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Quantitative Estimates of ABH Secretion in Saliva of Human Twins

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Abstract. Blood and saliva samples of 122 like-sexed twin pairs (65 MZ and 57 DZ) living in Chandigarh (India) were analyzed for ABH polymorphism. The results indicated that ABH secretions were independent of ABO blood groups though there was an indication of higher incidence of non-secretors among 'O' blood group twin individuals. No significant differences were observed between twins and singletons in secretor gene frequency estimates. The quantitative data revealed that mean titre scores for H substances were lower than that for A and B substances. F test contrasting intra-pair variance between zygosities for ABH quantitative secretions was highly significant indicating stronger genetic component of variation. The results suggested that quantitative assay of ABH secretions would be a better indicator for zygosity determination than mere qualitative differentiation.

Key words: ABH polymorphism, Saliva, Zygosity diagnosis, Twins.

INTRODUCTION

ABH blood group substances were discovered in saliva byYamakami [33] and in other body fluids by Hartman[10]. Since then tremendous genetic interest has been generated in this polymorphism. Schiff and Sasaki [24] have worked out its inheritance patterns. The trait is controlled by a pair of alleles Se and se, the secretor allele (Se) being dominant. A large number of human populations have been investigated for this polymorphism to draw out anthropological comparisons [17, 31].

Genetic variations observed in human populations are attributed interalia to differential selection pressures operating on these populations. ABH secretion in twins is no exception to this rule. After the classical anthropological studies, the emphasis has now shifted to explore the possibility and extent of associations of ABH secretion with diseases. A wide array of such associations has been reported and hypotheses forwarded [7, 8, 11, 20, 21, 30-32].

A quantitative estimation of blood group substances secreted in saliva has not received adequate attention. The quantitative differences among humans make them either strong secretors or weak secretors. To the best of our knowledge, no study has been conducted on the quantitative estimates of these secreted antigenic substances on Indian twins and hence the present investigation.

MATERIALS AND METHODS

Blood and saliva samples of 122 like-sexed twin pairs (244 individuals) were collected from Chandigarh, India. The twin registry here has been developed since 1975 and is being updated from time to time. These twins have been the source for various anthropological/genetic epidemiological investigations [25-29].

The zygosity of the twins was established on the basis of the similarity method for various genetic markers: A1A2BO, CcDEe, MN, Kell and Duffy blood group systems, PTC tasting ability, ABH secretion and dermatoglyphics. However, all the twin pairs were not subjected to all these tests. In about 80 pairs of twins, the zygosity was determined by the similarity for A1A2BO, Rh, PTC tasting ability, ABH secretion, dermatoglyphics and photographs. This zygosity analysis resulted in 65 MZ and 57 DZ twin pairs.

To collect saliva sample, the subject was first requested to rinse his/her mouth and then a cotton swab was given to be put below the tongue. When the swab became saturated with saliva, its contents were squeezed into the test tube with clean hands. To inactivate salivary enzymes and bacterial contaminations, these test tubes were put in a hot boiling water bath for 10-20 minutes. When the samples cooled down, these were centrifused for about 10 minutes at 2000 rpm. A clear supernatent fluid was transferred to another test tube leaving behind the sedimented material.

The standard antisera A,B and H were used. These were first subjected to titration following standard procedures [16]. The titre of the serum was expressed as the reciprocal of the greatest dilution causing visual agglutination. These antisera were diluted to give a final titre of 1:4 before use in the inhibition test. Dilution of antisera is essential in inhibition technique for determining the secretor status because the use of potent antiserum could give false results. As after inhibition of weakly secreted blood group substances in certain cases, there would be still some antibodies left behind to give agglutination with red cells of the corresponding blood group. Hence the right titre is the sine-qua-non for the potent test. Such a titre is the greatest dilution of the antiserum which still has adequate antibodies present to react and inhibit the corresponding antigens.

The quantitative assay of ABH secretions was done by the serial double dilution technique. In this technique, serial dilutions of saliva were prepared in small appropriately numbered tubes. A measured amount of saliva was put in the first two test tubes. From the second tube onwards, an equal volume of isotonic saline was put. The saline and saliva in the second tube were mixed thoroughly and one half of the mixture was transferred to the third tube. This process was repeated for each subsequent tube and in this process one half of the contents were discarded from the last tube. Thus a measured quantity of serial dilutions of saliva were prepared from 1 through 1:256. A drop from each of these tubes was

transferred to the cavities of VDRL slides. Then a drop of appropriately diluted antiserum was added to each cavity. These slides were covered with the petridishes to avoid evaporation as it could disturb the carefully controlled titre. The slides were left undistrubed for 30 minutes to allow inhibition. After that one drop of red blood cells of the corresponding blood group was added to each of the test cavities. After about 10 minutes, the results were recorded. Agglutination could be seen in all the cavities in case of non-secretors, while no agglutination was visible in secretors in various cavities depending upon the inhibition titre of the individual. The inhibition titres were given appropriate scores called titre scores starting from 0 to 9, where 0 was for the non-secretor and 9 for 1:256 serial dilution strength. Appropriate controls were set to ensure that standard diluted antisera gave reactions expected of them and did not give false results.

The data so collected were analyzed by subjecting to suitable statistical tests like mean, standard deviation, chi-square, t-test, analysis of variance and F-tests to draw out conclusions.

RESULTS

The data presented in Table 1 show distribution of secretor types in different blood group categories among MZ and DZ twins. The highest incidence of non-secretors is observed among O blood group individuals in both MZ and DZ twins. But chi-square test fails to reveal statistically significant differences in secretor status with reference to ABO blood groups. So it can be concluded that ABH secretion is independent of the blood group types in both zygosities.

Table 1 - Distribution of secretor types in relation to ABO blood groups in twins

Zygosity	Secretor status	Blood group				Total
		0	A	B	AB	
MZ	Secretor	11	15	24	4	54
	%	64.71	93.75	85.71	100.00	83.08
	Non-secretor	6	1	4	0	11
	%	35.29	6.25	14.29	0.00	16.92
		$\chi^2 = 6.25$	P > 0.10			
	Secretor	34	18	31	8	91
	%	75.56	85.71	81.58	80.00	79.82
DZ	Non-secretor	11	3	7	2	23
	%	24.44	14.29	18.42	20.00	20.18
		$\chi^2 = 1.01$		P > 0.70		

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The gene frequency estimates in twins and general Chandigarh population are presented in Table 2. The gene frequency of secretor allele (Se) is higher than its recessive

Table 2 - Gene frequency estimates in twins and general population of Chandigarh

Category	N	Gene frequencies		Expected genotypic frequencies		
		Se	se	SeSe	Sese	sese
MZ twins	65	0.589	0.411	0.347	0.484	0.169
DZ twins	114	0.551	0.449	0.304	0.495	0.202
Total	179	0.564	0.436	0.318	0.492	0.190
General population*	1806	0.593	0.407	0.352	0.483	0.166

* Pooled published data [1, 4]

Note: Chi-square between twins and general population is 0.693 with d.f. = 1 and P > 0.30.

Table 3 - Distribution of titre scores for ABH secretion among MZ and DZ twins

Titre	H substance			A substance			B substance		
score	MZ	DZ	Total	MZ	DZ	Total	MZ	DZ	Total
0	8 (30.77)	7 (29.17)	15 (30.00)	2 (6.67)	4 (21.05)	6 (12.24)	10 (21.74)	7 (26.92)	17 (23.61)
1	2 (7.69)	3 (12.50)	5 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (2.17)	1 (3.85)	2 (2.78)
2	6 (23.08)	1 (4.17)	7 (14.00)	2 (6.67)	1 (5.26)	3 (6.12)	0 (0.00)	0 (0.00)	0 (0.00)
3	1 (3.85)	5 (20.83)	6 (12.00)	5 (16.67)	0 (0.00)	5 (10.20)	1 (2.17)	0 (0.00)	1 (1.39)
4	4 (15.38)	3 (12.50)	7 (14.00)	5 (16.67)	0 (0.00)	5 (10.20)	3 (6.52)	3 (11.54)	6 (8.33)
5	0 (0.00)	3 (12.50)	3 (6.00)	3 (10.00)	1 (5.26)	4 (8.16)	3 (6.52)	3 (11.54)	6 (8.33)
6	1 (3.85)	2 (8.33)	3 (6.00)	1 (3.33)	4 (21.05)	5 (10.20)	10 (21.74)	5 (19.23)	15 (20.83)
7	1 (3.85)	0 (0.00)	1 (2.00)	7 (23.33)	2 (10.53)	9 (18.37)	6 (13.04)	2 (7.69)	8 (11.11)
8	2 (7.69)	0 (0.00)	2 (4.00)	4 (13.33)	4 (21.05)	8 (16.33)	7 (15.22)	3 (11.54)	10 (13.89)
9	1 (13.85)	0 (0.00)	1 (2.00)	1 (3.33)	3 (15.79)	4 (8.16)	5 (10.87)	2 (7.69)	7 (9,72)
Total	26 (100.01)	24 (100.00)	50 (100.00)	30 (100.00)	19 (99.99)	49 (99.98)	46 (99.99)	26 (100.00)	72 (99.99)
Mean	2.73	2.46	2.6	5.00	5.47	5.18	5.09	4.38	4.83
SD	2.82	2.11	2.48	2.45	3.34	2.80	3.16	3.20	3.17
χ^2	13.725			15.904			2.73		
df	9			9			9		
P	> 0.10			> 0.05			> 0.90		

Figures in parentheses are percentages

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Table 4 - Intra-pair differences in titre scores of ABH secretion among MZ and DZ twins

Difference in Titre scores	Twins		
	MZ	DZ	
		Concordant	Discordant
0	25 (51.0)	4 (22.2)	1 (7.7)
1	19 (38.8)	6 (33.3)	2 (15.4)
2	5 (10.2)	1 (5.6)	0 (0.0)
3	–	3 (16.7)	2 (15.4)
4	–	1 (5.6)	2 (15.4)
5	–	0 (0.0)	2 (15.4)
6	–	2 (11.1)	2 (15.4)
7	–	1 (5.6)	2 (15.4)
Within-pair mean squares	0.398	4.833	10.462

Figures in the parentheses are percentages

counterpart (se) in both MZ and DZ twins as well as in general Chandigarh population. This general population basically represents singleton population. No statistically significant differences have been observed between twins and singletons in gene frequency estimates.

MZ and DZ twins have been compared for quantitative estimates of their ABH secretions in Table 3. The mean titre score of H substance is the lowest when compared with that of A and B substances in both MZ and DZ twins. The observed differences in frequency distribution of titre scores between zygosities do not reach statistically significant level at 5% probability for any of the three ABH secretion types.

The intrapair differences in the titre scores of secreted antigens of MZ and DZ twins are given in Table 4. DZ twins are further sub-divided into concordant and discordant pairs on the basis of their ABO blood groups. The table shows that 41.94% of DZ twin pairs are discordant for the type of antigens. Within-pair mean squares (WMS) of the titre scores are very low in MZ than DZ twin pairs. F-Fest contrasting the two zygosities for this quantitative trait is highly significant at 0.1% level of probability. Hence, it can be concluded that amount of secreted antigens is significantly under genetic control.

DISCUSSION

The genes A, B, H and Se can be called transforming genes because they control certain stages in conversion of precursor substance to specific secreted products. Their allelic forms O, h and se can be considered as inactive genes because they do not play any part in conversion of precursor substance. Chemically, ABH antigens on red cells are glycol-

ipids, but in secretors they are partly glycoproteins [9]. Biochemical and genetic studies [12,18] have refuted the classical regulatory gene model for the Se locus and advocated a structural gene model that encodes for a distinct α [1,2] fucosyltransferase [Se enzyme]. Recent molecular genetic studies [13,23] have located the secretor gene [FUT 2] and a pseudogene of FUT 2 on the chromosome 19q. These recent studies have generated a keen interest in the secretor locus.

The dichotomous division of individuals into secretors and non-secretors would depend upon their quantitative levels of ABH substances secreted in saliva and other body fluids. The quantitative assay of these substances would differentiate secretors further as strong and weak secretors. However this further division is arbitrary because infact there is continuous variation of the strength of these substances from one extreme to another. If quantitatively, the level of secreted antigens is lower than the optimum threshold level, then that individual would be called non-secretor. The results of the present study vividly throw light on these aspects. It can be further emphasized that studies on ABH secretion should include quantitative aspects of these secretions. The observed results of higher incidence of non-secretors among O blood group individuals may be traced to the differences in titre scores of H substances as compared with that of A and B substances. The frequency distribution of titre-scores of these substances shown in Table 3 reveals preponderance of lower scores in case of H substance than A and B substances.

Natural selection has usually been invoked as one of the agencies that maintain genetic polymorphism depending upon the prevailing environmental conditions. This hypothesis has been candidly accepted to be operative for ABH polymorphism too. The environmental upheavals witnessed in this century have changed many balanced polymorphisms into transient ones. Advanced western or urban human populations have seen increase in incidence of many genetic disorders/traits. For example, colour blindness is one such genetic disorder whose incidence has increased in western/urban populations than that in nomads. Selection relaxation hypothesis has been forwarded to explain this trend [19]. Similar trend has been reported for ABH secretions as there has been an increase in the frequency of non-secretors in advanced urban societies than their primitive counterparts and non-human primates and selection-relaxation hypothesis has been invoked to explain the trend as for colour blindness [2]. The ABH substances in saliva and gastric juices are said to have protective function against lectins present in raw food. The former tends to neutralize the deleterious effect of lectins on gastric mucosa. It is a common knowledge that raw food constitutes a major part of diet of nomads and non-human primates, while urban people consume highly processed food items.

It can be inferred from the foregone discussion that selection seems to be operative at ABH secretion locus. Our next aim is to investigate whether this selection is randomly operating on twins and singletons or not. This issue also stems from an evidence of higher prenatal mortality of twins than singletons and difference in perinatal mortality rates between zygosities. For example, a study on spontaneously aborted complete embryos and foetuses revealed higher embryonic and foetal mortality in MZ than DZ twins and further in twins than singletons [14]. However, Boklage [6] contests these claims of differential mortality rates between zygosities and forcefully argues that excess mortality of twins is not confined to MZ twins though it is concentrated in like-sexed twin pairs. The other motivating factor for undertaking the comparison was the reports that indicate differences between twins and singletons for many biological traits/disorders [5, 25, 30].

Contrary to the above expressed apprehensions, the results of comparison between zygosities for qualitative as well as quantitative estimates of ABH secretion are not significant (Table 2 and 3). Unfortunately the comparable quantitative data on ABH secretion among singletons/general population are not available. However, there is one unpublished report [22], where comparable quantitative data are available on three endogamous population groups. Since there were no significant differences among them, their data were pooled and reanalyzed following the models used in the present study to make the two comparable. Moreover, the twin sample also included individuals from these three endogamous groups. The results of this comparison are presented in Table 5.

Table 5 shows that mean titre score of H substance is the lowest and that of A substance being the highest in both twins and singletons. The overall pattern is similar between zygosities and between twins and singletons. The higher value of standard deviation among twins than singletons shows greater variation in the former. The differences between twins and singletons in their mean titre scores are not significant for A and B substances but significant for H substance. This can be attributed to chance factors. These results indicate that natural selection has been randomly operating among zygosities and between twins and singletons for ABH secretion locus. The differential perinatal mortality rates among them have not caused any significant difference on qualitative as well as quantitative levels of ABH secretion.

Intra-pair variations in titre scores yield significant results. More than 50% of MZ cotwins have identical titres, while only one fifth of concordant DZ cotwins have identical titres. None of MZ cotwins have difference of 3 or more in their titre scores, while the picture is very different in DZ twins. These results clearly indicate that quantitative secretion of ABH substances is under strong genetic influence though environmental or other biological factors may interact to cause intra pair differences in titre scores of MZ twin pairs. These results have many implications and these are briefly discussed in the following text.

In like-sexed twins, concordant for the type of ABH secreted substances, the quantitative differences in titre scores may be used in zygosity diagnosis. If difference in titre scores of two members of a twin pair is more than 3, then that pair can be safely diagnosed as DZ.

Table 5 - Comparison of mean titre score between twins and singletons

Type of substance	Titre score						
	Twins			Singletons			t-test
	N	$\bar{X}$	SD	N	$\bar{X}$	SD	
H	49	2.60	2.48	102	3.80	1.89	3.02*
A	49	5.18	2.80	1 17	5.62	1.09	1.07
B	72	4.83	3.17	165	4.94	1.48	0.28

* $P < 0.05$

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In another instance, where cotwins of a pair have titre scores of 0 and 1, they would be classified as non-secretor and secretor respectively. Such a pair would be ordinarily called DZ. But if we review our results, we would note that some cotwins of MZ twin pairs show a difference of two in their titre scores. In the light of this, it may not be appropriate to label the above said twin pair as DZ. It will be prudent to decide the zygosity of such a pair on the basis of concordance for other genetic markers. So it can be concluded that if ABH secretion is to be employed as one of the genetic marker in diagnosis of zygosity, then it will be indispensable to do its quantitative assay.

Aberrant secretors are those who secrete A and / or B substances without secreting H or vice versa [15]. No attempt has been made in this study to investigate this aspect for many reasons. For example, it has been argued that non-secretors of H and secretors of A or B are mainly due to small amount of H antigen and this could be detected if appropriate reagent like human anti H (from Oh persons) or sheep anti H is used in the test [3]. So in this study, O blood group individuals were tested for secretion of H, while A and B blood group individuals for A and B respectively. The twins having AB blood groups were tested for both A and B. In total, 15 twin individuals were investigated for quantitative secretions of both A and B substances. Among them only three individuals were secretors for A and non-secretors for B. In fact two of the three cases of such aberrant secretors were members of a MZ twin pair who had titre strength of 3 for A substances. So both the members of the MZ twin pair were identical in their aberrant behaviour. While the DZ twin pair was discordant for this condition. In a large series of twin data, quantitative differences in the titre scores may shed more light on aberrant secretors. In weak secretors, such an aberrancy may be more common. But this hypothesis should be considered as tentative till we have more data on this aspect.

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