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PALEOSEROLOGICAL STUDY OF HUMAN BONE REMAINS FROM THE DEVÍN-CASTLE SITE (SLOVAKIA)

ABSTRACT: Fifty skeletal remains of a part of the ancient Slavonic population from the Devín-castle site (Slovakia), dated to the l0th – l3th century, were blood typed. A modification of the AE method was used. The bones investigated showed a high reactibility. The deviation from genetic equilibrium, according to the H-W rule points to a high incidence of AB phenotypes, similarly to that shown by a comparison with the frequencies obtained for the recent population in the same region.

KEY WORDS: Paleoserology – Absorption– Elution method – Devín site

INTRODUCTION

Since the early days of paleoserology which date back to around 1934, when the pioneers Boyd and Boyd (1934) started blood typing Egyptian mummies, a number of biochemical techniques have been developed and modified, which can be used to determine blood group specificity of mummified soft tissue and bone (Lengyel 1982, Paoli *et al.* 1986, Borgognini Tarli *et al.* 1986, Lee *et al.* 1989, Borgognini Tarli *et al.* 1993). From these techniques the most used are haemaglutination-inhibition (HI), absorption-elution (AE) and fluorescent-antibody (FA) method.

In the present paper we are reporting the determination of ABO antigens in skeletal remains using AE test. We attempted to confirm the usefulness, reproducibility and reliability of the method and to acquire additional biological data from the skeletal material that already has been studied by classical methods of anthropology.

MATERIAL AND METHODS

The material studied included skeletal remains of 50 individuals from archaeological finds at the locality Devín-castle, close to the city of Bratislava (*Figure 1*), dated to 10th–13th century. Detailed information on the anthropological investigation of the material has been

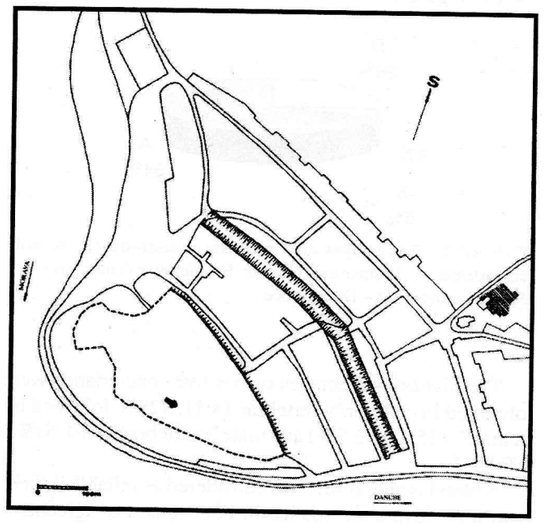

FIGURE 1. The Devín-castle site (Slovakia).

published (Poláčeková 1991). The sex has been determined in a standard way the using the method of Ferembach *et al.* 1979.

Bone powder obtained by crushing the spongiosa of the vertebral body was used as the substrate. This material is adequate with respect to the antigen remainders being protected by a layer of compact bone tissue (Lengyel 1975).

Modified procedure of the absorption-elution test (according to Paoli *et al.* 1986) was used for blood typing. The principle of the test is based on antigen-antibody conjugation with subsequent separation of substrate-bound antibodies at a critical temperature of +56°C. The modification consisted in the use of AGH serum added after elution into the cooled supernatant together with an aliquot of the washed red blood cells.

A pair of AE tests was used as a direct control. AE tests were run repeatedly on the same bone tissue samples to verify the reliability of the results.

RESULTS

The sample included 50 individuals. *Table 1* shows the sample distribution according to the sex; the majority of both the males and females in the sample were adults.

The blood groups repeatedly determined using the AE test were discordant in 13 cases (26%). Positive concordance allowing to determine the respective blood group was obtained in 32 instances (64%), negative concordance was obtained for 3 cases (6%), thereof in one case there was mixed ABH agglutination at the first and the repeated test, and in 2 cases the bone material did not react at all. Non-discordance (i.e. the first test being successful in determining the blood group while the repeated one was not, or vice versa) occurred in 2 cases (4%), (*Figure 2*).

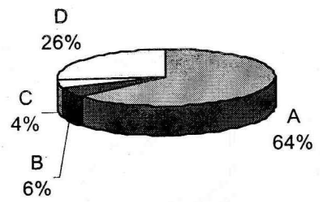

FIGURE 2. Percentages of concordance, discordance and non-discordance. A – Positive concordance; B – Negative concordance; C – Non-discordance; D – Discordance.

The highest percentages of positive concordance were observed in children's vertebrae (8:11, 72%), followed by female's (15:24, 62.5 %) and male's vertebrae (8:14, 57%), (*Table 1*).

A blood typing result was considered as reliable if there was a positive concordance in both runs, i.e. agreement between the first and the second test. This was achieved in a total of 32 individuals (64%). The same technique used repeatedly to determine the blood group in another 18 individuals yielded discordant results or the blood group was successfully determined in one test series while the repeated test provided no conclusive result, non-specific reaction or no reaction at all.

Table 2 shows the observed phenotype and gene frequencies. The parameter D/σ used to evaluate genetic equilibrium points to excessive incidence of the AB phenotype as compared to expected values. If, however, the time elapsed is accounted for, the parameter D/σ can be used to check the rough deviation from the frequency due to e.g. bacterial degradation or false positive reaction, rather than to evaluate the actual genetic equilibrium (Borgognini Tarli, Paoli 1982).

DISCUSSION AND CONCLUSIONS

Reliability and reproducibility of the AE technique were checked directly, i.e. by running AE test repeatedly for the entire material investigated. The results were compared with those obtained by Vondráková (1994) using the same technique (AE) (*Table 3*).

The reported percentage of positive concordance is higher than in Vondráková (1994), however the difference in N of samples is evident. According to our experience the relatively high percentage of positive concordance (66%) gives evidence of good reproducibility of the AE technique.

Comparing the two different techniques, AE (present results) and HI (results obtained in laboratory in Pisa) (Borgognini Tarli, Paoli 1981), exhibit a higher percentage of discordance in the case of AE method. The reason may be in the fact that HI uses twice-purified water extract (the bone powder is purified before extraction and the water extract after extraction). This results in a higher percentage of negative reactions (higher percentage of negative concordance for HI as compared to AE), on the other hand however, there is lower percentage of discordance.

The detection of ABO antigens in dependence on the sex is unambiguously most positive in the case of children. The high percentage of cases in which a determination could be made may be due to the high intensity of haematoporesis in the bone marrow of children, and thus to a higher chance of the bone tissue to become saturated by blood group antigens.

The differences are evident if comparing the distributions of blood groups and gene frequencies obtained by ABO analysis of bone remains using the AE test with the phenotype and gene frequencies of the recent population of the same region (*Table 4*).

A comparison with the frequencies for the present population of the region points to a high frequency of the AB phenotype with low frequencies of both the 0 and the B phenotype in the historical bone sample. Naturally, also the sample size (N) plays a role, N for the historical sample

TABLE 1. Sample distribution and percentage of positive concordance according to the sex.

	Males	Females	Children	Undetermined
N (individuals)	14	24	11	1
Percent distribution	28%	48%	22%	3%
N (positive concordance) : N (individuals)	8:14	15:24	8:11	–
Percent of positive concordance	57%	62.5%	72%	–

TABLE 2. Phenotype and gene frequencies of the sample.

N	O	A	B	AB	p	q	r	D/σ
32	1	14	3	14				
					0.602	0.294	0.105	–1.4
100%	3.125%	43.750%	9.375%	43.750%				

TABLE 3. Percentage of positive and negative concordance, discordance and non-discordance: A comparison of results from two different samples and laboratories using AE technique.

Authors	Vondráková 1994 AE test	Present results AE test
Sample dating	10–11 A.D.	10–13 A.D.
N (samples)	23	50
N (tests)	46	100
Positive concordance	26.1%	66.0%
Negative concordance	30.4%	4.0%
Discordance	34.8%	26.0%
Non-discordance	8.7%	4.0%

TABLE 4. Comparison of phenotype and gene frequencies of the historical and recent populations from the same region.

	N	O	A	B	AB	p	q	r	D/σ
Present results	32	3.125	43.75	9.375	43.75	60.17	29.35	10.48	–1.4
Mourant *et al.* (1976)	5.110	33.4	39.2	19.2	8.2	27.99	14.80	57.71	–0.24

being small due to time limitations as well as to limitations concerning chemicals and equipment. Also, there has been no other sample of the same time period analysed to which our results might have been compared. Further investigations on skeletal remains of the same time period would be needed to check whether the high frequencies of AB and low frequencies of 0 and B phenotypes observed are random or whether they actually are representative for the given population.

Paleoserology deals with the problems concerning blood typing. Certain problems may have been due to individuals with weak antigens A_2 and A_2B. E.g. Lengyel (1975), who investigated fresh autoptic samples, could correctly classify all individuals with the A_1 phenotype in the blood group A, while only 95.16% of those with the A_2 phenotype were identified as having blood group A. The remaining individuals with blood group A_2 were falsely assigned blood group 0 (34.22%) or AB (1.61%). This is associated with the weak serologic activity of the A_2 phenotype. This ratio is shifted towards higher percentages of falsely classified group A_2 individuals with the increasing chronological age of the individual and with the increasing proportion of errors in determining historical skeletal material. Probably, the higher percentages of the AB phenotype in our material were partly due to erroneous classification of A_2 individuals into the AB group.

The most frequently discussed issues in paleoserologic investigations of historical anthropological material concern degradation changes caused by certain bacteria acting on bones while in earth (Thieme, Otten 1975, Prokop, Uhlenbruck 1969, Watkins 1972, Lengyel 1975, Borgognini Tarli, Paoli 1982), and contamination by pseudoantigens and additional antigens (yielding pseudospecific reactions) present in the viruses, plants and animals (e.g. *E.coli* presents with the B antigen). Based on this it can be assumed that the high AB frequencies observed may also be a result of the above mentioned contaminating substances.

It follows from the present results and their discussion that the AE technique can be successfully used to determine blood groups of the AB0 system. At the same time, we could confirm that the AE method requires lesser amounts

of skeletal material and is therefore suitable for the investigation of partial and/or damaged skeletons (provided the organic components of the bone tissue are preserved) as well as for any situation where destruction of skeletal remains is to be kept at a minimum.

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