of the vaginal secretion stains tested in this study. Similarly GLO-I and G-6-PD isozymes could not be detected in any vaginal secretion stains tested in this study. Schwed and Stock (1982) and Bryson et al (1988) also showedthe absence of GLO-I isozymes from vaginal secretions.

CONCLUSION

On the basis of above parameters, the vaginal secretion stains can be conclusively identified but cannot be individualised. For the individualisation of vaginal secretion stains,more polymorphic genetic parameters should be studied.

REFERENCES

Ablett PJ (1983) The identification of the precise conditions for seminal acid phosphatase and vaginal acid phosphatase separation by isoelectric focussing patterns. J Forens Sci Soc 23 (3):255-258

Bargagna M, Sabelli M,Giacomelli C (1982) The detection of Rh antigens (D,C,E,e) on bloodstains by a micro-elution technique using low ionic strength solution (LISS) and papain treated cells. Forens Sci Int 19 (2):197-203

Bryson CK, Garlo AM, Piner SC (1988) Vaginal swabs:endogenous and post-coital components. J Forens Sci (In press)

Chowdhuri S (1980) Examination of Biological Stains. Controller of Publications, Government of India, Delhi, India

Culliford BJ (1971) The Examination and typing of Blood stains in a Crime Laboratory. US Government Printing Office, Wasington DC

Garlo AM (1985) PGM and EsD activity in post-coital vaginal swabs. J Forens Sci Soc 25:301-311

Graves MH, White JM, Fitzpatrick FA, Kuo MC (1978) A comparison of absorption-inhibition and absorptio-elution methods in the detection of ABO(H) antigens found in vaginal samples submitted in sexual assault cases. J Forens Sci 23 (2):345-352

Jenkins GC, Brown J, Lincoln PJ, Dodd BE (1972) The problem of aquired B antigen in forensic serology. J Forens Sci Soc 12:597-603

Joshi S, sharma VK, Cheema DPS, Kaur G (1986) A comparison of absorption-inhibition and absorption -elution technique for the detection of ABO antigens from human vaginal secretion stains. Proceedings of Silver Jubilee Celebrations Conference held at Punjabi University,Patiala

Kind SS (1960) Absorption-elution grouping of dried blood stains on fabrics. Nature 185:789-790

Low B, Masseter L (1974) Anti-globulin test in low ionic strength salt solution for rapid antibody screening and cross-matching. Vox Sang 26 (1):53-61

Martin PD (1981) A and B blood group antigens in vaginal swab extract. Report II. Metropolitan Police Forensic Science Laboratory, London

Martin PD, Cheshire S (1982) ABH antigens and protein levels from vaginal swab extracts. 10th International Congress of the Forensic Haemogenetics, Munich:253-257

Rothwell TJ, Harvey Kj (1978) The limitation of Lugol's Iodine Staining technique for the identification of vaginal epithelial cells. J Forens Sci Soc 18:181-184

Sato M, Ito H, Tsutsumi H, Aoki M, Ktsumata Y, Sato K,Yada S, Mizutani S (1983) Isozyme typing of human semen, vaginal secretion and their mixture. Acta Crim Japon 49 (4):157-165

Scheward W, Stock D (1982) Nachewis von Erblichen enzymeigenschaften im vaginal sekert. Z Rechtsmad 89:115-118

Sehajpal PK, Sandhu HS, Tewari K, Sharma VK (1986) Detection of glyoxalase-I isozymes in human blood stains by a rapid method. Ann of Biolog 2 (1):90-92

Spencer N, Hopkinson DA, Harris H (1964) Phosphoglucomutase polymorphism in man. Nature 204: 742-745

World Health Organisation (WHO) Scientific group on standardisation of procedure for the study of Glucose-6-phosphate dehydrogenase, 1967: Nomenclature of Glucose-6-phosphate dehydrogenase in man. Bull World Health Organisation 36:319-322