NEW POSSIBILITIES FOR DETERMINING THE ABO BLOOD GROUP IN FORENSIC MEDICAL PRACTICE

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Abstract. *Based on the data on the modern distribution of blood groups according to the AB0 system, the distribution of blood groups according to the AB0 system among the population of Tashkent city was studied within the framework of a forensic medical examination. The distribution of blood groups was: 0αβ(I) – 2310 (26%), Aβ(II) – 3613 (40.7%), Bα(III) – 1944 (22%) and AB0(IV) – 998 (11.3%). In forensic medical practice, methods for determining human blood group are used: Schiff, anti-A and anti-B co-clones and Gel technologies, and various ingredients are used: alpha and beta isohemagglutinating sera, heteroimmune sera anti-A, anti-B, anti-0(H), monoclonal antibodies anti-A, anti-B, as well as phytagglutinins.*

Keywords: *system AB0, Schiff's method, tsoliclons, gel technology blood, agglutinogens, blood group, agglutinins, phytoagglutinins*

Introduction.

The development of forensic hematology is closely associated with advances in a number of scientific disciplines, primarily hematology and immunology. The examination of blood is of exceptional importance in forensic investigations of deaths classified as violent (homicide, suicide, accidents), as well as in cases of sexual crimes (rape, sodomy, etc.). During life, when the integrity of tissues is disrupted, blood may come into contact with various objects and serve as physical evidence revealing the true circumstances of the case. In such situations, a fundamentally important question must be resolved – the possible origin of the blood (found on certain objects) from a particular individual. For this purpose, liquid blood samples taken both from a corpse and from a living person are examined for comparison. First of all, the blood group of the AB0 system is determined, which is based on the detection of agglutinogens and agglutinins. [1,5,8,14].

Determining the blood group of a living person presents no difficulty. However, during the examination of liquid blood from a corpse, this is not always possible. [4,6]. This is primarily due to the poor preservation of agglutinins, which rapidly degrade after death and under the influence of various factors. In addition, erythrocytes also undergo changes. Under the influence of certain factors, they may either deform or dissolve [3,17,22,24].

These factors may lead to hemolysis, putrefaction of the blood, coagulation or, conversely, dilution, and other alterations. In such cases, agglutinins and often agglutinogens cannot be detected, meaning that the blood group cannot be determined [10]. Therefore, in such cases the blood is first dried and then subjected to examination using more labor-intensive and time-consuming methods. Moreover, as reported in the literature, drying has a negative effect on the course of the reaction. It significantly reduces the absorption capacity of agglutinogens A and B [7,20,23].

The study of group-specific substances is carried out more deeply by immunochemists and biochemists, while forensic specialists utilize their achievements for their own purposes. Advances, particularly those related to group-specific substances of the AB0 system, make it possible to attempt differentiation within the AB0 system under the influence of certain external and internal factors. The current state of research on the blood group of cadaveric blood is largely focused on this issue. In order to resolve these questions, a more detailed study of the properties and characteristics of this erythrocyte system (AB0) is required, taking into account recent scientific works [6]. It is also necessary to investigate postmortem changes occurring in blood and to determine the possibilities for identifying blood groups under such conditions. This requires a comprehensive study of the

characteristics of cadaveric blood, agglutinogens, and agglutinins both under normal conditions and under the influence of various factors [6,12,19]. The AB0 blood group system derives its name from the antigens present in erythrocytes. This blood group system is considered the classical blood group system. It is the only serological system that, in addition to agglutinogens, also contains agglutinins. Therefore, in forensic medical examination of physical evidence, the blood group of liquid blood, including cadaveric blood, is determined based on both agglutinogens and agglutinins. For this purpose, various reagents are used: isohemagglutinating sera alpha and beta, heteroimmune sera anti-A, anti-B, anti-0, monoclonal antibodies anti-A and anti-B, as well as agglutinins of plant origin (phytoagglutinins) or animal origin (protectins). Each of these reagents has its own advantages [2,4.9,11,18].

Material and methods of research.

In the present study, 360 blood samples obtained from living individuals and cadavers (2017–2020), as well as 148 archival forensic examination materials from the forensic biological laboratory of the Tashkent branch of the Republican Scientific and Practical Center of Forensic Medical Examination, were used. In forensic medical practice, modern methods are applied to determine the human blood group: 1. the agglutination reaction using the Schiff method with anti-A and anti-B cyclones (tsoliklons) for blood group determination; 2. the Across Gel card technology for determining blood groups.

In the study, 22 blood samples from individuals aged 25–55 years were examined. The agglutination reaction using the Schiff method was applied during the examination. Agglutinins and agglutinogens in liquid blood are determined by the Schiff method (agglutination reaction), which is based on the interaction between agglutinogens and agglutinins. In this reaction, agglutinogens are identified using standard isohemagglutinating sera α and β, while agglutinins are determined using standard erythrocytes of groups A and B. For this purpose, four special agglutination laboratory plates are used. On two of them the letters “A” and “B” are written with a glass marker, and on the other two the symbols “α” and “β” are indicated. According to the corresponding markings, four drops of a 1% suspension of standard group A and B erythrocytes are placed into the plates labeled “A” and “B”, while two drops of standard sera α and β are added to the plates labeled “α” and “β”. The examined blood sample is centrifuged, and the serum is separated from the erythrocytes, from which a 1% suspension is prepared. Four drops of the 1% erythrocyte suspension from the examined blood are added to the plates containing α and β sera, while two drops of the serum from the examined blood are added to the plates containing standard erythrocytes A and B. The mixture of erythrocytes and serum is centrifuged at 1500 rpm for 4 minutes, after which it is thoroughly shaken. The reaction results are evaluated both macroscopically and microscopically. After the examination of the liquid blood samples, stains are prepared from them on indifferent carrier materials (clean gauze).

Results and discussion.

In the study, 22 blood samples from individuals aged 25–55 years were examined. In the control samples with known blood groups $0_{\alpha\beta}$ (I), A_{β}(II), B_{α}(III), and AB_0 (IV), no agglutination was observed. In the known blood sample of group $0_{\alpha\beta}$(I), agglutination was observed only with anti-H phytoagglutinin, indicating the presence of the 0(H) antigen. In the known blood sample of group A_{β}(II), agglutination occurred only with anti-A phytoagglutinin, confirming the presence of antigen A. In the known blood sample of group B_{α}(III), agglutination was observed only with anti-B phytoagglutinin, indicating the presence of antigen B. In the known blood sample of group AB_0 (IV), agglutination occurred with both anti-A and anti-B phytoagglutinins, demonstrating the presence of both A and B antigens. Among the examined study samples, agglutination with anti-B isosera was observed only in samples No.1,4,5,12,13, and 15-17, indicating the presence of antigen B. In samples No.2,6,8,14 and 18, agglutination occurred only with anti-H phytoagglutinin, indicating the presence of antigen 0(H), which suggests that this blood could originate from individuals with blood group $0_{\alpha\beta}$(I). In samples No. 3, 7, 9, 19, and 21, agglutination was observed only with anti-A iso-serum, indicating that the blood may originate from individuals with blood group A_{β} (II). In samples No.

10,11,20, and 22, agglutination occurred with both anti-A and anti-B iso-sera, demonstrating the presence of antigens A and B, which indicates that the blood may originate from individuals with blood group AB0 (IV). When determining the group affiliation of blood, only antigens A, B, and 0 (H), as well as agglutinins α and β, are identified. Based on the results of the forensic medical examination, information about the detected antigens and agglutinins in blood samples taken from unidentified cadavers submitted for examination is provided, and the corresponding blood group is indicated in the conclusion of the forensic biological examination. An algorithm for determining the blood group using anti-A and anti-B cyclones (tsoliklons) was also developed. Procedure: 1**.** One large drop of the tsoliklon reagent is placed in each well of the plate; 2. Using a capillary, a small drop of blood (0.01 ml) is placed next to the tsoliklon reagent, ensuring that the capillary does not come into direct contact with the reagent; 3. The blood and the tsoliklon reagent are mixed using separate sticks. 4. The plate is gently shaken, and agglutination is observed for 2.5 minutes; 5.One drop of physiological saline is added with a pipette into the wells where agglutination has occurred; 6. The plate is shaken again and the agglutination reaction is observed. Results in blood group determination using tsoliclons: - If no agglutination is observed in any well - Group $0_{\alpha\beta}$(I); - Agglutination with anti-A tsoliklon - Group A_{β}(II); - Agglutination with anti-B tsoliklon - Group B_{α}(III); Agglutination with both anti-A and anti-B tsoliklons – Group AB_0(IV).

Application of the gel filtration method in practice: 1. Rapid and accurate determination of blood group and Rh factor, including in complex cases; 2. Extensive typing of erythrocyte antigens across all known systems; 3. Screening and identification of alloantibodies against erythrocytes; 4. Individual selection of blood components for transfusion; 5. Diagnosis of post-transfusion complications and hemolytic disease of the newborn; 6. Detection of rare antigens (up to 76 different test profiles). Advantages of gel technology: -The gel filtration principle combined with the use of monoclonal antibodies ensures high sensitivity and specificity; -All reagents are standardized in dosage and their quality is controlled; - Simple interpretation of results: positive reaction (cells remain in the upper part of the gel), negative reaction (cells sediment at the bottom); -Ease of use: testing time is 2-5 times faster for antibody screening and compatibility testing; no need for erythrocyte washing; results can be automatically evaluated and photo-documented; -Rapid training of laboratory personnel; -Reduction of errors associated with traditional methods; Test results can be copied for archival purposes or used as teaching material in diagnostically difficult cases; -The ability to use very small amounts of blood is of significant importance; - Reduces the risk of infection for personnel when working with potentially infected samples; -Plastic cards are made from biodegradable materials, are easy to recycle, and do not pollute the environment; -The test system is stable and can be stored at room temperature for up to one year.

The use of gel technologies makes it possible to perform a set of tests that were previously available only in reference laboratories within routine practice. The advantages of gel technology demonstrate the transition from manual procedures to automated technologies. This new stage in the development of laboratory diagnostics is characterized by a continuous increase in data obtained due to improvements in laboratory equipment. Correct determination of human blood groups according to the AB0 system is of great importance for preventing post-transfusion complications.

Conclusion.

1. The properties and characteristics of phytoagglutinins depend on the plant’s growth environment and the time of seed collection.

2. In the practice of forensic medicine, liquid human blood according to the AB0 system 1. agglutination on a flat surface with the help of monoclonal antibodies; 2. agglutination in test tubes; 3. microplate agglutination reactions were used.

3. The use of modern Across Gel card technology to determine a person's blood type is considered a more reliable and preferred method in the practice of forensic biology experts.

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