REVIEW ARTICLE

ERYTHROCYTE SEDEMENTATION RATE: A USEFUL TOOL IN DIAGNOSTIC APPROACH

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ABSTRACT
Erythrocytes Sedimentation Rate (ESR) is one of the most common lab tests requested by physicians across the globe. Although it doesn't enjoy the status of being called a diagnostic test,even then it is in continuous use as a good indicator in conditions like infections and inflammations; for screening and prognosis of certain diseases and also for monitoring the course of an existing disease.It is a good indicator to estimate the quantitative change in plasma that may occur in a lot of clinical conditions ranging from simple to much serious disorders such as inflammation, infection, pregnancy, obesity and even malignancies. In certain hematological conditions such as polycythaemia, sickle cell anaemia, leukemia, hypoproteinaemia as well as few other conditions like hyper viscosity, congestive heart failure etc. Physicians does rely on ESR as an indicator to some extent.

Current review is done to check its clinical importance, different aspects of ESR usage, methods by which it is performed and advancements in the test.

Introduction
Erythrocytes Sedimentation Rate (ESR) basically measures the speed of red cells to settle down in a vertically mounted standardized open-ended tube (length 30 cm)for one hour in autologous plasma; thus the name sedimentation rate[1].It is an inexpensive, low-cost, low-technology and less cumbersomeprocedure. Since ESR fluctuation is observed in quite a large number of conditions, it is consideredas a highly non-specific parameter and therefore it cannot be used as a pin-point diagnostic test for any particular disease[2].

There is another parameter very commonly ordered by the physicians; C-reactive protein (CRP), but it certainly is less cost-effective than ESR. CRP, just like ESR, is also used to assess inflammatory responses. Although ESR has certain limitations as compared to CRP in a number of inflammatory conditions such as in rheumatoid arthritis, yet it is widely considered as an indicator of acute phase reaction that indirectly measures the extent of inflammation. This is because the acute-phase plasma proteins (such as fibrinogen) cause the red cells to settle down quickly. Nonetheless ESR is a helpful predictive toolin inflammatory diseases, helpsin indications of certain diseases, in monitoring the progress of specific conditions and also helps as a prognostic tool in malignancies[2].

ESR was not commonly used as a laboratory test until 1897, when a Polish physician named Edmund FaustynBiernacki devised a clear method of performing ESR and also described its clinical significance[3]. This progressive work was left incomplete due to his death at the age of 45 years. During his studies, he analyzed that ESR varies with age and sex and its sedimentation rate is fast in patients with low red blood cell count and slow in individuals who have fever[4]. A Swedish hematologistRobert Sanno Fåhræus, in 1918, working on ESR after seven years of Edmund

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Faustyn Biernacki's death, attempted the same principle on pregnant and non-pregnant women to judge time difference of erythrocyte sedimentation[3, 5]. His studies caused ESR to become a widely used pregnancy test for some time. In 1921 a Swedish scientist Alf VilhelmAlbertsonWestergren's developed a new methodto perform and utilize this test while he was doing research on the patients with pulmonary tuberculosis[6]. This method got so much popularity that it is still in use around the globe.

Mechanism of Erythrocyte Sedimentation

ESR allows the red cells to settle down in autologous plasma for one hour, which basically indicates the plasma to blood cells ratio.An increased ratio (more plasma than red cells) will cause a fast sedimentation of erythrocytes, thus a high ESR will be observed and *vice versa*. This shows that the rate of red cell sedimentation in ESR does not only dependupon red cell count and red cell shape, but alsoupon the specific gravity between plasma and red cells; which can be affected by the rouleaux formation of red cells [3].

There are three phases of ESR; rouleaux formation phase, decantation phase and packing of red cell.

a) Rouleaux formation phase: It is the preliminary stage that lasts approximately for 10 minutes. During this phase, red cells settle down as a stack of coins called rouleaux formation, packing them as aggregates.

b) Decantation phase (Rapid fall): This phase lasts approximately for 40 minutes. During this phase the red cells fall at a much higher and at a constant rate.

c) Packing of red cell (Slow fall):This phase also lasts for approximately 10 minutes. During this final phase the aggregated red cells pack themselves steadilyat the bottom of the tube[7].

Normal Values

ESR values, even in healthy individuals, constantly change and do not remain the same throughout the course of life. There is a progressive increase in ESR normal value with advancement in age[8]. Therefore, researchers formulated a method to determine normal values for all age groups in accordance with the gender. The formulae are given as under;

- Normal ESR for men = age in years / 2
- Normal ESR for women = age in years + 10 / 2 [9,10]

Mean values and upper limitsin 95% of normal individuals are shown in table1.

Table 1: Normal values of ESR

Age range (years)	ESR mean (mm/1st hour)
Newborn	0-2
10-19	8
20-29	10.8
30-39	10.4
40-49	13.6
PREGNANCY	
Early gestation	48 (62 if anemic)
Later gestation	70 (95 if anemic)

Factors affecting ESR

ESR result is sensitive to quite a few things. An increase or decrease in ESR result can be observed due to a number of factors that include;

a) Plasma elements affecting ESR
b) Red cell elements affecting ESR
c) Drugs affecting ESR
d) Conditions associated with raised ESR

Table 2: Plasma elements affecting ESR[7, 11]

Decreased	Increased
Increased albumin	Decreased albumin
Decreased fibrinogen	Increased fibrinogen
Hyperviscosity	Globulins

Table 3: Red cell elements affecting ESR[7, 11]

	Decreased	Increased
Size	Microcytes	Macrocytes
Quantity	Polycythaemia	Anaemia
Morphology	Sicke cells, spherocytes, schistocytes	
Others	Hemolysis, hemolytic anemia	

Table 4: Drugs affecting ESR[7, 11]

Decreased	Increased
Cortisone	IVIG
NSAIDs	Heparin
Valproic acid	Oral contraceptives

Table 5: Conditions associated with raised ESR[7]

System	Conditions
Renal	
	Renal failure
	Glomerulonephritis
Rheumatological	
	Acute rheumatic fever
	Systemic lupus erythematosus
	Sarcoidosis
	Osteomyelitis
Others	
	Congestive heart failure
	Obesity
	Pregnancy
	Inflammatory bowel syndrome/Coeliac disease
	Interstitial lung disease

Methods of ESR Estimation

1. Westergren's method:
It is by far the most widely used and the most well reputed ESR estimation method. International Council for Standardization in Hematology (ICSH) established this method[12] and it is now accepted by Clinical and Laboratory Standards Institute (CLSI)[13,14,15]. The recommended apparatus is a straight glass tube, 30 cm in length and 2.55 cm in diameter[1]. The bore must be uniform to 0.05 mm throughout the length of tube.To perform this test, anticoagulated blood should be collected in 1:4 ratios, which means 1.6 ml blood and 0.4ml anticoagulant (sodium citrate). The Westergren's tube should be filled up to zero mark and should be placed vertically in Westergren's rack for 1hour. After one hour (1st hour) the column of the plasma is read to report the ESR value[1, 16, and 17].

2. Wintrobe's method:
For this method, anticoagulated blood should be takenin EDTA (Ethylenediaminetetraacetic acid). With the help of pasture pipette the Wintrobe's tube is filled up to "zero" mark and placed vertically in a rack for one hour. After one hour, the reading is noted at which the column of the plasma is residing [17].

3. Semiquantitative Slide Method:
ESR can also be demonstrated by putting a drop of citrated blood on a slide.

The slide is placed at an angle of 45^{O} such that the drop comes down steadily by gravity and leaves a trail behind. The slide is then allowed to dry at room temperature. It is then stained with Leishman's stain (4-5 drops) and is left for 2 minutes. Distilled water, almost double the amount, is poured and again the slide is left for 8 minutes. The slide is then washed in running tap water and allowed to dry. Upon examining under the microscope the red cell aggregates are observed. The aggregation is reported in grades as A, B, C and D. "A" being the least aggregated while "D" has maximum aggregation. But this method certainly is not the recommended one and needs further assessment and evaluation in order to call it appropriate[18].

4. Automated method:
In this method tube is held at an angle of $18^{o}$to the vertical in the instrument which also maintains temperature. After 20 minutes, the height of red cells is measured by using light transmission. To make a comparison of this method with Westergren's method, mathematical correction is applied. Although it's a fast and fully automated technique, scientists are still perplexed as the results are sometimes comparable and sometimes not. Nevertheless, it is a common technique observed by labs worldwide nowadays. Much work is being done to improve this technique and continuous comparison with Westergren's method is being applied [19].

5. Portable microfluidic system:
In 2016 Turkish scientists devised a new technology to measure ESR in accordance with erythrocytes aggregates. This method gives the ESR result in just 2 minutes via a small finger prick. According to them 40 c l whole blood is filled in a disposable polycarbonate cartridge illuminated by a near infrared emitting diaode.By using a solenoid pinch valve, the erythrocytes are disaggregated under the effect of a mechanical shear force. After the aggregation is completed, a photo detector is used to measure the transmitted light coming through the cartridge for 1 min and 30 seconds. At complete disaggregation, the intensity level is at its lowest and *vice versa*[20].

Quality control of ESR
Routinely standardized methods can be used for quality control. Whole blood preparation can be assessed for daily control on automated system (e.g. ESR-Chex or Ves-Matic Cube 200) [21]. Daily calculation of cumulative mean of 100 specimens every day in a regular situation should be done. Less than 15% coefficient of variation is a reasonable indicator for routine instrument monitoring[22].

Discussion
ESR is in use for almost a century in clinical practice, although its specificity is still controversial. Use of

ESR basically quantifies inflammatory changes which occur during infectious, inflammatory and neoplastic disorders. Increase or decrease of certain protein production is the reflection of inflammatory or necrotic changes in the body. The change in the degree of protein occurs due to many factors such as acute and chronic infections, tumors, degenerative and autoimmune diseases such as rheumatoid arthritis[22].

The pathophysiological elevation of ESR may be due to anaemia, known fibrinogen protein[7, 23], hypercholesterolemia[5], intense obesity [1, 8], pregnancy [3], old age [9, 10]and malignancies and renal diseases [24]. The effect of anaemia is mediated by the alteration in the ratio of erythrocytes to plasma, which favors rouleaux formation. Fibrinogen may or may not be the cause. For example, increase in ESR in certain diseases can be observed due to elevated serum level of non-fibrinogen proteins like M proteins, Macroglobulins, and RBC agglutinins, while an elevated ESR in renal failure maybe attributed to elevated serum fibrinogen levels[4, 7, and 8]. On the other hand, intense obesity also demonstrates a high ESR which is most likely due to the elevated fibrinogen levels [1, 8].

Above mentioned diseases and their relative ESR values clearly demonstrate that ESR is a parameter that does not depend on any single factor. This makes it quite unreliable. On the other hand, in conditions like multiple myeloma, Waldernstrom gammaglobinaemia, rheumatoidarthritis, polymyalgia rheumatica, temporal arteritis etc., the significance of ESR cannot be ruled out[5,6]. In the said conditions ESR still has diagnostic significance which is not debatable. In many other circumstances brief or persistent rise of ESR is also used to observe disease activity and response to treatment. Slight increase of ESR is usually found in acute inflammation, infection, pregnancy[4,7] and in people of advanced age [9,10], while a bit more aggressive rise is seen in conditions in which globulin protein is increased which is due to severe infection or inflammation.

Pathophysiological decrease of ESR may be found in clinical conditions like, sickle cell anemia,polycythemia, dysfibrinogenaemia, afibrinogenaemia, high serum bile salt level and in hematologicaldiseases which cause red cell morphological abnormalities[7]. Common morphologic changes of RBCs can hold up pellet formation which causes ESR to be affected. Abnormal or irregular shapedRBCs (e.g., sickle cells)do not settle down quickly,resulting in a decreased ESR. Spherocytes, anisocytosis, and poikilocytosis also impede with the stacking of red cell, causing the same[7, 10]. Adecreased ESR is not clinically important in some conditions likepolycythemia and leukocytosis, while on the other hand it is quite significant in hereditary disorders such as sickle cell anaemia and hereditary spherocytosis[7].

Regarding malignancies, it is a general perception that ESR is clinically significant in hematologicalneoplasms only;it is equally valuable in some non-hematological malignancies as well, such as prostate cancer, ovarian cancer and breast carcinoma. An elevated ESR among these malignancies is the sign of poor prognosis and advancing disease[25].

ESR can also be used to predict coronary heart disease as well as can be used as an indicator of atherosclerosis that maybe caused due to rheumatoid arthritis [26]. ESR along withCRP and lactic acid play a vital role in monitoring the patients who are at high risk of developing cerebral infarction due to internal carotid artery occlusion [27].

Conclusion

New techniques and ways are continuously being explored to perform this test. Scientists are working hard to find the methods to use less amount of blood and get quick results. Since the comparison of new techniques is always done with Westergren's method, looks like until something really applausable does not come up, Westergren's method is by far the gold standard technique to perform this test.

Keeping all the aspects discussed above in mind, it can therefore be concluded that although ESR is not treated as a diagnostic or a well-reputed parameter, yet it becomes quite handy in a lot of situations. Physicians do rely on this test and get help from this parameter in a number of conditions, which makes ESR a really useful tool in diagnostic approach.

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