Extremely elevated erythrocyte sedimentation rate

Etiology at a tertiary care center in Saudi Arabia

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ABSTRACT

الأهداف: تحديد أسباب سرعة تثقل كريات الدم الحمراء المفرط لدى المراهقين والبالغين في مركز للرعاية الصحية من الدرجة الثالثة.

الطريقة: أُجريت هذه الدراسة المقطعية الاسترجاعية التي تعتمد على الملاحظة في مدينة الملك عبدالعزيز الطبية، الرياض، المملكة العربية السعودية وذلك خلال الفترة من يونيو 2007م إلى أكتوبر 2008م. لقد قمنا باستخدام طريقة ويسترغرين من أجل تحديد سرعة تثقل كريات الدم الحمراء وذلك في المراهقين والبالغين الذين تبلغ أعمارهم أو تزيد عن 12 عاماً، وقد شملت الدراسة مرضى العيادات الخارجية والداخلية والذين يعانون من بعض المشاكل الطبية والجراحية والنسائية، حيث تم الرجوع إلى كافة السجلات الالكترونية والورقية للمرضى كلاً على حدة وبدون تكرار للحالات المتعاقبة، وكانت هذه الحالات تشكو من سرعة تثقل كريات الدم الحمراء بمعدل يزيد عن أو يساوي 100مليمتر/ساعة، وتم تحديد العوامل المسببة لهذا الاضطراب.

النتائج: لقد قمنا بإجراء 44,366 اختباراً لتعيين سرعة تثقل كريات الدم الحمراء في هذا المركز، وسجل 1864 (4.2%) من هذه الاختبارات قيماً تزيد عن أو تساوي 100 مليمتر/ساعة وكانت هذه الاختبارات تخص 567 مريضاً. ولقد وجدنا أن مسببات هذا الاضطراب في 508 مريضاً ممن انطبقت عليهم الدراسة كانت كالتالي: الالتهابات (38.6%)، أمراض المناعة الذاتية (15.9%)، الأمراض الخبيثة (15.4%)، أسباب متفرقة (10.2%)، الإصابات النسيجية بفعل نقص التروية أو الإصابات الرضحية (8.7%)، أمراض الكلى (8.4%). فيما وجدنا أن هناك أيضاً عشرة مسببات أخرى وهي: التهاب المفاصل الروماتويدي (7.3%)، التهاب العظام والنقي (6.9%)، السل (5.5%)، الإصابات الرضحية (5.3%)، أورام لمفاوية وإنتان مجهول السبب (5.1%) لكل منهما، التهابات المسالك البولية (4.7%)، التهاب المفاصل الإنتاني (3.1%)، خراجات (2.8%)، الحمل (2.2%). وقد تعذر معرفة أسباب سرعة تثقل كريات الدم الحمراء في 14 مريضاً (2.4%).

خاتمة: أشارت الدراسة إلى أن هناك سبباً كامناً وراء إصابة المرضى بسرعة تثقل كريات الدم الحمراء، ولذلك يتعين إجراء الفحوص اللازمة من أجل الكشف عن المسببات والوصول إلى التشخيص الصحيح.

Objectives: To evaluate the etiology of extremely elevated erythrocyte sedimentation rate (ESR) in adolescents and adults at a tertiary care center.

Methods: This retrospective, cross-sectional, observational study was carried out at King Abdulaziz Medical City, Riyadh, Saudi Arabia using the Westergren method of determining ESR in adolescents and adults aged ≥12 years. The patients included inpatients and outpatients with medical, surgical, and gynecological problems. During a period from June 2007 to October 2008, consecutive, non-repetitive patients with ESR ≥100 mm/hour were evaluated for possible etiology by checking the electronic and paper data file of each patient.

Results: During the study period, out of the 44,366 ESR tests carried out at this center, 1864 (4.2%) had an ESR ≥100 mm/hour belonging to 567 patients. Out of 508 patients fulfilling the study criteria, the main associated causes included: infections (38.6%), autoimmune diseases (15.9%), malignancy (15.4%), miscellaneous causes (10.2%), ischemic tissue injury or trauma (8.7%), and renal diseases (8.4%). Ten common individual causes included: rheumatoid arthritis (7.3%), osteomyelitis (6.9%), tuberculosis (5.5%), trauma (5.3%), lymphoma and sepsis of unknown origin (5.1%) each, urinary tract infection (4.7%), septic arthritis (3.1%), abscesses (2.8%), and pregnancy (2.2%). Fourteen (2.4%) patients had no known cause.

Conclusion: Most of the patients with extreme ESR elevation have an underlying cause and a focused evaluation of such patients needs to be carried out to reach a diagnosis.

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The erythrocyte sedimentation rate (ESR) determination is a commonly performed laboratory test and is known as an acute-phase reactant test, namely, it reacts to acute conditions in the body, such as infection, or trauma. Edmund Biernacki and Robin Fahraeus have been credited with the discovery of ESR, although it had been described much earlier by John Hunter.[1] Some studies suggest that the test may be useful as a "sickness index" or screening tool for some specific diseases. An extremely elevated ESR (EEESR) defined as equal to or greater than 100 mm per hour, is associated with a low false-positive rate and a 90% predictive value for serious underlying disease, most often infection, collagen vascular disease, or metastatic malignancy.[2] An EEESR carries a poor prognosis. In one study, such inpatients had a mortality of 12% at one month, and 34% at 6 months. A follow up ESR of >60 mm/hr at one month in these patients (in the absence of rheumatoid disease or paraproteinemia) was found to carry a poor prognosis.[3] In the pre-HIV era, a review found an EEESR to be most commonly associated with infections, followed by malignancy, inflammatory/collagen disease, renal, and miscellaneous diseases.[4] In Saudi Arabia, data on ESR are available from small studies in children and dialysis patients.[5,6] Hence, this study was carried out to provide further information on the etiological relationship of EEESR in Saudi patients in a tertiary care center.

Methods. After approval from the institutional research committee, a cross-sectional, observational, retrospective study was carried out on adolescents and adult patients. Selection criteria were: all patients aged ≥12 with ESR equal to or more than 100 mm per hour (mm/hour) tested at King Abdulaziz Medical City, Riyadh, Saudi Arabia from June 2007 to October 2008. This hospital is a tertiary care teaching referral center in Riyadh, Saudi Arabia with 1000 beds. Non-repetitive, outpatients and inpatients medical, surgical, and gynecologic cases that had been thoroughly investigated were included. Patients with no follow up investigations were excluded. The ESR at this hospital is carried out on whole blood samples obtained through standard venous venipuncture techniques in 4 mls BD Vacutainer tubes (BD, Plymouth, UK) or Vacuette tubes (Greiner Bio-one, Kremsmunster, Austria) with di- or tri- potassium EDTA. The test is performed using an automated TEST 1 THL Analyser (Alifax, Padova, Italy). This automated ESR analyzer measures the capacity of the red cells to aggregate photometrically over a very short period of time and translates the results into traditional units as mm/hr. Results between 0-149 mm/hour are reported as exact figures, whereas those having ESR equal to or more than 150 are reported as ≥150 mm/hour. The clinical data of this patient population were studied to evaluate the underlying diseases associated with these ESR values. The cause of the EEESR in each patient was evaluated by checking the available CBC, differential count, chemistry, urinalysis, histopathology data, microbiology results, serology, protein electrophoresis, special staining or flow cytometry, plain x-rays, endoscopic studies, and CT or MRI scanning results. Sepsis of unknown origin was diagnosed when the patient had 2 or more criteria of systemic inflammatory response syndrome (SIRS) with positive blood cultures, but without obvious identifiable localizing infection.[7] Patients having more than one possible cause of EEESR were evaluated for any change from previous ESR, or any acute event during the period of the highest ESR reading and disease related to the new change in ESR was identified as a cause. Only patients, who had been investigated thoroughly and had no identifiable cause for EEESR despite thorough investigations, were considered as idiopathic.

The data were computed using Microsoft Excel 2007 and by descriptive statistics total numbers, percentage (%), and mean values of different groups of patients were calculated.

Results. Out of a total of 44,366 samples tested for ESR in adolescents and adults ≥12 years during the study period in this hospital, 1864 samples (4.2%) had ESR ≥100 mm/hour. These samples belonged to 567 patients, with most of the patients having more than one test (mean 3.3 tests per patient). Fifty-nine patients were excluded from the study, 45 patients did not follow up after the initial blood test, while for 15 patients the files were not available. The final data belonged to 508 patients and the main causes of EEESR in these patients are summarized in **Table 1**. The 10 most common individual causes of EEESR are summarized in **Table 2**. Main infections included osteomyelitis, tuberculosis, and urinary tract infections as shown in **Table 3**. Autoimmune diseases, malignancies, and miscellaneous conditions were the second, third, and fourth most common groups with EEESR as summarized in **Table 4**. Kidney diseases included 43 (8.4%) patients with EEESR, of these, 20 (47%) had end stage renal disease (ESRD) on dialysis, while 23 (53%) had various stages of pre-end stage chronic kidney disease (CKD). Trauma and ischemic tissue injury were the cause of EEESR in 44 (8.7%) cases. Trauma induced by accidents or by surgical operations was the cause in 27 patients. Among them, 6 patients had fractures, 7 had soft tissue, muscle, or deep organ trauma, while 14 patients had undergone different operations in the last 6 months. Seventeen patients had ischemic tissue injury with manifestations of cardiovascular or cerebrovascular

Table 1 - Main causes of extremely elevated erythrocyte sedimentation rate with gender distribution (N=508)

Cause	Male n (%)	Female n (%)	Total n (%)	Mean age of all patients (years)
Infections	116 (22.8)	80 (15.7)	196 (38.6)	56.6
Autoimmune diseases	18 (3.5)	63 (12.4)	81 (15.9)	40.0
Malignancy	35 (6.9)	43 (8.5)	78 (15.4)	55.6
Miscellaneous causes	18 (3.5)	34 (6.7)	52 (10.2)	45.5
Kidney disease	20 (3.9)	23 (4.5)	43 (8.4)	61.0
Trauma and ischemic tissue injury	22 (4.3)	22 (4.3)	44 (8.7)	60.1
Idiopathic	6 (1.2)	8 (1.6)	14 (2.8)	43.4
Total	235 (46.3)	273 (53.7)	508 (100)	53.4

Table 2 - Ten most common causes of extremely elevated erythrocyte sedimentation rate (N-508).

Individual causes	N	(%)
Rheumatoid arthritis	37	(7.3)
Osteomyelitis	35	(6.9)
Tuberculosis	28	(5.5)
Trauma (accidental & operative)	27	(5.3)
Lymphoma	26	(5.1)
Sepsis of unknown origin	26	(5.1)
Urinary tract infections	24	(4.7)
Septic arthritis	16	(3.1)
Abscesses	14	(2.8)
Pregnancy	11	(2.2)

Table 3 - Extremely elevated erythrocyte sedimentation due to infections (N=196).

Type of infection	N	(%)
Osteomyelitis	35	(17.9)
Tuberculosis	28	(14.3)
Sepsis from unknown site	26	(13.3)
Urinary tract infections	24	(12.2)
Wound infections	19	(9.7)
Septic arthritis	16	(8.2)
Abscesses	14	(7.1)
Pneumonia	7	(3.6)
Infected gangrene	7	(3.6)
Biliary infections	5	(2.6)
Infective endocarditis	4	(2.0)
HIV /AIDS	3	(1.5)
Other infections	8	(4.1)

HIV/AIDS - Human immunodeficiency virus/Acquired immune deficiency syndrome. Other infections in 8 patients included 2 cases of bacterial peritonitis with one case each of malignant otitis media, septic abortion, brucellosis, invasive fungal infection, and cutaneous leishmaniasis.

Table 4 - Autoimmune diseases, malignancies and miscellaneous causes of extremely elevated erythrocyte sedimentation rate (N=312).

Cause	N	(%)
Autoimmune diseases (n=81)		
Rheumatoid arthritis	37	(45.7)
Systemic lupus erythematosis	23	(28.4)
Rheumatic fever	6	(7.4)
Juvenile chronic arthritis	3	(3.7)
Vasculitis	2	(2.5)
Mixed connective tissue disease (MCTD)	1	(1.2)
Other autoimmune diseases*	9	(11.0)
Malignancies (n=78)		
Lymphomas	26	(33.3)
Multiple myeloma	10	(12.8)
Leukemias†	9	(11.1)
Carcinoma colon and rectum	7	(9.0)
Hepatocellular carcinoma	4	(5.0)
Other cancers‡	22	(28.2)
Miscellaneous causes (n=52)		
Pregnancy	11	(21.2)
Inflammatory bowel disease	11	(21.2)
Status post-transplantation	9	(17.3)
Interstitial lung disease	7	(13.5)
Myelodysplastic syndrome	2	(3.8)
Intra-uterine fetal death	2	(3.8)
Benign tumors	2	(3.8)
Other miscellaneous causes§	8	(15.4)

*Other autoimmune diseases included 2 cases of polymyalgia rheumatica, and one case each of scleroderma, polyarteritis nodoa. Hoshimoto's thyroiditis, Grave's disease, giant cell arteritis, autoimmune hepatitis, and primary biliary cirrhosis. †Out of 9 cases of leukemias, 6 were acute myelogenous leukemia, 2 were acute lymphoid leukemia and one was chronic lymphocytic leukemia. ‡Out of 22 other cancers, 2 each were carcinoma thyroid, carcinoma lung and carcinoma prostate, 3 cases of metastatic cancer with unknown primary, one case each of cancer of gall bladder, duodenum, breast, endometrium, kidney, adrenal, nasopharynx, brain, neck, testis and one case each of Kaposi sarcoma, leiomyosarcoma and spindle cell cancer. §Other miscellaneous causes included: 2 cases of diabetic Charcot joints, one case each of psoriasis, glycogen storage disease, Rosai Dorfman's disease, intracerebral hemorrhage and lumbar spinal stenosis.

disease. Of these, ischemic heart disease was the cause of EEESR in 6 patients, myocardial infarction in 5 patients, peripheral vascular disease in 2 patients, ischemic stroke in 3 patients and myocarditis in one patient. Fourteen (2.8%) patients had no identifiable cause of EEESR, which could be grouped to any of the different categories and were termed idiopathic.

Discussion. The search for simple diagnostic markers that can be assessed anywhere at low cost is important particularly in developing countries. An EEESR is associated with a low false-positive rate for a serious underlying disease. The conditions found in this situation have varied in individual populations, depending on patient's age, inpatient versus outpatient status, and the discipline of patients studied. In most studies, infection has been the leading cause of an extremely elevated value, followed by collagen vascular disease, and malignant tumors.[5] Renal disease has also been a notable etiologic factor.[6,8] In our study, the

main associated causes included infections in 38.6%, autoimmune diseases in 15.9%, and malignancy in 15.4%. There is limited comparative Saudi data in adolescents and adults. However, in Saudi children with EEESR,[5] infections were the most common cause (49.5%), followed by connective tissue diseases (26.3%), malignancy (12.1%), and renal diseases (8.1%). Compared to our study, data from adult inpatients in South Africa,[9] most of whom were HIV positive, detected infection to be present in 91.6%, malignancy in 3.8%, renal disease in 3.1%, inflammatory/collagen diseases in 2.9%, and miscellaneous disease in 4.9% of the cases with EEESR.

In our study, the main infections responsible for EEESR were osteomyelitis, tuberculosis, and sepsis of unknown origin, urinary tract infections, and septic arthritis. Our results indicate the infections as the main cause of EEESR. This is in agreement with other similar studies.[4,5,9] Osteomyelitis is a severe infection of bone associated with EEESR. Long term antibiotics and laboratory monitoring of white blood cells and erythrocyte sedimentation rate with at least 12 months follow up have resulted in excellent results.[10] Improved diagnostic accuracy for osteomyelitis in diabetic foot can be achieved by clinical and laboratory findings. A combination of ulcer depth with serum inflammatory markers is likely to be a sensitive strategy that may allow greater detection of early diabetic osteomyelitis.[11] The ESR has been found to be abnormal in patients who have septic arthritis due to an infected joint prosthesis. However, although useful in prosthetic hip and knee infections, ESR has poor sensitivity for the diagnosis of shoulder implant. Therefore, its performance is variable.[12] In our study, lymphoma, multiple myeloma, and leukemia were the most common malignancies associated with EEESR. In various cancers, a high ESR has been found to correlate with an overall poor prognosis, for example, Hodgkin's Disease, gastric carcinoma, renal cell carcinoma, chronic lymphocytic leukemia, breast cancer, colorectal cancer, and prostate cancer.[13,14] A sedimentation rate greater than 100 mm/hr usually indicates metastasis in patients with solid tumors.[2] Several European studies of patients with Hodgkin's disease have indicated that an elevated ESR may still be a superior predictor of early relapse, especially if the value remains elevated after chemotherapy or fails to drop to a normal level within 6 months after therapy.[15] However, more modern tests should be used as criterion for diagnosing relapsed Hodgkin's disease. Renal disease was responsible for 43 (8.4%) cases of EEESR. In a study of Saudi patients on hemodialysis, 32% of the patients had EEESR, and the rise in ESR was associated with elevated plasma fibrinogen with no relationship to anemia.[6] In our study, 25 (4.9%) had EEESR due to trauma associated with accidents or surgical operations. The ESR usually increases to high levels after major surgical operations or extensive trauma, and often returns to normal within 6 months. The ESR rises acutely immediately postoperatively after intramedullary nailing of a long bone fracture.[16] However, the prevalence of EEESR in trauma or after different surgical operations is not known. The ESR has been proposed to be a simple test that can independently predict the risk of developing coronary heart disease (CHD). In a cohort of 7,988 men and 8,685 women who participated in The Reykjavik Study in Iceland,[17] ESR was an independent predictor of the risk of developing CHD. Raised ESR has been associated with a poorer prognosis in patients with coronary artery disease, unstable angina, and myocardial infarction.[18,19] However, whether this increased mortality varies directly with the degree of rise in ESR is not known. A persistently elevated ESR in someone who has had a stroke or transient ischemic attack may imply ongoing thrombosis/fibrinolysis.[20] In our study, 16 (3.1%) of the patients had stable ischemic heart disease, peripheral vascular disease, acute coronary syndrome, or a stroke. In our study, 14 (2.8%) had no obvious known cause despite extensive investigations with follow up periods of 3 months to 3 years. This compares to 2.4% in a pooled data,[4] and 0.2% cases in a South African study[9] of EEESR. The proportion of these cases depends on the nature of the study (prospective or retrospective), the intensiveness of investigations, duration of follow up, and inclusion of well-investigated cases. Being a retrospective study, several factors could not be evaluated. We could not assess many aspects, which could have been possible in a prospective study. We could not find out the reason for asking for ESR by the treating doctors or the treatment given in each patient. The follow up period was not uniform and the prognosis of patients was not known in all cases. This type of work will need a multidisciplinary collaborative effort in a well-planned prospective study, which is not available in the literature at the moment. Our study has tried to find out the causes of EEESR in Saudi population in a tertiary care setting.

We conclude that most of the patients with EEESR have an underlying cause and a focused evaluation of such patients needs to be carried out to reach a diagnosis.

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References

1. Madrenas J, Potter P, Cairns E. Giving credit where credit is due: John Hunter and the discovery of erythrocyte sedimentation rate. ***Lancet*** 2005; 366: 2140-2141.

2. Osei-Bimpong A, Meek JH, Lewis SM. ESR or CRP? A comparison of their clinical utility. ***Hematology*** 2007; 12: 353-357.
3. Hameed MA, Waqas S. Physiological basis and clinical utility of erythrocyte sedimentation rate. ***Pak J Med Sci*** 2006; 22: 214-218.
4. Levay PF, Viljoen M. Erythrocyte sedimentation rates equal to or above 100 mm/hr in the pre-AIDS era. ***S Afr Med J*** 2002; 92: 286-287.
5. Abbag FI, Qahtani JM. Extreme elevation of the erythrocyte sedimentation rate in children. ***Ann Saudi Med*** 2007; 27: 175-178.
6. Al-Homrany. The significance of extreme elevation of erythrocyte sedimentation rate in hemodialysis patients. ***Saudi J Kidney Dis Transplant*** 2002; 13: 141-145.
7. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. ***Crit Care Med*** 2003; 31: 1250-1256.
8. Steinvil A, Shapira I, Arbel Y, Justo D, Berliner S, Rogowski O. Determinants of the erythrocyte sedimentation rate in the era of microinflammation: excluding subjects with elevated C-reactive protein levels. ***Am J Clin Pathol*** 2008; 129: 486-491.
9. Levay PF, Retief JH. Causes of high erythrocyte sedimentation rates in an inpatient population. ***S Afr Med J*** 2005; 95: 45-46.
10. Ilyas AM, Mudgal CS. Management of medullary osteomyelitis of the humerus. ***Tech Hand Up Extrem Surg*** 2008; 12: 144-149.
11. Fleischer AE, Didyk AA, Woods JB, Burns SE, Wrobel JS, Armstrong DG. Combined clinical and laboratory testing improves diagnostic accuracy for osteomyelitis in the diabetic foot. ***J Foot Ankle Surg*** 2009; 48: 39-46.
12. Piper KE, Fernendez-Sampedro M, Steckelberg KE, Mandrekar JN, Karau MJ, Steckelberg JM, et al. C-reactive protein, erythrocyte sedimentation rate and orthopaedic implant infection. ***PLoS One*** 2010; 22: e9358.
13. Cha CH, Park CJ, Cha YJ, Kim HK, Kim DH; Honghoon, et al. Erythrocyte sedimentation rate measurements by TEST 1 better reflect inflammation than do those by the Westergren method in patients with malignancy, autoimmune disease, or infection. ***Am J Clin Path*** 2009; 131: 189-194.
14. Castellote Varona FJ. [Extreme elevated erythrocyte sedimentation vs. C-reactive protein.] ***An Med Interna*** 2008; 25: 250-251. Spanish.
15. Bien E, Balceska A. Serum soluble interleukin-2 receptor, beta 2-microglobulin, lactate dehydrogenase and erythrocyte sedimentation rate in children with Hodgkin's lymphoma. ***Scand J Immunol*** 2009; 70: 490-500.
16. Garnavos C, Xirou ST, Nikolatos A, Kanakaris N, Tzortzi P, Balbouzis T, et al. Alteration of body temperature, erythrocyte sedimentation rate, and C-reactive protein after reamed intramedullary nailing: a prospective study. ***J Orthop Trauma*** 2005; 19: 323-328.
17. Andresdottir MB, Sigfusson N, Sigvaldason H, Gudnason V. Erythrocyte sedimentation rate, an independent predictor of coronary heart disease in men and women: The Reykjavik Study. ***Am J Epidemiol*** 2003; 158: 844-851.
18. Wu LA, Tan SL, Wright RS. Kopecky SL, Burritt MF, Santrach PJ, Frye RL. Prognostic value of the erythrocyte sedimentation rate in patients with unstable angina. ***Arch Pathol Lab Med*** 2002; 126: 772.
19. Timmer JR, Ottervanger JP, Hoorntje JC, De Boer MJ, Suryapranata H, Van't Hof AW, et al. Prognostic value of erythrocyte sedimentation rate in ST segment elevation myocardial infarction: interaction with hyperglycaemia. ***J Internal Med*** 2005; 257: 423-429.
20. Swartz JE, Jacobson BF, Connor MD, Bernstein PL, Fritz VU. Erythrocyte sedimentation rate as a marker of inflammation and ongoing coagulation in stroke and transient ischaemic attack. ***S Afr Med J*** 2005; 95: 607-612.

Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.