Reference values for erythrocyte sedimentation rate in King Chulalongkorn Memorial Hospital using modified Westergren method

Yuwaree Vanavanitkun* Narudee Bhokaisawan*

Atchara Kalayanachati** Navapun Charuruks*

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Background

: The erythrocyte sedimentation rate (ESR) is still widely used as a nonspecific test for the acute phase inflammatory response. Since increment of biohazard risk to laboratory staff and inconvenience of the method have led to safer and more convenience methods for performing the ESR. However, the reference values on ESR for the local subjects are not established yet.

Objective

: To establish the local reference values for the ESR using modified Westergren method, a disposable ESR system, base on the International Committee for Standardization in Haematology (ICSH) recommendation on reference values.

Material and methods

: Three hundred eighty seven healthy subjects consist of 205 (53 %) female and 182 (47 %) male were randomly selected from the annual check up clinic at King Chulalongkorn Memorial Hospital. For safety and convenience in performing the ESR test, a modified Westergren method using disposable sedimentation tube was selected for this study.

* Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn
** Epidemiology Department, King Chulalongkorn Memorial Hospital

Results   :   *The reference range and mean ESR levels are 1-19 and 7.3 mm./hour for male and 4-23 and 12.4 mm./hour for female, respectively. We divided our subjects into two groups, the young adult group (20-40 years old), and the middle age group (41-60 years old). We founded that reference levels for ESR is 1-13 and 1-16 mm./hour for the young and the middle age male group, 5-20 and 4-21 mm./hour for the young and the middle age female group, respectively. From our results, we could demonstrate a statistical significant difference of ESR levels between different sex (p = 0.00) but not for age groups. However, we founded that there was a small increase of ESR with these two groups (p = 0.16 for male and p = 0.67 for female). In addition, to learn more about ESR levels at different age, we also divided the subjects to be 5-years interval groups. Again we could only demonstrate the increasing trend of ESR with age.*

Conclusion   :   *From this study, we concluded that there is a statistical difference of ESR mean between male and female and in all age groups the ESR values were higher in female than in male. Although we could not demonstrate a statistical significant difference of ESR levels between different age groups, but a small increase of ESR with age had been noticed. In addition, our study demonstrated the higher reference levels of ESR when compared to the western studies.*

Key words   :   *ESR, Reference level, Modified Westergren method.*

ยุวรีย์ วนาวณิชย์กุล, นฤดี โภไคศวรรย์, อัจฉรา กัลยาณชาติ, นวพรรณ จารุรักษ์. ค่าอ้างอิงของ
Erythrocyte Sedimentation Rate ของโรงพยาบาลจุฬาลงกรณ์ โดยวิธี Modified Westergren
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ที่มา : *Erythrocyte Sedimentation Rate (ESR) เป็น nonspecific test ที่มีการนำมา
ใช้อย่างแพร่หลายในการพิจารณาผลของการอักเสบเฉียบพลัน เนื่องจากความ
ไม่สะดวกและปลอดภัยของวิธีการแบบเดิมต่อเจ้าหน้าที่ในห้องปฏิบัติการ จึงมี
การพัฒนาวิธีการที่ปลอดภัยกว่าและสะดวกกว่าในการตรวจหาค่า ESR อย่างไร
ก็ตามค่าอ้างอิงของ ESB ในโรงพยาบาลจุฬาลงกรณ์ ยังไม่มีการศึกษา*

วัตถุประสงค์ : *เพื่อหาค่าอ้างอิงของ Erythrocyte Sedimentation Rate สำหรับใช้ใน
โรงพยาบาลจุฬาลงกรณ์ โดยวิธี Modified Westergren Method (ระบบ
disposable) ตามคำแนะนำของ International Committee for Stardardization
in Haematology (ICSH)*

วิธีการวิจัย : *คนไทยซึ่งมีสุขภาพดีจำนวน 387 ราย ประกอบด้วยเพศหญิง 205 คน (53 %)
เพศชาย 182 คน (47 %) ได้รับการสุ่มเลือดจากผู้ที่เข้ารับการตรวจสุขภาพที่
โรงพยาบาลจุฬาลงกรณ์ เพื่อทำการตรวจหาค่า ESR โดยวิธีการ Modified
Westergren ซึ่งใช้วัสดุที่ใช้แล้วทิ้ง เพื่อความปลอดภัยและสะดวกของบุคลากร*

ผลการวิจัย : *ค่าอ้างอิงของ ESR ในเพศชาย เท่ากับ 1-19 มม./ชม. เฉลี่ย 7.3 มม./ชม.
ค่าอ้างอิงของ ESR ในเพศหญิง เท่ากับ 4-23 มม./ชม. เฉลี่ย 12.4 มม./ชม.
ได้มีการแบ่งกลุ่มอายุเป็น 2 กลุ่ม กลุ่มแรก 20-40 ปี กลุ่มที่สอง 41-60 ปี พบว่า
ค่าอ้างอิงของ ESR ในเพศชาย อายุ 20-40 ปี และอายุ 41-60 ปี เท่ากับ 1-13
และ 1-16 มม./ชม. ตามลำดับ ค่าอ้างอิงของ ESR ในเพศหญิง อายุ 20-40 ปี
และอายุ 41-60 ปี เท่ากับ 5-20 และ 4-21 มม./ชม. ตามลำดับ จากผลการ
วิเคราะห์พบว่ามีความแตกต่างอย่างมีนัยสำคัญของค่าอ้างอิง ESR ระหว่าง
เพศชายและเพศหญิง แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญในระหว่างกลุ่ม
อายุ อย่างไรก็ตามพบว่ามีการเพิ่มขึ้นของค่า ESR เล็กน้อย ระหว่าง 2 กลุ่ม
อายุ ($p = 0.16$ ในเพศชายและ $p = 0.67$ ในเพศหญิง) นอกจากนั้นเพื่อศึกษา
ค่าอ้างอิง ESR ตามระดับอายุต่าง ๆ ได้ทำการแบ่งกลุ่มอายุทุก 5 ปี ซึ่งพบว่า
ค่าอ้างอิงของ ESR มีแนวโน้มเพิ่มขึ้นตามอายุ*

วิเคราะห์สรุปผล   : *จากการศึกษา สรุปได้ว่า ค่าอ้างอิงของ ESR มีความแตกต่างอย่างมีนัยสำคัญ*
ทางสถิติระหว่างเพศชายและหญิง และในทุกช่วงอายุ ค่าอ้างอิงของ ESR ใน
เพศหญิงสูงกว่าเพศชาย แต่อย่างไรก็ตามไม่สามารถแสดงความแตกต่างอย่าง
อย่างมีนัยสำคัญทางสถิติ ถึงความแตกต่างในระหว่างช่วงอายุ แต่สังเกตพบว่า
แนวโน้มที่จะมีค่า ESR เพิ่มมากขึ้น ตามอายุที่เพิ่มขึ้น นอกจากนี้การศึกษาพบ
ว่าค่าอ้างอิงของ ESR ในโรงพยาบาลจุฬาลงกรณ์ มีค่าอ้างอิงสูงกว่าค่าอ้างอิงที่
ศึกษาโดยประเทศตะวันตก

คำจำกัดความ   : *ESR, ค่าปกติ, Modified Westergren Method.*

Erythrocyte sedimentation rate (ESR) is a measure of the degree of setting of erythrocytes in plasma in an anticoagulated whole-blood specimen during a specified period of time.[1] The ESR test has long been used in clinical laboratories because it is simple, inexpensive and useful.[2] Although ESR is a nonspecific and insensitive laboratory test. It is still considered as a reliable indirect indicator of the acute phase inflammatory response. It is actually increased in the presence of infectious disease and acute or chronic inflammatory process. ESR is widely ascertained to monitor the course of many rheumatic and collagen-vascular diseases and their response to therapy, and it is commonly used as a diagnostic marker either or classification criteria for temporal artritis and polymyalgia rheumatica. It is used as prognostic index for monitoring disease activity as well as establishing remission in patients with rheumatoid arthritis as well.[3,4]

The ESR method selected by the International Committee for Standardization in Haematology (ICSH)[1] is based on that of Westergren. Subsequent ICSH guidelines[5] allow for the use of alternative ESR techniques, provided that comparability with the Westergren ESR is achieved. Increased awareness of biohazard risk to laboratory staff has led to safer methods for performing the ESR. Several modified methods have been developed such as; disposable sedimentation tubes,[3] vacuum extraction systems,[6] micromethods,[3] etc.

Values for "normal" ESR are not given in this international recommendation, as they may be influenced by local conditions. The objective of this study is to establish the local reference values for the ESR using modified Westergren method, a disposable

ESR system, base on the ICSH recommendation on reference values.[7]

Materials and methods

Samples obtained from 387 (male 182, female 205) subjects who attended the annual check up program of King Chulalongkorn Memorial Hospital. All blood samples were collected in 3.8 % sodium citrate with ratio of one volume of sodium citrate to four volumes of blood (Greiner VACETTE®, 2 ml., item no. 454073/ lot no. 090012). Using a specially prepared vaccuum collection tube. Samples were stored at room temperature (20-25°C). The test had been set up within 4 hours of venepuncture.

ESR is measured by modified Westergren method using plastic Westergren pipette (Greiner VACETTE®, item no. 72711/ lot no. 000077). The level of blood is adjusted to the "o" mark the tube is then placed to a holding device in a strictly vertical position. Under room temperature, not exposed to direct sunlight, and free from vibrations and draught and left for 60 min. After this period the distance from the bottom of the surface meniscus to the top of the column of sedimenting erythrocytes is read in mm and recorded as ESR value.

The age, gender, history of illness and medication of each subject were noted at the time of sample collection. Physical examination were performed by physician. All subjects had fasted at least twelve hours before venepuncture as the sample for lipid profiles were also collected at the same time.

The inclusion criteria were that the subjects should not receive medication or have history of illness during 3 months before the study. All of them had normal results of physical examinations and routine

tests on blood and urine. The exclusion criteria were those subjects receiving drug therapy, having medical illness, having abnormal physical examinations and having abnormal routine test on blood and urine. Pregnant women were also excluded.

The arithmatic mean (X), standard deviation (SD), and 5th and 95th percentiles of each parameter were calculated. The reference ranges for ESR were estimated by using nonparametric statistics (percentiles) because ESR values did not conform to true gaussian distribution. The unpaired t-test was used to test the significance between male and female for ESR mean at different ages. The factors with p-value < 0.05 were considered statistically significant.

Results

A total of 387 healthy subjects were analysed, 205 (53 %) females and 182 (47 %) males, age between 22-60 years old, mean 40.51, standard deviation 10.11 years. Subjects' characteristics and ESR results were demonstrated in Table 1. The reference range and mean level of male ESR is 1-19 mm./hour and 7.3 mm./hour, and female ESR is 4-23 mm./hour and 12.4 mm./hour, respectively. We also divided our subjects into two groups, the young adult group (20-40 years old), and the middle age group (41-60 years old) (Table 1). We founded that reference levels for ESR in this study is 1-13 mm./hour for the young male group, 1-16 mm./hour for the middle age male group, 5-20 mm./hour for the young female group, and 4-21 mm./hour for the middle age female group, respectively (Table 1). The results had shown that all mean and reference levels of ESR in female were higher than in male. The reference level at different age was also calculated and shown in Table 2 and Figure 1. The difference between sex is statistically significant at almost every age group (Table 2).

Table 1. Subjects' characteristics and ESR results.

Subjects' Characteristics and Results	Male			Female		
	20 - 40 years old	41 - 60 years old	Total	20 - 40 years old	41 - 60 years old	Total
1. Number	92	90	182	100	105	205
2. Age (years)						
range	22 - 40	41 - 60	22 - 60	22 - 40	41 - 59	22 - 59
mean	31.62	49.76	40.58	32.22	48.30	40.43
SD	5.24	5.55	10.57	5.53	5.38	9.71
3. ESR (mm./hour)						
range	0 - 15	0 - 25	0 - 25	2 - 24	1 - 29	1 - 29
mean	6.83	7.82	7.32	12.29	12.50	12.45
SD	3.98	5.49	4.80	4.82	6.05	5.43
Reference range	1-13	1-16	1-19	5-20	4-21	4-23

Table 2. ESR reference levels at different age between male and female is shown, notice that ESR levels in female is higher than male at all different age. P-value of ESR mean between male and female is also calculated. P-value <0.05 is considered statistically significant.

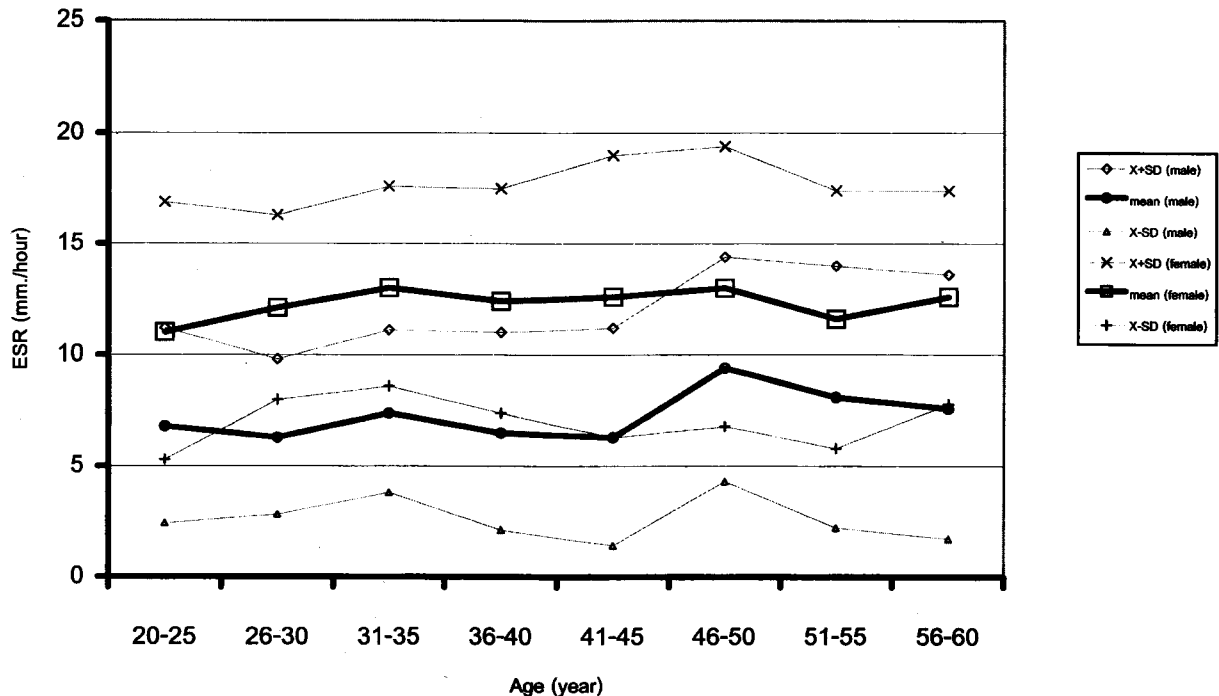| Age (years)	Male			Female			P-value
	number	ESR mean	ESR range	number	ESR mean	ESR range	
20 - 25	16	6.8	2 - 14	16	11.0	4 - 2	0.03*
26 - 30	21	6.3	2 - 13	24	12.1	6 - 18	0.00*
31 - 35	29	7.4	2 - 14	24	13.0	7 - 22	0.00*
36 - 40	26	6.5	1 - 15	36	12.4	5 - 20	0.00*
41 - 45	24	6.3	1 - 18	38	12.6	4 - 24	0.00*
46 - 50	21	9.4	4 - 20	35	13.0	4 - 25	0.05*
51 - 55	27	8.1	2 - 20	16	11.6	3 - 21	0.06
56 - 60	18	7.6	1 - 21	16	12.6	5.22	0.01*

*p-value < 0.05

Figure 1. The local ESR mean in male and female at different age.

Discussion

According to the ICSH recommendation on reference values, we have established locally reference levels for ESR [2] using a plastic disposable 200 mm. ESR tube (Greiner VACETTE®) to reduce the biohazard risk. This method correlated closely (r = 0.9637, p = 0.679) with the standard Westergren method. [8]

The reference levels for ESR in this study is 1-19 mm./hour for male and 4-23 mm./hour for female, respectively (Table 1). Our reference results is higher than previous study reported by Westergren that stated an upper limit of 5 mm./hour for men and 10-15 mm./hour for female. [9] Since several factors affecting the ESR such as; plasma proteins, packed cell volume (PCV), viscosity of the plasma, temperature, sex, etc. The explanation of this should be mainly the difference of race and life's style such as; food and environment, etc. All of these differences influence mainly to PCV that claimed to be a very sensitive factor affect to ESR. [3] The different methods should not effect the results since previous study reported the closely correlation between both methods. [8] The mean level for ESR is 7.3 mm./hour for male and 12.4 mm./hour for female, respectively (Table 1). We found that there was statistical significance between sex (p = 0.00). This is not out of our expectation since there were papers [2-3, 5] reported the difference of ESR level according to sex. The explanation about this event is the physiologic difference between both sexes and this is the most important reason. [3] The examples of the physiologic difference between both sexes such as; hormonal effect, plasma protein, PCV, etc. Since some studies [5, 9] had reported a significant difference

between age, we divided our subjects into two groups, the young adult group (20-40 years old), and the middle age group (41-60 years old) (Table 1). We found that reference levels for ESR in this study is 1-13 mm./ hour for the young male group, 1-16 mm./hour for the middle age male group, 5-20 mm./hour for the young female group, and 4-21 mm./hour for the middle age female group, respectively (Table 1). From our results, we could not demonstrate a statistical significant difference of ESR levels between different age groups. However we found that there was a small increase of ESR with these two groups (p = 0.16 for male and p = 0.67 for female). In addition, to learn more about ESR levels at different age, we also divided the subjects to be 5-years interval groups (Table 2). Again we could only demonstrate the increasing trend of ESR with age. One reason may used to explain our result is a subject number of our study. The further expansion of the subjects should be continued. In addition, the further study about ESR levels using modified Westergren method should be revised and set in pathological states that affect it.

From this study, we concluded that there is a statistical difference of ESR mean between male and female. We found that in all age groups the ESR values were higher in female than in male. Although we could not demonstrate a statistical significant difference of ESR levels between different age groups, but a small increase of ESR with age had been noticed (Table 2). In addition, our study demonstrated the higher reference levels of ESR when compared to the western studies. Race and life's style should be the main reasons of this difference and it reminds us to establish the local reference values in accordance with the ICSH recommendation on reference values.

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