RESEARCH ARTICLE

Reliability of Postmortem ABO Blood Grouping: A Study of 100 Cases

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Abstract

Blood grouping has been one of the cornerstones for identification of biological materials in forensic investigations. Antigens of the ABO system can be detected even prior to birth. ABO blood groups can also be detected after death for a long period in many body tissues (teeth, bones, etc.). Blood has its own forensic value in many medico-legal issues. The present study looked at a total number of 100 autopsy cases brought for medico-legal investigations in the mortuary of the Department of Forensic Medicine in collaboration with the Department of Pathology at the Postgraduate Institute of Medical Sciences (PGIMS), Rohtak (Haryana, India). The blood samples were collected from right ventricle without any anticoagulant. ABO blood grouping was performed by direct haemagglutination technique using monoclonal antisera. Reverse blood grouping was also attempted. Our results showed that ABO blood group antigens can be detected from blood fluid after death, even in decomposed bodies with an estimated post-mortem interval (EPMI) of <85 hours. Our results also show that as post-mortem interval increases beyond a certain limit there is a decrease in antigens on RBCs in the post-mortem blood.

Keywords: Blood, ABO blood groups, decomposed bodies, postmortem interval, forensic investigations.

Introduction

Human identification is a mainstay of civilization and identification of unknown individuals has always been of paramount importance to society. Establishing identity is an imperative aspect of any forensic investigative procedure. A doctor's role as a forensic expert goes hand in hand with the police officer in establishing the identity of individuals in many cases [1].

Blood grouping has been one of the cornerstones for identification of biological materials in forensic investigations. Blood grouping plays an important role in serological identification in cases of disputed paternity or maternity. Blood and other body fluids collected from victims and suspects play an important role in identification and in the investigation of criminal cases. The degree of specificity shown by various blood group combinations is akin to that of fingerprints; when an individual has a rare blood group, he can be identified with a high degree of certainty [2].

Blood is a very important entity in medico-legal practice, which alone or with other trace evidences plays a key role in solving different problems. No other bodily fluids or tissues have such a divergent medico-legal importance [3]. A careful laboratory investigation of blood, semen, saliva or their stains can yield information useful in a court of law with varying degrees of reliability [4.] Nowadays, biological evidence generally involves the comparison of DNA; but DNA analysis can be an expensive, complex and more time consuming [3].

Antigens of the ABO system can be detected even prior to birth. Their strength after birth until about three years of age and thereafter remains unchanged. Distribution of ABO blood group antigens in the Indian population is an order of O (38.5%)>B (32.0%)>A (23.0%)>AB (6.5%) [5].

Among the many challenges often faced by forensic pathologists is the estimation of the post-mortem interval (PMI) in cases where the time of death is uncertain or even completely unknown. The accuracy of such estimates may range from within a few hours in cases of recent death to weeks or even months when only decomposed or fragmentary remains are available [6]. The standard blood grouping techniques successfully determined the ABO group of red cells from cadavers, even those extracted many hours after death, and the results agreed with those given by tests on the same patients before death [7].

Post-mortem changes, in particular the putrefactive changes, make ABO blood grouping difficult and the interpretation of the results even more so. However, a combination of the traditional serological approach (Absorption elution technique) and the immunohistological technique (Indirect immune peroxidase reaction) is useful in such situations [8]. The present study aimed to determine the period of time for which ABO blood group typing from blood taken from decomposed body could be assessed.

Materials and Methods

The present study was conducted in the department of Forensic Medicine in collaboration with the Department of Pathology at PGIMS, Rohtak (Haryana - India). This study looked at a total number of 100 bodies brought for autopsy in the mortuary of the Department of Forensic Medicine. Bodies were categorized as: 1) Fresh having an estimated postmortem interval (PMI) of <2 days; 2) early decomposition (ED) with an estimated PMI of 2-3 days and; 3) advanced decomposition (AD) having an estimated PMI of >3 days.

Bodies were divided into 2 groups: identified and unidentified. Dead bodies that were completely charred, had ruptured or lacerated heart and putrefied bodies in which the heart was empty or contained clotted blood were excluded from study.

The right ventricle was punctured and a blood sample was collected in a syringe without

dissecting the ventricle to prevent admixing of any other body fluid (Figure 1).

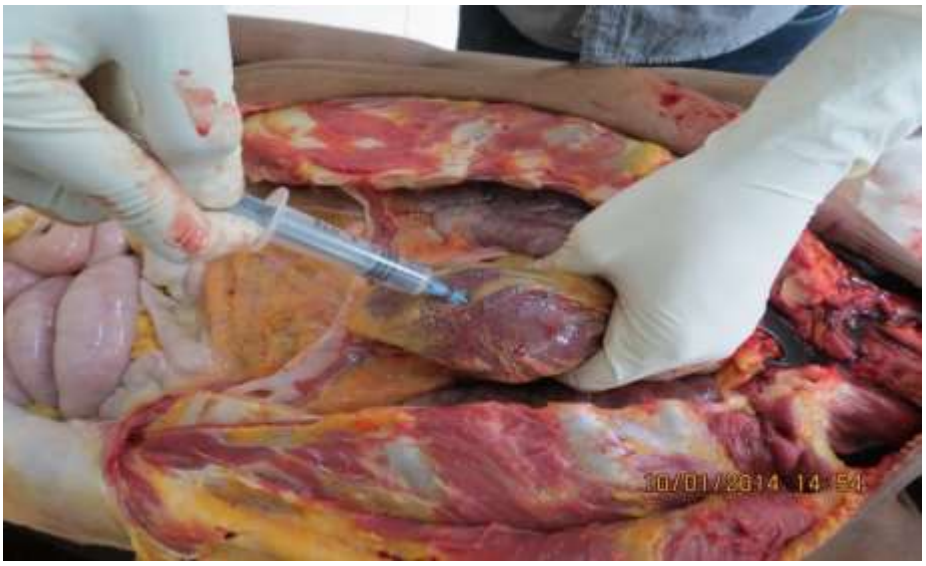

Figure 1: Procedure for collection of blood from cadaver

The blood sample was collected without any anticoagulant. ABO blood grouping was performed immediately in the morgue using the direct haemagglutination method on glass slides using monoclonal anti-A, anti-B and anti-D sera. (Figure 2, Figure 3 & Figure 4) Blood samples were taken from each corpse, irrespective of condition, site of recovery of the body, or the cause and time since death.

Figure 2: Procedure for blood group typing

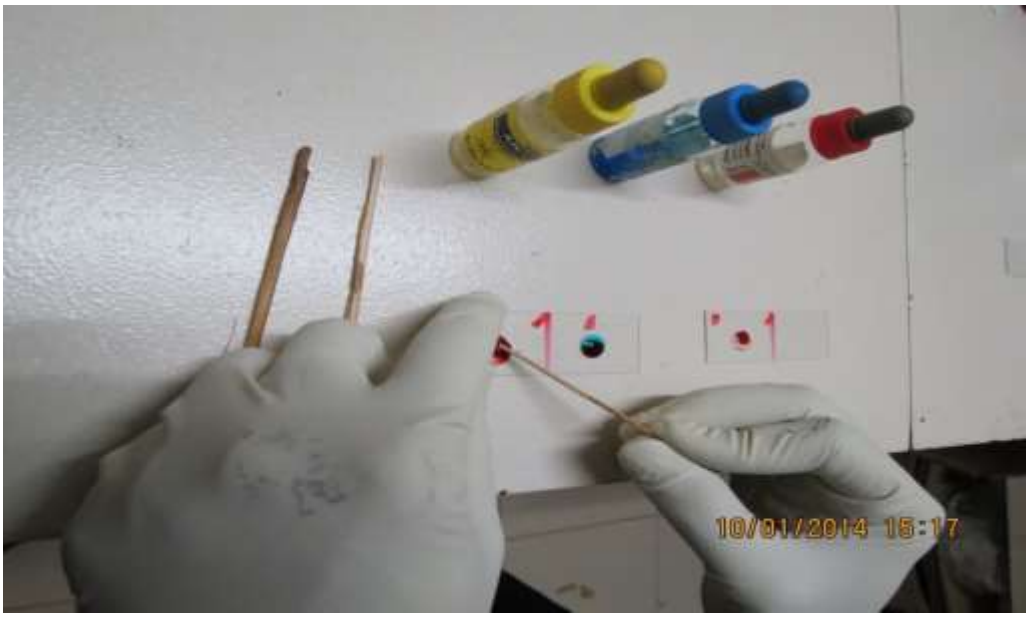

Figure 3: Procedure for blood group typing

Figure 4: Procedure for blood group typing

The blood samples which agglutinate with Antisera-A was blood group A, with Antisera-B was blood group B, with both antisera would be AB. The blood sample which was negative for both antigens A and B then it had possibility of either blood group O or blood that was lysed (i.e. disintegration of antigen).

To differentiate between these two possibilities as mentioned above, reverse typing (slide or tile method) was done. For reverse typing, serum was separated from the blood sample by centrifugation at 3000 rpm for 8 minutes at room temperature. The fresh sample of red blood cell antigens, i.e. 3-5% group A, B and O

RBC suspension in normal saline (0.9%) was collected from the blood bank of PGIMS, Rohtak.

Reverse blood grouping were performed as per the standard laboratory procedure. Briefly, one drop of serum sample was placed in each well of a sterilized glass cavity slide and then mixed with one drop of each 3-5% suspension of group A, B and O red cells. Agglutination was recorded 2-3 minutes after mixing; weak agglutination was confirmed under low power magnification if needed. Group O serum agglutinated A and B cells, group A serum agglutinated only B cells, group B serum agglutinated only A cells and group AB serum did not agglutinate any cell suspension. Results were recorded in Microsoft Excel spreadsheet and the data was analyzed using SPSS version 20.0. The association of demographic variables with time interval between death and autopsy was compared by using a Chi-Square test. A p-value of less than 0.05 was considered statistically significant.

Results

Our study group comprised of 70 male and 30 female corpses of various age groups (Table 1). Post-mortem ABO blood group antigens were detected in 84/100bodies (84%), of which 69 (82.1%) were fresh, 10 (11.9%) were in the stage of early decomposition (ED) and 5 (6.0%) were in the stage of advanced decomposition (AD). In 76 (90.5%) cases, the ante-mortem blood group was unknown and only in 8 (9.5%) cases the ante-mortem blood group was known.

Table 1- Age and gender distribution of cases

Age group (in years)	Male	Female	Total
Up to 10	1	0	1
11-20	5	3	8
21-30	15	15	30
31-40	22	8	30
41-50	14	4	18
51-60	9	0	9
≥ 61	4	0	4
Total	70	30	100

Table 2- Distribution of cases in which ABO blood group antigens were detected with respect to season and place of body recovery

Season	Place of Body Recovery			Total
	Open	Water	Buried	
Summer	20/20	1/5	0	21/25
Winter	13/13	0/4	0	13/17
Temperate	17/17	3/7	0	20/24
Rainy	29/29	1/4	0/1	30/34
Total	79	5/20	0/1	84/100

The table 2 shows that in the total of 84 cases in which a blood group was detected, 30 cases (35.7%) were recovered in the rainy season. Most of these cases were recovered in the open (29 cases, 34.5 %) and one from water (1.2%); whereas 21 cases (25.0%) were recovered in the summer season, mostly from the open (20 cases, 23.8 %) and one from water (1.2%); 20 cases (23.8%) were recovered in the temperate season of which 17 cases (20.2%) were recovered from the open and 3 cases (3.6%) from water; all the 13 cases (15.5%) recovered in winter were recovered from the open. By applying the Pearson Chi-Square test, there was a non-significant (P>0.05) relation between the detection of a blood group and the season and place in which the corpses were recovered.

Table 3 - Distribution of cases according to the duration of cold storage of bodies and detection of ABO blood group antigens

Cold storage interval (CSI) in hours	Blood group antigens detected	Blood group antigens not detected	Total
0	**34**	**10**	**44**
1-12	**8**	**0**	**8**
13-24	**22**	**0**	**22**
25-36	**5**	**0**	**5**
37-48	**12**	**0**	**12**
49-60	**0**	**0**	**0**
61-72	**3**	**6**	**9**
Total	**84**	**16**	**100**

Table 3 shows the distribution of cases according to the duration (time in hours) of storage of bodies at the morgue and detection of ABO blood group antigens. Of the 44 cases which were not stored in the morgue cold rooms, 34 (77%) cases (fresh/identified/ claimed corpses) showed a positive result for ABO blood group antigens, while 10 decomposed bodies did not show any agglutination reaction for the presence of blood group antigens. Fifty-six cases were stored in the cold storage for different time intervals. The crucial Cold Storage Interval (CSI) was 1-48 hours wherein all 47 (100%) bodies in this interval showed positive results. However, for 61-72 hrs, 3 out of 9 bodies (33%) also showed a positive agglutination reaction for ABO antigens.

Table 4- Distribution of cases according to estimated postmortem interval (EPMI) and the detection of ABO blood group antigens

EPMI in hours	ABO antigens detected	ABO antigens not detected	Total
0-12	28	0	28
13-24	27	0	27
25-36	13	0	13
37-48	1	0	1
49-60	6	0	6
61-72	7	0	7
73-84	2	0	2
85-96	0	8	8
≥ 97	0	8	8
Total	**84**	**16**	**100**

*ABO blood group antigens were detected in 2 cases of EPMI 73-84 hours (in advanced decomposition), and these 2 cases were stored in a cold storage room for CSI 72 hours.

The maximum number of cases in which ABO blood group antigens could be detected were from the group with an Estimated Post-mortem Interval (EPMI) of 0-12 hours (28 out of 100 cases), next was the EPMI of 13-24 hours (27 out of 100 cases); while in cases of EPMI beyond 85 hours, no blood group antigens could be detected in the samples. Eight samples were of EPMI 85-96 hours and 8 samples were ≥ 97 hours of EPMI, which showed no result.

Table 4 shows that in all the cases of early decomposition, ABO blood group antigens were detected when bodies were stored in cold storage for 48 hours. The maximum period for which the body remained at room temperature after death was ≥ 24 hours (EPMI-CSI). Irrespective of the season, all the cases of early decomposition showed positive results.

Table 5: Cases of early decomposition related to season, estimated postmortem interval (EPMI), CSI (cold storage interval) and EPMI-CSI

Sr. No.	Season	Place of body recovery	EPMI in hours	CSI in hours	EPMI-CSI in hours
1	Rainy	Open	53	48	5
2	Rainy	Open	60	48	12
3	Rainy	Open	52	48	4
4	Temperate	Water	60	48	12
5	Temperate	Open	72	48	24
6	Summer	Water	72	48	24
7	Summer	Open	72	48	24
8	Summer	Open	72	48	24
9	Rainy	Open	60	48	12
10	Rainy	Water	60	48	12

Table 6- Cases of advanced decomposition related to season, EPMI, CSI and EPMI-CSI in which ABO blood group antigens were detected

Sr. No.	Season	Body recovered from	EPMI in hours	CSI in hours	EPMI-CSI in hours
1	Summer	Open	72	48	24
2	Rainy	Open	72	65	7
3	Rainy	Open	83	72	11
4	Temperate	Water	84	72	12
5	Temperate	Water	72	48	24

Table 6 shows that even those bodies which were in an advanced stage of putrefaction showed positive results if stored in a cold storage room for a considerable period of time. The maximum period for which the body remained at room temperature was ≤ 24 hours (EPMI-CSI). Irrespective of the season and their place of recovery, all the cases which were stored in cold storage showed positive results.

Discussion

Blood grouping has an important role in serological identification of disputed paternity or maternity. The degree of specificity shown by various blood group combinations is akin to that of fingerprints; when an individual has a rare blood group, he can be identified with a high degree of certainty.[2]

In the present study, ABO blood group typing could not be done irrespective of body stored in cold storage and with a considerable CSI beyond the EPMI of 85 hours. Cases with an EPMI of less than 85 hours with the body being stored in cold storage with considerable CSI showed positive results irrespective of the condition of the body, weather or season, age, sex and the place from which the body was recovered, i.e., open, water and buried. Septicaemia had a negative effect on blood group detection in the dead bodies as it enhanced decomposition, but this was overcome when the body was stored in cold storage for a significant period of time as seen in this study. In all the 11 cases of septicaemia showed positive results, i.e. blood group antigens were detected.

In the present study, ABO blood typing was done on 100 autopsy cases. This is well in agreement with the pioneering work done by Enticknap (1957) 7. In the present study, no distinction was attempted in inclusion of the cases; all cases irrespective of age, gender, site of recovery, season and cause of death were included. In contrast, Enticknap's subjects had either died of malignant diseases or post-operations; one death was due to bacterial endocarditis, another had infections before death.

We detected ABO blood groups in 84/100 of the cases. Enticknap, on the other hand, could detect blood groups in 95 cadavers. It is, however, pertinent to note that all the cadavers in the Enticknap series were kept (refrigerated) at 7^0C continuously from 3-140 hours. In contrast, 56 cadavers (56/100, 56%) in the present study were kept in cold storage from 4-72 hours; the rest (44/100, 46%) were kept at room temperature from 3-264 hours before collection of blood (Table 7).

Table 7- Comparison between the study done by Enticknap and the present study

S. No.	Parameters	Enticknap Study (1957)	Present study
1.	Total numbers of cases in study	100	100
2.	Number of cases in which post-mortem blood group typing was positive	95 (95%)	84 (84%)
3.	Total number of cadavers kept at low temperature	100 (100%) at 7^0C (Refrigerated)	56 (56%) at 4^0C (Cold storage room)
4.	Cold Storage Interval	From 3-140 hours	From 4-72 hours
5.	Maximum time in hours at which blood groups could be detected	Not described	84 hours

Padosch et al (2007) attempted a medico-legal assessment of blood transfusion errors by analysing pre-transfusion and cadaver patient blood samples of 3-days (72 hours) post-mortem interval. They detected IgG loaded erythrocytes in both the samples and demonstrated ABO incompatible RBCs in the cadaver blood [8]. The present study analyzed post-mortem blood at various post-mortem intervals and detected ABO blood groups in a cadaver with an estimated post-mortem interval of 84 hours.

In the study conducted by Nagano et al (1975), the ABH antigens activity decreased when heated above 120-130ºC for 60 minutes [10]. Nishi et al studied the serological thermo-stability of blood group active glycolipids by heating them at 120ºC for 30 minutes or 140ºC for 5 minutes in saline and observed that exposure of ABH antigens to elevated temperatures did not affect their antigenic activity and that ABH antigens were remarkably heat-stable [11]. In the present study, blood group antigens were detected even in the blood samples taken from decomposed bodies (<85 hours of EPMI) which were stored in a cold storage room (4ºC). Therefore, it can be concluded that a low temperature for a considerable period of time decreases lysis of RBCs in post-mortem blood during the process of putrefaction.

The maximum Estimated Post-mortem Time Interval (EPMI) in which ABO blood grouping was performed was 0-12 hours (28 out of 100 cases), followed by 13-24 hours (27 out of 100 cases). Beyond 85 hours EPMI, no blood group substances could be detected in the samples. Eight samples were taken with an EPMI of 85-96 hours and 8 samples were taken ≥ 97 hours of EPMI, which showed no results. The present study shows that as post-mortem interval increases beyond a certain limit there is a decrease in antigens on RBCs (destruction) in the post-mortem blood. Mudd (1986) demonstrated that ABO blood group antigens could also be detected even in old blood stains by haemagglutination assay using V-bottom microplates in conjunction with an absorption-elution procedure [12]. These are complex techniques and require higher tools and costly equipment's but the method used in this study, i.e. Direct haemagglutination by slide method, is simple, cheap and easy to perform and can be used as a preliminary test to identify blood groups in unknown decomposed bodies.

Shah et al conducted a cross sectional study of 29 cases to find out the post-mortem survival period of RBCs, the duration until which they remained intact & feasibility of grouping of post mortem blood by simple tube agglutination method. They concluded that time since death can be inferred from RBCs at autopsy and routine tube agglutination technique can be used for blood grouping on

post mortem samples. They also stated that blood grouping can be done with reasonable accuracy till intact RBCs are demonstrable in the samples i.e. up to 19 hours. The present study, however, could demonstrate post mortem blood groups in the samples up to 84 hours after death using direct agglutination slide method [13].

Mehta et al conducted a study on 500 post mortem blood samples using slide method for detecting blood groups in post mortem blood. They could detect the blood groups in 457 cases (91 %) while in 43 cases the blood groups could not be detected which were all having time since death > 72 hours 14.

The above two studies used stored blood for the blood group detection. However, the present study was conducted on blood of all post mortem duration including decomposed bodies. Further, no stored blood was used and blood collected from the bodies was subjected to testing.

Conclusion

This study showed that CSI has an important role in the preservation of blood group antigens, while seasonal variations are of little value. There is no role of age and sex in the detection of blood group antigens; post-mortem blood group detection depends upon Estimated Post-mortem Interval and Cold Storage Interval. This is exemplified by the fact that the EPMI of < 85 hours showed all results as positive, including all cases of early decomposition and 5 cases of advanced decomposition with the bodies being stored in cold storage room at a temperature of 4^0C. In all cases of EPMI >85 hours, no positive results were obtained, even after a significant CSI. Therefore, it can be concluded that ABO blood group antigens can be detected from liquid blood even in decomposed bodies of EPMI of <85 hours provided bodies are stored at a cold storage room temperature of 4^0C.

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