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Comparative study of the measurement of erythrocyte sedimentation rate (ESR) by blood gas capillary and Wintrobe tubes

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Rationale : *The erythrocyte sedimentation rate (ESR), is a measure of the settling of red blood cells in a vertically aligned tube during one hour. This measurement is an indication of inflammation which increases in many diseases. The Wintrobe macro method is an established standard ESR method which requires a large amount of blood from the patient. The method is therefore not suitable for young infants.*

Objective : *To compare the measurement of ESR by micro-method using blood gas capillary tube with the Wintrobe method in pediatric patients.*

Materials and Methods : *Blood samples were collected from 102 children pediatric out patient clinic for the measurement of ESR. There were 46 males and 56 females from 6-15 years of age. For each subject, a sample of venous blood and a sample of capillary blood were collected to test for ESR values using the Wintrobe and the blood gas methods.*

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Result : *There was no significant difference of ESR between the blood gas capillary tube and the Wintrobe method (25.8 ± 17.9 and 25.5 ± 14.2 mm./hr.) from the same subject. A significant positive correlation of ESR values between each method ($r = 0.78$; $p < 0.001$) as well as an intra class correlation coefficient (ICC) of 0.77 were found. However, the ESR values for capillary blood using the blood gas tube (35.2 ± 15.5 mm./hr.) were significantly higher than those for venous blood using the Wintrobe tube (25.2 ± 14.2). There was a high correlation ($r = 0.86$; $p < 0.001$) and intra class correlation coefficient of 0.70. There was no significant difference in ESR values between each method for venous blood. However, the blood gas capillary capillary tube resulted in a higher ESR than the Wintrobe method when the ESR value was lower than 40 mm/hr. For the ESR values above 40 mm/hr, the measurements from both methods were not statistically different.*

Conclusion : *The ESR values obtained by the blood gas method were significantly correlated to the standard Wintrobe method. Therefore, the estimation of the ESR can be performed alternatively by the blood gas method. This blood gas micro-method is suitable for and is also useful as an alternative screening test in routine laboratory investigation for abnormal ESR.*

Keyword : *Wintrobe method.*

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อาวีวรรณ วัชรศีชร, นวพร ศุภนุช, อุมาภรณ์ ขำมณี, เตือนใจ อินทุโสมา, ยง ภู่วรวรรณ. การศึกษาเปรียบเทียบอัตราการตกของเม็ดเลือดแดงโดยใช้หลอด Blood gas กับวิธีของ Wintrobe. จุฬาลงกรณ์เวชสาร 2546 ก.ค; 47(7): 401 – 9

ปัญหา : *การวัดค่าอัตราการตกของเม็ดเลือดแดง (Erythrocyte Sedimentation Rate, ESR) ยังเป็นการทดสอบที่มีคุณค่า ใช้กันอย่างกว้างขวางในการวินิจฉัยแยกโรค เนื่องจากวิธีเดิมเป็นวิธีมาตรฐานคือ วิธี Wintrobe ต้องใช้เลือด 1-2 มิลลิลิตร (วิธีแมคโคร) และหลอดราคาแพง ขณะที่วิธีไมโครใช้เลือดน้อยเพียงใช้เลือดจากปลายนิ้ว เป็นวิธีที่ง่ายกว่า และราคาไม่แพง*

วัตถุประสงค์ : *ศึกษาค่า ESR จากเลือดผู้ป่วยเด็ก โดยใช้หลอด Blood gas เปรียบเทียบกับหลอด Wintrobe*

วิธีการและผลการศึกษา : *ศึกษาค่า ESR จากเลือดผู้ป่วยเด็กอายุระหว่าง 6 -15 ปี จำนวน 102 ราย เป็นชาย 46 ราย และหญิง 56 ราย โดยเจาะเลือดจากหลอดเลือดและปลายนิ้ว พบว่า เมื่อเจาะเลือดจากหลอดเลือดของผู้ป่วยคนเดียวกันใส่หลอด Blood gas เปรียบเทียบกับหลอด Wintrobe ได้ค่า ESR ใกล้เคียงกันและไม่แตกต่างกันทางสถิติ (25.8 $\pm$ 17.9 และ 25.5 $\pm$ 14.2 มม./ชม.ตามลำดับ) และยังพบความสัมพันธ์เชิงตรง (positive correlation) ของค่า ESR ระหว่างการใช้หลอดทั้ง 2 ชนิดนี้ โดยมีค่าสัมประสิทธิ์สัมพันธ์ (r) เท่ากับ 0.78 ($p < 0.001$) และค่า intraclass correlation (Icc) เท่ากับ 0.77 อย่างไรก็ตาม เมื่อเจาะเลือดจากปลายนิ้วของผู้ป่วยคนเดียวกัน วัดค่า ESR พบว่า ค่าที่ได้จากหลอด Blood gas สูงกว่าหลอด Wintrobe และแตกต่างกันอย่างมีนัยสำคัญทางสถิติ (35.2 $\pm$ 15.5 และ 25.5 $\pm$ 14.2 มม./ชมตามลำดับ $p < 0.001$) แต่กลับพบความสัมพันธ์ของทั้ง 2 วิธีสูง โดยมี r เท่ากับ 0.86 ($p< 0.001$) และค่า ICC เท่ากับ0.70*

นอกจากนี้ เมื่อพิจารณาข้อมูลทั้งหมดตามระดับค่า ESR 3 ระดับ วิเคราะห์ค่าสถิติเปรียบเทียบระหว่างวิธีใช้หลอด Blood gas และวิธีใช้หลอด Wintrobe เมื่อเจาะจากหลอดเลือดไม่มีความแตกต่างกัน แสดงให้เห็นว่าสามารถใช้หลอด Blood gas แทนหลอด Wintrobe ได้ ในทางตรงกันข้ามพบว่าค่า ESR ที่ระดับ 0-20 และ 21-40 มม./ชม. การใช้หลอด Blood gas ที่เจาะเลือดจากปลายนิ้วได้ค่าสูงกว่าการใช้หลอด Wintrobe อย่างมีนัยสำคัญทางสถิติ แต่ค่า ESR ที่ระดับ > 40 มม. /ชม. ค่าที่ได้จากหลอดทั้ง 2 วิธี ไม่มีความแตกต่างกันทางสถิติ

สรุป : *สามารถใช้หลอด Blood gas เป็นวิธีไมโครวิเคราะห์ค่า ESR ได้ และเมื่อเปรียบเทียบค่า ESR ที่วิเคราะห์ด้วยหลอด Blood gas กับวิธีมาตรฐานที่ใช้หลอด Wintrobe วิธีแมคโคร พบว่ามีความสัมพันธ์กันทางบวกอย่างมีนัยสำคัญทางสถิติ วิธีไมโครนี้เหมาะสมสำหรับเป็นการตรวจสอบคัดกรองผู้ป่วยเด็กเล็ก*

Erythrocyte Sedimentation Rate (ESR) is the height of plasma above dropped red blood cell in a test tube within a limited time. It is measured in term of millimeters per hour. To measure ESR, the capillary tube is set vertically still. Gradually, the red blood cells begin to fall to the bottom of the tube. After an hour sharp, the height of plasma above the dropped red blood cells is measured.

ESR is still commonly examined until present days. Westergren method [1] was first used to find ESR in 1924 and was then standardized by ICSH [2,3] (International Committee for Standardization in Haematology) in 1973 and later improved in 1977. Hence, other acceptable methods such as Wintrobe[4] had to be compared with the set standard Westergren method with comparable results. In addition, there is also micro method[5] in which many kinds of tubes have been created in order to decrease the amount of blood needed for the testing. However, some types of tubes are not available and not widely used. The mechanism of erythrocyte sedimentation is called rouleaux formation[6,7] which is influenced by the following factors:

1. Quantity of the plasma proteins: The ESR rises when fibrinogen[8-10] coats the plasma membrane, red blood cells increase weight and agglutinate easily. On the other hand, albumin[6], cholesterol and lecithin[8,9] reduce the ESR value.

2. Shape, size and amount of the red blood cells :

2.1 Shape:[11,12] a decreased ESR is associated with a number of blood disease in which red blood cells have irregular shapes that causes slower settling such as sickle cells and spherocytic cells.

2.2 Size:[11,13] macrocytosis increases the ESR because the macrocytic red cells with a smaller surface-to-volume ratio settle more rapidly.

2.3 Amount: in patients with polycythemia[8,13] too many red blood cells decrease the compactness of the rouleaux network and artifactually lower the ESR. In anemia,[6,8,9] with reduced hematocrit, the velocity of the upward flow of plasma is altered so that red blood cell aggregates fall faster.

3. Anticoagulant: higher level of anticoagulant used in blood collection results in lower ESR.[9,11] Heparin causes higher ESR[13,14] while EDTA in proper amount has no impact.[6]

4. Conditions of experimental equipment, method and environment.

4.1 A capillary tube

- A long tube causes red blood cells to drop faster.[8,14]

- The diameter of the tube can affect the ESR values only if it is less than 2 millimeters.[9,15] Red blood cells drop slower in a narrower tube,[8] while they fall faster in a wider tube.[14]

4.2 Angle of the settling tube:[6,8,11] a tilted ESR tube[16] causes an artificial elevation of ESR.

4.3 Before the experiment, the blood sample has to be mixed gently with the anticoagulant.[12,14]

4.4 Room temperature: When the room temperature is raised, the ESR is also raised.[8,11] Maintain room temperature at 70 to 79° F or 22 to 27° C

4.5 Perform test within three hours after blood is drawn.[15]

The objective of this study is to compare the measurement of ESR by micro-method with the

standard method (Wintrobe). The micro-method needs small amount of capillary blood and is convenient for young infants.

Material and method

Population study

Venous and capillary blood samples were collected from 102 patients from the pediatric outpatient clinic with permissions from either the patients or their parents. The subjects included 46 males and 56 females with diversified diseases. Their age group ranged from 6 to15 years.

Methods

EDTA was the anticoagulant used for the collection of venous blood; 0.06 volume of 235 mM EDTA was added into every 3 volumes of blood.

Type of tubes: a standardized Wintrobe and a blood gas capillary tubes.

The determination of ESR was performed by the following methods:

Method 1: capillary and venous blood were tested for ESR in the blood gas capillary tube. The blood samples were filled into the vertically aligned tubes.

Method 2: venous blood samples were tested for ESR by the blood gas capillary and Wintrobe tubes[13] in the similar way.

In both methods, the distance that the column of blood had fallen or the height of plasma in an hour was recorded and reported in millimeters/hour. The ESR in blood gas capillary tube employed a specific rule of measurement with millimeter scale which was measured from the lowest point at the curve of the plasma surface to the highest point at the curve of dropped red blood cells.

Data analysis

The data were analyzed statistically for the means, standard deviations and intra-class correlation coefficient.

Wintrobe tube Blood gas capillary tube

Figure 1. Comparison of Wintrobe and blood gas capillary tubes. The scale on the Wintrobe tube represents 1 mm per each unit and blood sample has to be filled up to the 100 mm. The blood gas capillary tube has no scale. It is 125 mm in length and 1.55 mm in diameter and has to be coated with heparin on the inside before use. The blood gas tube has to be filled up to the top with blood sample.

Result

In this study, there was no significant difference of ESR between that of the blood gas capillary tube and that of the Wintrobe tube (25.8 ± 17.9 and 25.5 ± 14.2 mm./hr, respectively) using the venous blood from the same subject. A significant positive correlation of ESR values between each methods ($r = 0.78$; $p < 0.001$) as well as an intra-class correlation coefficient (ICC) of 0.77 were found. However, the ESR values for capillary blood using the blood gas tube (35.2 ± 15.5 mm./hr) were significantly higher than those for venous blood using the Wintrobe tube (25.2 ± 14.2). There was a high correlation coefficient ($r = 0.86$; $p < 0.001$) and intra-class correlation coefficient of 0.70.

When the ESR values of all subjects were separated into 3 groups followed the previous study,[5] ranged from 0-20 mm/hr, 21-40 mm./hr and above 40 mm/hr, according to the classification of normal ESR value (<20 um/h) and highly abnormal value (>40 mm/ml) there was no significant difference in the ESR values between each method for venous blood. However, the blood gas capillary tube resulted in a higher ESR than the Wintrobe method when the ESR value was lower than 40 mm./hr. When the ESR values were above 40 mm./hr, the measurements of both methods were not statistically different.

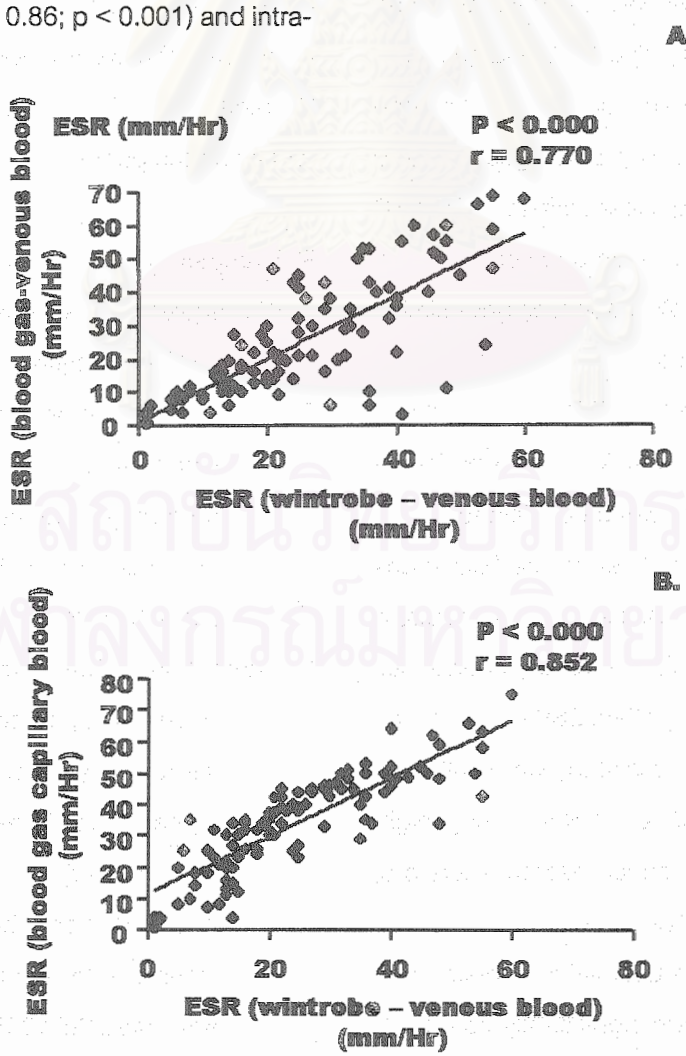

Figure 2. Correlation between ESR by blood gas capillary tube and Wintrobe tube from venous blood (A) and ESR from capillary blood using blood gas tube and from venous blood using Wintrobe tube (B).

Discussion

In previous study [5], which employed heparinized micro-hematocrit tubes (75 mm in length and 110 mm in diameter), capillary blood and Wintrobe method could be used interchangeably when ESR was below 40 mm./hr. In another study, the result of Wintrobe method was higher. For venous blood, the micro and Wintrobe method could be used interchangeably when ESR was below 20 mm./hr. However, in a different study, the result of Wintrobe method was higher.

In this study, the blood gas tube used for the measurement of ESR was 125 mm in length and 1.55 mm in diameter. If ESR was less than 40 mm/hr, measurements for capillary blood in blood gas tube, were higher than those for venous blood in Wintrobe tube. ESR from venous blood in blood gas tube and Wintrobe tube were not significant different. For ESR above 40 mm./hr, capillary blood and venous blood in both blood gas tube and Wintrobe tube gave similar results with a high correlation.

The phenomenon that ESR from blood gas tube was higher than that from Wintrobe tube for the values less than 40 mm./hr can be explained by two factors. The first one was that heparinized blood gas tubes causes red blood cells to settle faster, therefore ESR is higher.[13,14] Secondly, the variety of diseases of the pediatric patients may affect ESR values. Since all of the blood samples in this study were collected from pediatric patients, the result of the study might not be correctly applied to normal children. Therefore, further studies in normal children are recommended

In conclusion, the ESR values obtained by the blood gas method were significantly correlated with that of the standard Wintrobe method except for those from capillary blood sampling with ESR value less than 40mm./hr. Therefore, the estimation of the ESR can be performed alternatively by the blood gas method. This micro method is ideally suitable for young infants and it is also useful as a screening test in routine laboratory investigations. Also we suggest a further study which ESR from capillary blood in blood gas tube of normal children to be included for better interpretation and understanding of the ESR blood gas method.

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