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Identification of ABO Blood Group Specific Substances From Human Nails

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ABSTRACT This study reports the successful detection of ABO blood group specific substances from freshly cut finger and toe nails obtained from 123 'donors'. Both mixed haemagglutination and absorption-elution techniques were employed.

INTRODUCTION

Determination of ABO blood groups in finger and toe nails is a matter of immense medico-legal interest. In personal identification of decomposed bodies, dismembered murder victims, air crash and bomb attack victims, the ABO groups may be detected from human nails by the mixed-elution technique; the primary value being in demonstrating non-identity. Shimizu and Yamanauchi (1950) were the first to detect the presence of ABH substances in human thinly cut nail samples by absorption-inhibition technique.

In the three alcohol-extracted fractions from nails, they found that both carbohydrate and protein fractions contained ABH activities. Sugimura and Shimoda (1954) eluted ABH substances from nails in boiling water containing 0.5 per cent lead acetate wherein they showed the presence of ABO blood groups. Thoma (1955) employed the absorption-inhibition technique for determination on hair and nails using ultrasonic vibrations.

Subsequent investigations by Aoyama (1961), McWright (1961) and Ihara (1968) made it clear that human nails contain the blood group substances in quite high concentrations and can be detected by means of absorption-inhibition technique. Guth (1978), Itol and Habe (1980) and Mikami et al. (1982) compared the determination of ABO blood groups of nails, skin and hair from the same individual and found that among these nails gave results of the highest accuracy.

MATERIALS AND METHODS

Nails Samples : Finger nail and toe nail samples were included. Nail material was washed thrice in Sorenson phosphate buffered saline. To a few fragments of nails 0.25 ml of 0.1 per cent potassium hydroxide was added. The tubes were then placed in a water-bath at 37°C for ten minutes, during which period they were occasionally shaken vigorously.

Immediately after incubation 0.5 ml of 70 per cent ethanol was added and the contents were mixed thoroughly. Then the tubes were centrifuged at 2500 r.p.m. for five minutes. The deposit was washed three times with bovine albumin diluent and resuspended in diluent for further tests. Subsequently, the nail pieces were divided into three parts for each technique and treated with two drops of anti-A anti-B and anti-H (*Ulex europeaus*), respectively. A standard period of 48 hours for absorption at 4°C was used for nails through out the study.

Diluent : This was 22 per cent bovine serum albumin diluted 1.5 in 100 (v/v) in normal saline.

Papain Treatment of Red Cells : The papain was prepared according to Low's (1955) method.

(i) Absorption-Elution Technique : The absorption elution technique used was adapted from that of Nickolls and Pereira (1962).

After absorption the antisera was removed from each tube and contents were carefully washed four times with ice-cold saline. Final wash was made with albumin diluent to remove all traces of uncombined antibody. The washing process was spread over a period of two

hours. One drop of albumin diluent was added to each tube and they were placed in a hot water bath (56° C) for ten minutes, the tubes being agitated after an interval of about three minutes. After that the tubes were removed from the water-bath, the appropriate 0.5% indicator red cells (papain treated in case of H) were added in each tube and then centrifuged at 1500 r.p.m. for 2 minutes and the contents examined macroscopically for agglutination.

(ii) Mixed-Agglutination Technique : The mixed haemagglutination technique used was adapted form that of Coombs and Dodd (1961). All the steps were identical to as mentioned under absorption elution technique except that elution process at 56°C was omitted in this technique. After adding the appropriate indicator red cell diluted in bovine albumin diluent the tubes were left undisturbed for thirty minutes at 4°C. They were then gently tapped to resuspend. Slides were prepared by picking up the nail pieces with a wide bore pipette and placing them on a microscope slide and covering with a coverslip. The preparations were then examined under a low-power microscope. A score was given for both agglutinates-on-nails and free agglutinates, the range varying from 4 + to 1+. Reactions weaker than 1 + were recorded but were not taken as decisively indicative of the presence of a particular antigen in the nail material.

RESULTS AND DISCUSSION

A total of 123 nail samples of known blood group and secretor/non-secretor status were analysed periodically every fortnight over a period of 8 months. Thoma (1955) employed absorption-inhibition technique for ABO grouping of finger and toe nails. Sonic oscillations for 30 minutes at 1000 KHz were found to help expose the blood group substances in some way. Reliable results could be obtained with samples belonging to all four groups which were collected only from secretors. McWright applied the procedure to 15 mg amounts of nail substance, except that sonication was for 10 minutes and in the presence of antisera. Outteridge (1963) reported reliable results in the grouping of 105 nail samples. Yada et al. (1966) reported successful grouping of finger nail samples of about 1 mg by elution method including those from non secretors. The nail material was first scraped and then crushed prior to absorption. Thereafter, Kirst et al. (1971), Sakai and Takizara (1978), Sehajpal and Sharma (1982) and Garg (1983) modified the absorption-elution technique to enhance the test sensitivity.

Sugimara and Shimoda (1954) reported that ABO grouping of nail was possible only from secretor samples, while Shimizu and Yamanauchi (1950), Aoyama (1961), Yada et al. (1966) and Sehajpal and Sharma (1982) reported that ABO grouping was possible from both secretors and non-secretors and that there was no difference in antigenic activity between them. Mikami et al. (1982) detected the ABO blood groups from nails of new born and adult individuals and reported that both show the same reactivity. Mikami et al. (1982, 1983) also examined the effect on blood group activity of nail samples which were left in water and in soil and showed that ABO typing was possible even after six months but with relatively reduced reactivity. After one year, false reactions appeared in many cases, presumably due to bacterial attack. Moreover, they could detect ABO blood groups from nails which were subjected to high temperature (200°C) for 15 minutes suggesting that the blood group substances in human nails are highly thermostable and that nails from decomposed cadavers, drowned bodies and partially burned bodies can also be typed with high accuracy.

In the present study, satisfactory results could be obtained even after 8 months of storage from nail samples belonging to both secretors and non-secrectors. Absorption-elution technique proved to be more sensitive than mixed agglutination technique.

It may be important to note that no ultrasonic vibrations were given as has been recommended by Thoma (1955), McWright (1961) and Swinburne (1962).

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