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Group antibodies as factors specifically influencing erythrocytes sedimentation rate

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The use of the erythrocyte sedimentation rate (ESR) method has been recognized in monitoring the inflammation process, with a proven role of proinflammatory factors in the ESR increase. The aim. To reveal the role of group antibodies in the specific increase of ESR. Methods. For the study erythrocytes with EDTA anticoagulant were used. ESR was measured after the contact of erythrocytes with saline (negative control), standard anti-A and anti-B antibodies of the IgM class, polyclonal anti-A, anti- B, anti-A, B sera (experiment), as well as serum of group AB in a ratio 1 : 3: 0.2 ml of erythrocytes and 0.6 ml of saline or serum. The results were evaluated after one and 12 hours of incubation, at room temperature and at 4 °C. Results. Contact of anti-A IgM antibodies at a dilution of 1 : 30 with A erythrocytes led to an increase of ESR (from (3.25 ± 0.50) mm/h to (83.7 ± 1.60) mm/h) ($p < 0.001$) with a presence of a red precipitate. The contact of erythrocytes with polyclonal citrate plasma (or serum) in a ratio of 1 : 2 led to a similar increase in ESR in cases of the specific binding. Anti-A, B serum increased ESR of A erythrocytes up to (53.00 ± 2.64) mm/h ($p < 0.001$) with the presence of a red precipitate, while anti-B serum did not show such effect: (ESR — (5.25 ± 0.50) mm/h) ($p > 0.05$). It should be noted that the serum absorbed by the corresponding erythrocytes showed reduced ESR values. After the absorption of anti-A antibodies by A erythrocytes the serum lost the ability to specifically increase the ESR of A erythrocytes. Conclusions. Group antibodies are able to specifically promote ESR. The found ability can be reduced by the method of specific absorption of the serum. The physicians may consider the role of group specific autoimmune antibodies in developing high values of ESR. The therapy aimed to regulate the autoimmune humoral activation and specific absorption might be useful in normalization the ESR parameter.

Використання методу визначення швидкості осідання еритроцитів (ШОЕ) визнано для моніторингу запального процесу, при цьому досліджено роль прозапальних білків у підвищенні ШОЕ. Мета. Оцінити роль групових антитіл у специфічному підвищенні ШОЕ. Методи. Для дослідження використовували еритроцити з антикоагулянтом ЕДТА. ШОЕ вимірювали після контакту еритроцитів з фізіологічним розчином (негативний контроль), стандартними антитілами анти-А і анти-В класу IgM, поліклональними сироватками анти-А, анти-В, анти-А, В (дослід), а також сироваткою крові групи АВ у співвідношенні 1 : 3: 0,2 мл еритроцитів і 0,6 мл фізіологічного розчину або сироватки. Результати оцінювали через одну та 12 год інкубації за кімнатної температури та 4 °С. Результати. Контакт антитіл IgM анти-А у розведенні 1 : 30 з еритроцитами А призводив до підвищення ШОЕ (від (3,25 ± 0,50) до (83,7 ± 1,60)) мм/год ($p < 0{,}001$) з наявністю червоного осаду. Контакт еритроцитів з поліклональною цитратною плазмою (або сироваткою) у співвідношенні 1 : 2 призводив до аналогічного підвищення ШОЕ зі специфічним зв'язуванням. Анти-А, В сироватка підвищувала ШОЕ у А еритроцитів (до (53,00 ± 2,64) мм/год) ($p < 0{,}001$) з наявністю червоного осаду, а анти-В сироватка не мала такого ефекту: ШОЕ — (5,25 ± 0,50) мм/год ($p > 0{,}05$). Сироватка крові, поглинена відповідними еритроцитами, показала знижені значення ШОЕ. Після абсорбції анти-А-антитіл А-еритроцитами сироватка крові втратила здатність специфічно підвищувати ШОЕ у А еритроцитів. Висновки. Групові антитіла здатні специфічно підвищувати ШОЕ. Винайдена властивість може бути зменшена методом специфічної абсорбції сироваток. Лікарі можуть розглядати роль групових аутоімунних антитіл у підвищенні ШОЕ у пацієнтів. Для нормалізації цього показника може бути корисною терапія, спрямована на регуляцію аутоімунної гуморальної активації та специфічну абсорбцію. Ключові слова. Еритроцити, седиментація, підвищення, сироватка, антитіла.

Key words. Erythrocytes, sedimentation, increase, serum, antibody

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Introduction

The erythrocyte sedimentation rate (ESR) remains a frequently used method and has a long history of monitoring inflammatory diseases [1–3]. Currently, the recommendations of the International Council for Standardization in Hematology and the National Committee for Clinical Laboratory Standards for measuring ESR are based on the Westergren method using samples with the anticoagulant EDTA.

The values of ESR were studied depending on the blood groups of the ABO system in rheumatoid arthritis. Thus, in patients with blood group A the ESR level was lower than in patients with blood group O (12.46 mm/h and 21.5 mm/h, p = 0.02). Blood group O showed higher levels of ESR compared with the patients of other groups (p = 0.02) [4].

ESR is considered an indicator of inflammation, since it depends on the concentration of acute-phase proteins circulating in the blood (fibrinogen), which increase the dielectric constant of the blood and neutralize negative charges on the surface of erythrocytes [5–8].

ESR values are reported to be higher in women than in men and increase steadily with age. The median ESR of women is 2 times higher than that of men, and the median ESR of people over 65 years of age is 2 times higher than that of young people. Body mass index, the presence of metabolic syndrome and smoking are positively associated with high ESR. In persons with high physical activity ESR values were found to be lower than in persons with low physical activity.

Obesity and metabolic syndrome are assessed as pro-inflammatory conditions associated with an increase of ESR [2, 9].

Thus, no studies have been conducted to evaluate the effect of polyclonal antibodies on the ESR value.

The aim of the study was to evaluate the effect of polyclonal antibodies on the ESR value.

Material and methods

For the study erythrocytes with EDTA anticoagulant were used. ESR was measured after the contact with saline (negative control), standard anti-A and anti-B IgM antibodies, polyclonal anti-A, anti-B, anti-A, B sera and group AB sera in a ratio of 1:3 (0.2 ml of erythrocytes and 0.6 ml of saline or serum). The results were evaluated after one hour and 12 hours of incubation at room temperature, as well as at +4 °C, expressed in millimeters. The blood was taken into vacuum tubes containing EDTA (Becton Dickinson, Franklin Lakes, NJ). Evaluation of the erythrocytes size was performed by the software program ToupView. Student's coefficient and the Mann-Whitney test was used to analyze numerical variables.

Results and discussions

After the contact of anti-A, B citrate plasma with B erythrocytes the ESR increased (from (3.2 ± 0.40) mm to (55.75 ± 2.10) mm) (p < 0.001) with the appearance of a red precipitate (of (17.00 ± 0.50) mm). At the same time after absorption of the plasma by A erythrocytes and subsequent contact with B erythrocytes, no decrease in ESR was observed (55.10 ± 1.80) mm (p < 0.001), precipitate of (23.70 ± 1.20) mm, (52.40 ± 1.70) mm (p < 0.001) and precipitate of (27.40 ± 1.30) mm.

The contact of anti-A, B plasma with A erythrocytes led to an increase of ESR (from (3.25 ± 0.50) mm to (61.33 ± 1.52) mm (p < 0.001) and a precipitate of (10.66 ± 0.57) mm). However, after specific absorption by different samples of A erythrocytes in three cases anti-A, B plasma decreased the ability to sediment A erythrocytes, increasing the height

Table

ESR after the contact of erythrocytes with polyclonal sera

	normal saline	anti-A, B	anti-B	anti-A	anti-A, B plasma	anti-B plasma	anti-A, B citrate plasma	anti-B citrate plasma
A	3.57 ± 0.78 n = 10	52.60 ± 9.18 n = 16 p < 0.001	4.42 ± 0.75 n = 10 p > 0.05	43.50 ± 1.50 n = 20 p < 0.001	60.20 ± 10.30 n = 34 p < 0.001	23.30 ± 4.75 n = 7 p < 0.001	65.60 ± 8.43 n = 14 p < 0.001	22.70 ± 10.90 n = 7 p < 0.001
AB	4.20 ± 0.80 n = 10	—	32.10 ± 7.20 n = 7 p < 0.001	23.60 ± 7.18 n = 7 p < 0.001	—	—	63.10 ± 7.40 n = 7 p < 0.001	45.30 ± 7.6 n = 7 p < 0.001
O	3.61 ± 0.50 n = 10	—	2.80 ± 0.63 n = 13 p > 0.05	2.87 ± 0.64 n = 7 p > 0.05	12.00 ± 2.20 n = 7 p < 0.001	2.28 ± 0.48 n = 7 p > 0.05	20.00 ± 2.64 n = 7 p < 0.001	11.21 ± 4.70 n = 7 p < 0.001
B	3.40 ± 0.75 n = 7	62.80 ± 4.64 n = 14 p < 0.001	63.30 ± 2.00 n = 13 p < 0.001	2.50 ± 0.74 n = 7 p > 0.05	48.20 ± 3.25 n = 10 p < 0.001	73.50 ± 4.64 n = 17 p < 0.001	66.30 ± 4.91 n = 11 p < 0.001	51.66 ± 10.10 n = 7 p < 0.001

of the precipitate: (52.00 ± 1.41) mm, precipitate (21.60 ± 1.50) mm, ($p < 0.001$), (42.33 ± 0.57) mm precipitate (40.60 ± 1.15) mm, ($p < 0.001$), (53.55 ± 1.10) mm precipitate (21.33 ± 0.57) mm ($p < 0.001$).

It is noteworthy that in the absence of specific absorption: anti-A, B serum absorbed by B and O erythrocytes, when contacted with A erythrocytes, the ESR results did not differ from those obtained without absorption: anti-A, B serum and A erythrocytes (40.60 ± 1.10) mm ($p < 0.001$), precipitate of (29.60 ± 1.50) mm, after absorption by B erythrocytes and subsequent contact with A erythrocytes: (40.75 ± 0.95) mm ($p < 0.001$), precipitate of (23.00 ± 1.70) mm, after absorption by O erythrocytes: (30.60 ± 1.10) mm ($p < 0.001$), precipitate of (20.00 ± 1.40) mm.

When estimating the ESR of group B erythrocytes with anti-B serum, increased result (61.60 ± 1.52) mm ($p < 0.001$) and a precipitate of (21.00 ± 0.70) mm was noted; after absorption by erythrocytes with anti-B absorbing ability the height of the precipitate increased (to (30.60 ± 1.10) mm ($p < 0.001$), (33.50 ± 1.40) mm ($p < 0.001$), (42.00 ± 1.00) mm) ($p < 0.001$), whereas after absorption by erythrocytes without anti- B absorbing ability no increase of precipitate height was observed: (18.25 ± 1.25) mm ($p > 0.05$).

Specific absorption of antibodies by erythrocytes makes it possible to detect a greater height of the precipitate in the ESR reaction in comparison with unabsorbed serum. 12-hour contact of absorbed serum by the studied erythrocytes and non-absorbed serum (plasma) with standard erythrocytes makes it possible to detect the absorption capacity of erythrocytes and to reveal antigens.

Specific contact of anti-A IgM antibodies at a dilution of 1 : 30 with A erythrocytes (ratio of antibodies and erythrocytes 4 : 1) led to an increase of ESR (from (3.25 ± 0.50) mm to (84.30 ± 1.57) mm/h ($p < 0.001$), at a dilution of 1:60: (3.75 ± 0.50) mm/h ($p > 0.05$) with the presence of a red precipitate, at a dilution of 1 : 90: (3.20 ± 0.40) mm/h ($p > 0.05$) without precipitate).

Similarly, elevated ESR values ((90.60 ± 1.10) mm/h, $p < 0.001$) and the presence of a precipitate were detected when A erythrocytes contacted with anti-A IgM antibodies at a dilution of 1 : 30, while a decrease of ESR was detected after the contact of absorbed by A erythrocytes anti-A IgM antibodies (6.50 ± 0.57) mm/h ($p > 0.05$), that indicates the specificity of erythrocyte sedimentation under the influence of blood group antibodies.

At the same time, the contact of IgM anti-A antibodies at a dilution of 1 : 30 with O erythrocytes did not lead to an increase of ESR — (2.25 ± 0.50) mm/h ($p > 0.05$), with IgM-anti-A antibodies at a dilution of 1 : 60 — (2.23 ± 0.4) mm/h ($p > 0.05$), with anti- A IgM antibodies at a dilution of 1 : 90 — (2.28 ± 0.48) mm/h ($p > 0.05$), and with anti-A antibodies absorbed by O erythrocytes (4.80 ± 0.44) mm/h ($p > 0.05$).

Contact of erythrocytes with polyclonal serum in a 1 : 1 ratio resulted in a similar increase of ESR in a case of the specific binding (Table 1). Anti-A,B serum after the contact with A erythrocytes increased ESR of A erythrocytes (to (53.25 ± 2.38) mm/h) ($p < 0.001$) with the presence of a red precipitate, while anti-B serum did not increase ESR after the contact with A erythrocytes (5.25 ± 0.50) mm/h ($p > 0.05$).

Anti-A, B serum with A erythrocytes increased ESR from (4.75 ± 0.50) mm to (55.40 ± 1.26) mm/h ($p < 0.001$), as well as anti-A serum to (51.25 ± 1.95) mm/h ($p < 0.001$). The ESR value increased after the contact of A erythrocytes with anti-A, B serum (from (3.25 ± 0.50) mm to (37.10 ± 1.23) mm/h) ($p < 0.001$), as well as with anti-A serum to (10.66 ± 0.57) mm/h ($p < 0.01$).

Contact of A erythrocytes with anti-A, B serum led to an increase of ESR (from (3.42 ± 0.70) mm to (61.50 ± 1.29) mm/h) ($p < 0.001$), contact of A erythrocytes with anti-A, B serum led to an increase of ESR from (3.25 ± 0.50) mm to (51.66 ± 1.53) mm/h ($p < 0.001$) with a clear boundary between erythrocytes and serum, while after contact of A erythrocytes with anti-B serum an increase of ESR was not noted (16.00 ± 1.00) mm/h, without a clear border between erythrocytes and serum.

Contact of AB erythrocytes with anti-A serum led to an increase of ESR (from (3.75 ± 0.50) mm/h to (9.75 ± 0.95) mm/h ($p < 0.05$), as well as with anti-B serum to (11.00 ± 1.00) mm/h) ($p < 0.05$). After contact of anti-A serum with A erythrocytes in a ratio of 1 : 1, after 10 minutes, the ESR value increased to (32.30 ± 2.08) mm ($p < 0.001$), while with anti-B serum, the ESR value turned out to be half as much (15.60 ± 1.15) mm ($p < 0.001$) without a clear border. At the same time, after 60 minutes the ESR values with anti-A serum were increased (35.33 ± 1.52) mm/h ($p < 0.001$), as well as with anti-B serum (35.60 ± 1.10) mm/h ($p < 0.001$).

After the contact of erythrocytes with plasma in the cases of the specific binding higher ESR values as compared to those with serum were detected. Thus, after the contact of anti-A, B plasma with A erythrocytes the ESR value increased from (4.25 ± 0.50) mm/h to (73.30 ± 1.26) mm/h

($p < 0.001$), after contact with anti-B plasma the ESR value occurred to be less (33.40 ± 1.20) mm/h ($p < 0.001$).

Contact of anti-A, B serum with A erythrocytes and complement led to an increase of ESR (from (5.00 ± 0.57) mm/h to (52.60 ± 1.50) mm/h) ($p < 0.001$), after contact of anti-A serum with complement and A erythrocytes the ESR value increased to (50.52 ± 1.00) mm/h ($p < 0.001$). After contact of A-erythrocytes with anti-A, B serum and complement ESR increased to (64.60 ± 2.50) mm/h ($p < 0.001$), as well as after contact with anti-A, B plasma and complement (2 : 1) to (66.00 ± 2.60) mm/h ($p < 0.001$), while after contact with anti-B plasma and complement the ESR value was less (5.33 ± 1.50) mm/h ($p < 0.001$), without a clear border.

It should be noted that the serum absorbed by the corresponding erythrocytes showed reduced ESR values.

ESR of AB erythrocytes after contact with citrate anti-A, B plasma increased from (3.20 ± 0.50) mm/h to (25.40 ± 1.52) mm/h ($p < 0.001$), and when plasma was absorbed by A erythrocytes, the value decreased to (16.00 ± 1.00) mm/h ($p < 0.001$), however, after absorption by A group leukocytes the ESR was not decreased: (25.70 ± 1.20) mm/h ($p > 0.05$).

Other researchers have found a significant increase in the ESR in the blood of patients with rheumatoid arthritis, mediated by the influence of IL-1, that increases the movement of leukocytes from the bones into the bloodstream and an increase in their accumulation [9].

The forces, called zeta potential, are reduced by fibrinogen, alpha-2 macroglobulin and immunoglobulins, that lead to the increased ESR levels.

Determination of ESR is a laboratory test with time-tested significance. The usefulness of this test has diminished as new methods for assessing inflammation activity have been developed. The test remains useful in the specific diagnosis of temporal arteritis, polymyalgia rheumatica, rheumatoid arthritis, Hodgkin's disease recurrence, vascular collagenosis or metastatic malignancy [2, 3, 9].

The obtained results are in accordance with previous results [3], that showed decrease of electrokinetic potential of erythrocytes under the influence of specific polyclonal antibodies and may testify to the association of increased ESR with decreased zeta potential of erythrocytes. The results obtained by the researchers show that ESR increases as the number of red blood cells changes, and room temperature affects the sedimentation rate — with an increase of the room temperature the ESR increases [10].

The conducted study revealed the possibility of using the ESR method in the selection of a donor for transfusiological purposes in a blood type revealing. Thus, in the case of specific binding of antigen and antibody the ESR value becomes increased. The optimal ratio of EDTA-containing erythrocytes and serum was found to be 1 : 3 with the addition of sodium citrate. Citrated plasma with EDTA erythrocytes showed the strongest difference between specific and non-specific binding.

A clear difference in specific and non-specific binding was observed after 10 minutes of incubation. However, to determine the result incubation was carried out for one and 12 hours. The use of citrated plasma required an incubation period of one hour. Specific binding was determined by a pronounced border between erythrocytes and serum, the presence of a precipitate, and an increased ESR value. The absence of the specific binding of erythrocytes with antibodies was revealed by the diffuse border of erythrocytes and serum, the absence of precipitate and a smaller increase of ESR.

Conclusions

Group antibodies are able to specifically increase ESR, that can be reduced by specific absorption of the serum. An increase of ESR with a pronounced border between erythrocytes and serum and the presence of a red precipitate can serve as a sign of the specific binding of the serum and erythrocytes. Since in clinical practice unexplained cases of increased ESR occur, the physicians may consider the role of group specific autoimmune antibodies in developing high values of ESR. Thus, the therapy aimed to regulate the autoimmune humoral activation and specific absorption might be useful in normalization the ESR parameter.

Conflict of interest. The authors declare no conflict of interest.

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GROUP ANTIBODIES AS FACTORS SPECIFICALLY INFLUENCING ERYTHROCYTES SEDIMENTATION RATE

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