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ALTERATION IN THE ERYTHROCYTE SEDIMENTATION RATE IN DENGUE PATIENTS: ANALYSIS OF 200 CASES

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ABSTRACT

A study of the erythrocyte sedimentation rate during the first hour (ESR) in dengue patients would help determine how this parameter is affected by this disease, as well as whether it can be used for diagnosis. Two hundred cases of dengue attended at Sree Balaji medical college and Hospital, Chromepet, Chennai, were included. The ESR values were classified as normal or elevated and compared by gender and clinical form of the disease. Among the 200 cases ESR was normal in 81.25% (n = 162), while in 18.75% (n=38) it was elevated. In 83.92% (n=73) of the male dengue patients ESR was normal, while in 16.08% (n=14) it was elevated. Among female dengue patients, 79.17% (n=89) had normal ESR, and in 20.83% (n=24) ESR was elevated (p=0.05). Among patients with classic dengue, 77.28% (n=137) had normal ESR, while in 22.72% (n=40), it was elevated. Also in 85.81% (n=19) of patients with hemorrhagic dengue, ESR was normal, while in 14.19% (n=03) ESR was elevated (p=0.026). We concluded that ESR was within normal ranges in most dengue cases, independent of gender or clinical presentation. Given the high frequency of normality and the ease of determination of this parameter, ESR data can help in the differential diagnosis of dengue.

KEYWORDS: Dengue fever, erythrocyte sedimentation rate (ESR), diagnosis.

INTRODUCTION

Dengue is an arbovirosis caused by a virus of the genus *Flavivirus*; four serotypes have been identified (Den-1, 2, 3 and 4). This disease is typically found in tropical and subtropical regions, where environmental and socioeconomic conditions favor the development of this vector, the mosquito *Aedes aegypti*. It reproduces in clean still water; this fact is crucial for control strategies, since no effective dengue vaccine is available. Dengue can be presented in various clinical forms, varying from oligosymptomatic forms to serious forms, such as dengue shock syndrome.[4,7]

Erythrocyte sedimentation rate (ESR) during the first hour is a simple and low cost exam. It measures the rate of sedimentation of erythrocyte of a blood sample in a graduated test tube maintained in a vertical position during one hour. Though it is unspecific, this test has been widely used for the documentation of inflammatory, infectious and neoplasic processes and in the accompaniment of various pathologies, especially in regions with limited resources. The erythrocyte sedimentation rate can also be high in pathologies such as anemia, macrocytosis, diabetes mellitus and hypothyroidism, and as part of physiological processes, such as gestation, menstruation and aging. This justifies its being designated an nonspecific exam. Consequently, it is recommended that ESR not be interpreted as a diagnostic exam, but rather as an auxiliary tool to anmnese, physical exams and other complementary exams.[8-11]

Given the fact that ESR is maintained within normal limits in dengue patients, during epidemics, this easily-performed exam could become an important tool to help confirm clinical suspicion of dengue. During the fever stage of dengue, various bacterial infections can be part of the differential diagnosis; in this case erythrocyte sedimentation rate is quite useful, because the values remain unaltered in dengue patients.[12] Consequently, given the effect of dengue on morbidity and mortality and the impossibility of a more complete investigation when there are many suspected cases, ESR would be useful to help diagnose with diagnosis whenever this disease is suspected.

MATERIAL AND METHODS

We examined the speed of erythrocyte sedimentation rate (ESR) during the first hour in 200 patients suspected of having dengue, attended at SBMCH, Chromepet, Chennai, from May 2016-July 2016. These patients were initially examined in the clinic (anamnesis and a physical exam) and then included in the following laboratory protocol: the symptomatic patients attended from days

one to five after the symptoms began were referred for a full hemogram, aspartate transaminase (AST) and alanine transaminase (ALT) concentrations and ESR, along with virus isolation.The exams solicited for the symptomatic patients attended from days six to nine included a complete hemogram, along with AST, ALT and ESR dosages. Serologies for IgM and IgG ELISA for dengue were requested for patients who were asymptomatic from day 10 on. This protocol improved participation of the patients and successfully confirmed the diagnosis of dengue. After these routine procedures, the patients continued to be attended in the ambulatory clinic or were hospitalized, depending on their clinical manifestations.

Case Definition

Inclusion criteria were patients with symptoms and laboratory data compatible with dengue (fever, head ache, myalgia, arthralgia, prostration, retro-orbital pain, nauseas, vomiting, leucopenia, hemoconcentration and thrombocytopenia) and IGM ELISA serology solicited after day 10 of the initiation of symptoms, positive for dengue. Classification as hemorrhagic dengue was made according to criteria of the World Health Organization (WHO), though with some adaptations, these being: platelet counts $\leq$ 100,000/uL, positive loop test and/or signals of spontaneous bleeding and hemoconcentration, the latter defined based on a hematocrit (Ht) > 45% in men, H>40% in women and > 38% in children.[13] The criterion of a 20% increase over a previous value was not used, due to the impossibility of making comparisons, because most patients had no previous hematocrit data, besides the impossibility of doing serial hemograms because of the large number of patients.

Sample Description

From March to May 2016, 250 patients suspected to have dengue were attended at the SBMCH, Chennai. Among these, 25 patients did not return for serological confirmation, 25 tested negative for dengue by IgM ELISA and 25 arrived at the ambulatory only nine days after disease symptoms began. Consequently, 200 confirmed cases of dengue met the criteria for our study.

The ESR exam was done using the Westergren method; 1.6 mL of a mixture of venous blood was mixed with 0.4 mL of 3.8% sodium citrate solution in a graduated transparent tube with an internal diameter of 2.5 mm. The zero mark of the tube was at the upper extremity, 200 mm from pipette tip. The tube was then placed in a vertical position for one hour. A reading was made of the height of the plasma column that formed at the upper part of the pipette, expressed as millimeters per hour (mm/h).[10] The reference values vary according to age and gender (Table 1).

Table 1. Reference values for ESR.

Age	Male	Female
0-50	<15 mm/h	<20 mm/h
51-85	<20 mm/h	<30 mm/h
>85	<30 mm/h	<42 mm/h

Source: Sox H.C., Liang M.H. The erythrocyte sedimentation rate: guidelines for rational use. Ann Int Med 1986;104:515-23.

Based on Table 2, the ESR values were classified as normal, lower than or equal to the reference value, or above the reference value. Using this classification, the ESR profile of dengue was examined based on the following: 1-The sample was divided by sex, with later classification of ESR; 2- The sample was divided according to clinical presentation, as classic dengue or hemorrhagic dengue, and then classified according to ESR; 3- After grouping by sex, a new division of these subgroups was made according to clinical presentation, and then according to ESR. We used the chi-square test to test for differences between proportions in the various comparisons.

Table 2: Patients attended at SBMCH, Chromepet, Chennai from May 2016-July2016.

Total no. of suspect cases attended at SBMCH	250 Cases
Patients who did not return for serological confirmation	25
Patients with negative IgM ELISA serology for dengue	25
Patients who first came to SBMCH after 9th day of disease	25
Confirmed cases of dengue included in our study	200

Table 3: ESR profile in dengue patients, compared by gender, clinical presentation and interactions between these two factors.

	Normal ESR	**High ESR**	**Total**
IgM positive for dengue	81.25%(n=162)	18.75%(n=38)	100%(n=200)
Male	83.92%(n=73)	16.08%(n=14)	43.77%(n=87)
Female	79.17%(n=89)	20.83%(n=24)	56.23%(n=113)
Classic form	77.28%(n=137)	22.72%(n=40)	88.91%(n=177)
Hemorrhagic form	85.8%(n=19)	14.19%(n=03)	11.09%(n=23)
Male+C.D.	77.98%(n=62)	22.02(n=17)	90.52%(n=79)
Male+H.D.	89.86%(n=07)	10.14%(n=01)	9.43%(n=08)
Female+C.D.	76.59%(n=75)	23.41%(n=24)	87.78%(n=99)
Male+H.D.	81.76%(n=11)	18.24%(n=03)	12.22%(n=14).

RESULTS

Table 2 shows the groups of patients in our study.

The sample of 200 patients included 43.77% (n=87) males and 56.23% (n=113) females. When we grouped the patients by clinical presentation, we found 88.91% (n=179) with classic dengue and 11.09% (n=21) with hemorrhagic dengue. The mean age of the patients in our sample was 36.5 (+/-14.4) years. None of patients died during this period.

Among the patients with dengue 81.25% (n=162) had normal ESR and 18.75% (n=22) had high ESR (Table 3). Among male dengue patients, 83.92% (n=73) had normal ESR and 16.08% (n=13) had high ESR. Among female patients, 79.17% (n=89) had normal ESR and 20.83% (n=24) had high ESR. There was a significant tendency towards normal ESR (chi-square test, $p < 0.05$, Table 3).

When we grouped the patients according to clinical presentation, we found normal ESR levels in 77.28% (n=137) and high ESR in 22.72% (n=40) of the patients who had classic dengue. Among patients with hemorrhagic dengue, 85.81% (n=20) had normal ESR and 14.19% (n=3) had high ESR (p=0.026, Table 3).

When we analyzed the effects of gender and clinical presentation together, we observed that 76.59% (n=76) of the female patients with classic dengue had normal ESR, while 23.41% (n=23) had high ESR. Among female patients with hemorrhagic dengue, 81.76% (n=11) had normal ESR, while 18.24% (n=3) had high ESR. This difference was not significant (chi-square test, p=0.31, Table 3). Among male patients with classic dengue, 77.98% (n=62) had normal ESR and 22.02% (n=17) had high ESR, while among male patients with hemorrhagic dengue, 89.86% (n=7) had normal ESR, and 10.14% (n=1) had high ESR (p=0.035, Table 3).

DISCUSSION

The erythrocyte sedimentation rate (ESR) is a complementary exam that initially was developed to help diagnose pregnancy; currently it is more commonly used in Rheumatology, and it has become important for the diagnosis and accompaniment of diseases such as rheumatic arthritis, systemic lupus erythematosus and rheumatic disease.[8,10] The ESR exam is a low-cost and sensitive, though unspecific test for documenting inflammatory, infectious and neoplasic processes.[10] The speed at which erythrocyte sediment in the tube, affecting ESR values, depends on the volume and the morphology of the red blood cells and of the plasmatic proteins, which can be classified according to the following scale of values: fibrinogen (10), beta-globulin (5), alpha and gamma-globulins (2) and albumin (1). Consequently, pathologies that include reductions in plasmatic proteins, rigidity and cell morphology alterations, such as sickle cell anemia, hereditary spherocytosis and hypofibrinogen syndrome, and reduced erythrocyte sedimentation; consequently, ESR values generally are within normal limits.[8,10,11] On the other hand, diseases or physical alterations that present with hemodilution and increased asymmetric, high molecular weight plasma proteins, such as alcoholism, multiple myelomas and lymphomas, generally result in above-normal ESR values. This is because these plasmatic proteins bind to the cell membrane, reducing the repulsion potential between the erythrocytes (denominated zeta potential) facilitating the piling up and adherence of erythrocytes, named erythrocytes in *rouleaux*.[8,10]

In cases of dengue, the principal characteristic of the disease is the state of hemoconcentrarion of the patient. After being bitten by the female *Aedes aegypti* mosquito, the dengue virus is inoculated into the individual, initially infecting the histiocytes of the skin; then, they begin to multiply in local lymph nodes, in the smooth and striated muscle and in the fibroblasts. After this initial multiplication, a viremia develops within the plasma or within monocytes/macrophages. In the case of classic dengue, antibodies link to the E protein epitopes of the viral envelope by complement fixation or by blocking the receptors with viral neutralization. In cases of hemorrhagic dengue, the massive penetration of virus into the mononuclear phagocytic system provokes liberation of chemical mediators that stimulate basophils to secrete large quantities of histamine, activating the complement system and coagulation mechanisms. This increased liberation of histamine and activation of the complement system and of the coagulation mechanisms are responsible for diffuse endothelitis, which leads to increased vascular permeability, followed by plasma leakage to the third space, characterizing hemoconcentration in the patient.[4,6,13-15]

In a study of 180 patients with dengue hemorrhagic fever, it was observed that 77% had normal ESR (up to 20 mm/h) and 15% had slightly accelerated ESR (from 21 to 30 mm/h). Another 8% had a ESR of from 31 to 49 mm/h. The authors attributed this to the hemoconcentration and hypoalbuminemia found in dengue patients, as a consequence of the leaking out of plasma.[9] The blood of a healthy individual consists of approximately 60% plasma and 40% blood cells (erythrocytes, leucocytes and platelets), varying physiologically with sex and age.[16] Given this information, since plasma leakage leads to hemoconcentration, the blood of dengue patients has a proportional loss of plasma and blood cells, that is, while on one hand the relative percentage of blood cells increases, on the other hand the proportion comprised of plasma decreases. Consequently, when dosing ESR by the Westergren method, the reading of the column of plasma will be reduced; since this corresponds to the ESR value, the latter will be within normal limits.[9,10,17] Additionally, when there is hemorrhaging, the ESR values are also normal because of the hypophibrinogenemia in these patients. Mean ESR levels

in cases of shock were found to be 7.63mm/h, while in the other cases it was 13.87mm/h, showing that ESR is lower in more serious cases.[9] In this same study, the ESR values of 70 dengue patients were compared with those of patients affected by other viral and bacterial infections. The mean ESR in dengue patients was 10.71mm/h; in the group of patients with other viral infections it was 20.46mm/h, and in patients with bacterial infections, it was 34.81mm/h. Based on these results, we can see that ESR is an exam that should be interpreted within context, and never as isolated information; it can helpful in the initial diagnosis of dengue, principally in the fever phase, helping in differential diagnosis compared to bacterial diseases.[9]

In another study made by our group in Campos dos Goytacazes-RJ, differential diagnosis of dengue from anicteric leptospirosis was found to important, due to similar clinical manifestations. In the cases of anicteric leptospirosis, 88.89% of the patients had ESR values above 40 mm/h.[12] Comparing this information with what we found in our study, we can see that ESR behaves differently in these two pathologies, making it a useful tool for differentiation.

During our study, we found that a small percentage of patients had ESR values above the reference values; generally this was because these patients had diseases associated with dengue, most commonly, urinary infections and anemia. ESR was elevated in urinary infections due to the greater production of proteins by the liver in the acute phase and the consequent increased aggregation of erythrocytes, accelerating their sedimentation. It is a valid conclusion that the ESR value is proportional to the intensity of the inflammatory response. In the case of anemia, the sedimentation of erythrocytes is facilitated by a reduction in the number of erythrocytes relative to the volume of plasma.[8,10,11]

Among 200 cases that we studied, we observed that the ESR values in dengue patients, independent of the clinical form, were within normal limits for a great majority of the patients, especially in male patients (83.92%), when compared to female patients (79.17%). Based on analysis of ESR according to clinical condition, we observed that in 85.81% of patients with dengue hemorrhagic fever, the ESR values were within normal limits, while among the patients with classic dengue, the ESR values were normal in 77.28%. Similar to what has been found in other studies, we attribute these findings to the degree of hemoconcentration, low levels of albumin and fibrogen and the finding of disseminated intravascular coagulation in patients with dengue hemorrhagic fever, the main factors responsible for the decrease in ESR, especially in the more serious cases.[8,9] Another point that should be emphasized is the fact that hemoconcentration is translated in the laboratory as elevated hematocrit values. Martins et al. observed that the erythrocyte sedimentation process needs a longer time to occur when hematocrit levels are high (hemoconcentrated) and faster (greater sedimentation rate) when the hematocrits were small (hemodiluted).[17]

Analyzing the ESR values by clinical manifestation in males and females, we observed that ESR levels were normal in most patients of both sexes. The percentage of male patients with normal ESR values was greater than for female patients. We believe this to be a consequence of more common bacterial urinary infections and anemia in female patients; these are pathologies that increase ESR. Also, women normally have greater rates of hemosedimenation.[8,10]

CONCLUSIONS

Based on our results, we conclude that ESR is within normal limits in most cases of dengue, independent of its clinical manifestation; this is mainly due to the hemoconcentration found in dengue patients, along with hypoalbuminemy and hypofibrinogenemia This contrasts with the ESR profile in patients with bacterial diseases and should be incorporated into diagnostic exclusion criteria for dengue.

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