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Determining Post-Mortem Survival Period And Blood Group Antigenicity Of Red Blood Cells: A Cross-Sectional Study

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ABSTRACT

Background

Establishing identity of the individual and post-mortem clocking are of utmost priority among medico-legal issues when dealing with a dead body. The search for newer and more objective parameters and their validation continues. Keeping in mind the scarcity of expert hands and budget constraints of a developing country like India, the parameters should preferably be of such a nature that they are relatively inexpensive and can be incorporated into the routine work.

Aim

To find out the post-mortem survival period of RBC, the duration upto which RBC remain intact and to find out the feasibility of grouping of post-mortem blood by simple tube agglutination method.

Methods

A cross-sectional study was conducted during a span of six months (April 2013 to September 2013). Twenty nine cases were included in the study after applying the inclusion-exclusion criteria. Blood samples were collected and stained with Leishman's stain for microscopy. Wherever intact RBC were found, blood grouping with agglutination method was performed. The findings were confirmed by microscopy followed by cross-matching to gauge the accuracy.

Results

Intact RBC were observed in almost all cases up till 19 hours post-mortem. Tube agglutination test was positive in all samples with intact RBC. Blood grouping result matched with the previously known blood group of the person in almost all cases.

Conclusion

Survival period of RBC can be utilised in post-mortem clocking. Blood grouping using routine tube agglutination technique can be done till intact RBC is demonstrated in sample.

Keywords: survival period, RBC, intact, postmortem clocking, tube agglutination test, blood grouping

INTRODUCTION

Post-mortem clocking is a key objective of medicolegal autopsy. Physical and chemical changes takes place in dead bodies which are under continuous assessment since decades. These changes offer some help in estimating time since death. Several of them have been proved to be useful as supplementary data but none could replace the traditional triad of algor mortis, livor mortis and rigor mortis.[1] Literature search revealed only few studies conducted to evaluate the cellular changes occurring in blood after death for estimating time since death.[2,3] Numerous cells in blood show varying degree of post-mortem changes and these changes vary with regards to the post-mortem interval.[1]

Blood group determination of a known or unknown dead individual is regularly employed as part of a medico-legal investigation, as per the circumstances of the case. It helps in identification of an individual as well as to establish connection of a weapon used/recovered through blood group matching/crossmatching using modified absorption elution technique. In living individuals, blood grouping is done by conventional tube agglutination method which is easy to perform. This method has not been used to determine blood group in postmortem blood samples. On the basis of survival of red blood cells in post mortem blood, ABO & Rh [D] typing can be done as it is based on presence of surface antigens on red blood cells. As the method involves demonstration of clumping of red blood cells, ascertaining the intactness of RBC is mandatory for detection of these antigens.

We undertook this study to estimate the postmortem survival period of RBC, the duration of RBC intactness; feasibility of grouping of post-mortem blood by simple tube agglutination method and the associated changes which may facilitate in estimating post mortem interval.

MATERIAL AND METHODS

The study was conducted on samples of blood collected from dead bodies during autopsy examination at the mortuary of Pramukhswami Medical College & Shri Krishna Hospital, Karamsad, Anand, India. This cross sectional study was carried out over a period of 6 months [April 2013 to September 2013]. Prior approval of Institutional Human Research Ethics Committee [IHREC] was obtained.

Inclusion criteria: Only cases satisfying all of the following criteria were included in the study.

1. Dead individuals of either sex
2. Cases of any age
3. Deaths occurring at the institute wherein time of death is known
4. Cases with known blood group recorded in hospital or in any other record

Exclusion criteria: Cases which fell in any of the following categories were excluded from the study.

1. Cases who have received blood transfusion during hospitalization prior to death
2. Cases with any haemolytic disorder
3. Bodies exhibiting any external or internal sign of decomposition.

Twenty nine cases were found suitable during the study period for this research project.

5ml of fluid blood was collected from subclavian vein taking all aseptic precautions in an EDTA vial and a thin smear was immediately prepared. It was stained with Leishman's stain. The smear was then examined for intactness and alteration in shape, color and size of the RBC, using a simple light microscope. In cases where, RBC was found intact, blood grouping by standard tube agglutination method was done. Gross finding of clumping was confirmed by microscopy and results compared with the predetermined group to assess the efficacy of this method. The results along with time of collection and examination of samples were noted on a pre-prepared proforma and descriptive analysis was done.

RESULTS

Out of total 29 cases, 22 [75%] were in the age bracket of 21 – 50. The sex distribution in the sample was almost equal with M:F ratio being 14:15.The post-mortem interval varied from 2.5 hours to 19 hours [mean: 10.06 hours]. Intact RBC's could be observed in all the cases up to 19 hours postmortem, except in one case in which it was till 15 hours. 5 cases [17%] demonstrated both intact as well as broken RBC's. The earliest postmortem interval at which RBC's were found to be broken was 7 hours.

Light microscopy changes (change in shape or color of RBC's) were noted in 11 cases [37% cases]. These changes were found to be more in relation to the cause of death rather than time since death. Crenated RBC's were found in 4 cases, all of whom had died from injuries following mechanical trauma and received intravenous fluids. Rest 7 cases [out of 11] demonstrated uneven cell size or absence of central pallor; death in these cases was due to flame burns; one case had died from bite of a hemotoxic snake.

After preparing the slide, tube agglutination test was done on blood samples of all the cases within 30 minutes. It was observed that 28 had intact RBC's and only one had broken RBC's and the test was positive in all samples with intact RBC's. In 27 cases [96.42%], blood grouping result matched with the known blood group of the person determined antemortem. In only one case, though ABO typing matched, there was error with regard to Rh typing; antemortem blood group being B +ve while tube agglutination test on postmortem blood sample showing it to be B –ve. Repeat test failed to clear the discrepancy.

DISCUSSION

Comprehensive literature search yielded only one study[1] in which correlation between intactness of RBC and post mortem interval was sought. In this study, blood smears were examined to note the morphology of blood cells for correlation with time since death. Shape and morphology of RBC's were found normal upto 2 hour postmortem; after 3-4 hours postmortem, morphology changed from discoid to elliptical shape, to crenated margins to crumbled discs which was identifiable up-to 18 hours of post-mortem interval [PMI] followed by lysis with no cells being noted beyond 20 hours of PMI. Lysis was seen earliest at 8 hours PMI. In our study, intact RBC's could be found up to 19 hours of PMI. Lysis was seen earliest at 7 hours of PMI which is in accordance with the above study. Earliest change in shape was observed in above study at 3 – 4 hours PMI which is also comparable with our findings; starting at 3 hours PMI.

Another study conducted to establish the admissibility of the standard technique [of using Anti A, B, D antisera in tube agglutination method] to determine the ABO and Rh [D] groups of red cells from the cadaver revealed that even though the blood sample has been extracted several hours after death, the results are in agreement with the antemortem blood grouping of the individual.[4] Findings of present study are comparable with this study. ABO and Rh[D] grouping could be done on all 28 cases [intact RBC's] upto 19 hours PMI.

CONCLUSION

We conclude that time since death can be inferred from survival period of red blood cells at post mortem examination and routine tube agglutination technique can be used for blood grouping on post-mortem blood samples. It also establishes that blood grouping can be done with reasonable accuracy till intact RBC are demonstrated in sample (up to 19 hours of post-mortem interval).

LIMITATIONS OF THE STUDY

1. Due to time constraint small sample size was studied.
2. The smears were prepared only once during the postmortem examination. All postmortem were conducted within 19 hours of death; the data post 19 hours is not available.

FUNDING AND CONFLICT OF INTEREST

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DETERMINING POST-MORTEM SURVIVAL PERIOD OF RED BLOOD CELLS: A CROSS-SECTIONAL STUDY

PROFORMA FOR DOCUMENTATION OF DETAILS OF SUBJECTS

I. General particulars

1. Name :
2. Age :
3. Sex :
4. Time of death:
5. Known blood group

II. Observations

1. Time of collection of blood sample
2. Time of preparation of slide
3. Time of performing the tube agglutination method
4. Result of blood smear:

1. RBC: Intact/ broken/ altered shape _____
2. WBC: Intact/ broken/ altered shape _____

1. Result of tube agglutination method for blood grouping:

1. Agglutination: Positive/ negative; If positive, result _____
2. Slide confirmation: Positive/ altered; if altered, result _____

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