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Study of Serum Protein Gel Electrophoresis Pattern in Relation to Elevated Erythrocyte Sedimentation Rate.

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ABSTRACT

ESR is the most frequently used laboratory test in cases of acute phase or chronic inflammatory conditions. In these conditions, there are consequent changes noted in the serum protein fractions which are studied with the help of electrophoresis. It is seen that the ESR is greatly influenced by the extent to which the red cells form rouleaux. The rouleaux formation and red cell clumping are mainly controlled by the concentrations of fibrinogen and other acute phase proteins; e.g. haptoglobin, ceruloplasmin, alpha-1 antiglycoprotein, alpha-1 antitrypsin and C-reactive protein. Rouleaux formation is retarded by Albumin and accelerated by raised concentrations of serum Globulin or unusually altered Albumin:Globulin (A:G) ratio. This study is done in view of determining the electrophoretic changes occurring in serum protein levels in cases of elevated ESRs more than 30mm/ first hr.

Keywords: ESR, Get electrophoresis, Serum protein, hemoglobin

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INTRODUCTION

The Erythrocyte Sedimentation rate (ESR) is a relatively nonspecific test that is frequently ordered during the diagnosis and monitoring of disease. Several factors influence the sedimentation rate [1]. Disease-related factors that affect the ESR include the plasma immunoglobulin and fibrinogen concentrations, and the presence and degree of anemia. Factors unrelated to disease process that may affect ESR values include age, sex and drug therapy[2].

The erythrocyte sedimentation rate is greatly influenced by the extent to which the red cells form rouleaux, which sediment faster than single cells. The rouleaux formation and red cell clumping are mainly controlled by the concentrations of fibrinogen and other acute phase proteins, example, α1-antitrypsin, haptoglobin, ceruloplasmin, α1-acid glycoprotein and C-reactive protein. Rouleaux formation is retarded by albumin, with raised concentration of serum globulin and unusually altered albumin:globulin ratio (A:G)[3].

Serum contains over hundred individual proteins, each with a specific set of functions and subject to specific variation in concentration under different pathologic conditions [4]. Since the introduction of moving-boundary electrophoresis by Tiselius [5] and the subsequent use of zone electrophoresis, serum proteins have been fractionated based on their electrical charge into five classical fractions: albumin, alpha1, alpha2, beta and gamma proteins. Each of these electrophoretic zones (except for albumin) normally contain two or more components. Approximately fifteen serum proteins have been studied extensively because they can be measured easily [6].

Thus, erythrocyte sedimentation rate is the most frequently used laboratory test in cases of acute phase or chronic inflammatory conditions along with consequent changes noted in the serum protein fractions which are studied with the help of gel electrophoresis.

MATERIALS AND METHOD

This study was conducted prospectively in the department of Pathology, A.J Institute of Medical Sciences Laboratory. Blood samples from 100 patients were obtained with erythrocyte sedimentation rate of more than 30mm/first hour. In case of patients with anemia, the ESR of >30mm/first hr, obtained after anemia correction. From these patients, under aseptic precautions, using a citrate containing vacutainer (for ESR) and plain vacutainer (for electrophoresis), 4ml blood was drawn in each and processed for ESR, Total proteins and protein electrophoresis respectively.

Freshly collected serum & serum stored at 2-6 degree were used. 1:7 dilution of serum with buffer solution used and processed with Biotec-Fischer SPE Agarose Gel Kit. Based on the graphs obtained and the values of individual proteins obtained on electreophoresis was documented and correlated with the elevated ESRs using Karl Pearsons correlation & Significance test.

RESULTS

An elevated ESR of 40mm or more at the end of first hour was recorded for 100 patients. Of the 100 patients involved, 68 were male and 32 females. Of the 68 males, 31 (45.6%) patients belonged to age group between 40-59years. (Table1). The mean age of the patients in this study was 45.52 with standard deviation of 14.68years. Therefore, Age ($\bar{x} \pm SD$) 45.52 ± 14.68. The minimum age being 14years and maximum 75years. The largest disease category with raised ESR was neoplastic diseases being 16 cases (23.5%) among males and 2cases (6.3%) among females. Among the 100 cases, with elevated ESRs 85 had ESR in the range between 40-100mm and 20 patients had minimally increased ESR, with values in the range between 40-60mm at the end of first hour. Out of the 15 cases with ESR, of 100mm and more at the end of first hour, 9 cases (60%) had infections and inflammation, 3 cases (20%) were of neoplasm, 1 case (6.67%) psychiatric disorder and 1 case (6.67%) alcoholic liver disease.

It was noted that, out of 100 patients 76 showed, albumin values less than 3.5gm/dl (normal: 3.5-5g/dl). Among these 76 patients, 53 were males and 23 females. Similarly, when the ESR values and albumin levels were taken into consideration, it was noted that majority of these patients with increased ESR had reduced albumin levels (<3.5g/dl) (Table 2). It is seen that, ESR is negatively correlated to total protein and

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their association is significant (p <0.05). Similarly, the hemoglobin and ESR are negatively correlated and this correlation is highly significant (p <0.01).(Table7)

Table 1: Age wise distribution of ESR

Age	No. of females (%)	No. of males (%)
1-19	2	2
20-39	9	19
40-59	14	31
60-79	7	16
Total	32 (32%)	68(68%)

Table 2: ESR distribution in relation to albumin levels

	Albumin		
ESR	<3.5	3.5-5.0	Total
40-100	62	23	85
>100	14	1	15
Total	76	24	100

Table 3: Comparison of Mean age & female sex distribution of cases with elevated ESR.

	Happe et al	KH Costenbader	This Study
Mean Age in years (S.D)	56.4 (13.1)	55.5 (13.9)	45.52 (14.68)
Female (%)	71 (54.6)	42 (76)	32 (32)

Table 4: Broad comparison between commonly diagnosed cases with elevated ESRs.

	Happe et al (n=117)	Cha et al (n=154)	This study (n=100)
Malignancy	8	69	18
Autoimmune disease	51	44	5
Infections & others	58	41	77

Table 5: Comparison showing commonly diagnosed conditions in relation to extremely elevated ESRs (>100mm/at the end of first hour).

	Kirekby&Leren[13] (n=348)	Bottiger&Molin[14] (n=13)	Zacharski&Kyle[15] (n=263)	This study (n=15)
Collagen disease	-	3 (23.07%)	-	-
Infections & inflammatory conditions	90 (25.86%)	1 (6.69%)	66 (25.1%)	9 (60%)
Pneumonia	82 (23.56%)	-	-	-
Malignancy	65 (18.68%)	5 (38.46%)	152 (57.79%)	3 (20%)
Psychiatric disorder	-	-	-	1 (6.67%)
Liver & Gall bladder disease	-	2 (15.38%)	-	1 (6.67%)
Renal disease	60 (17.24%)	2 (15.38%)	22 (8.37%)	1 (6.67%)
Miscellaneous	37 (10.63%)	-	15 (5.7%)	-
No definite diagnosis	14 (4.02%)	-	8 (3.04%)	-

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Table 6: Correlation between ESR and hemoglobin with serum protein levels obtained on gel electrophoresis (Karl Pearsons Correlation Coefficient=r)

	Total Protein (r)	Albumin (r)	Hb (r)	α1globulin (r)	α2globulin (r)	βglobulin (r)	γglobulin (r)
ESR	**-0.239**	**-0.199**	**-0.26**	**0.026**	**0.047**	**0.048**	**0.085**

Table 7: comparison of serum protein electrophoresis in patients with elevated ESRs

	Zacharski&Kyle[15] % with fraction		This study % with fraction	
	Reduced	Elevated	Reduced	elevated
Albumin	82	0	76	0
α1-Globulin	3	53	3	89
α2-Globulin	0.8	54	18	36
β- Globulin	0.8	18	47	27
γ- Globulin	8	31	63	15
Total protein	19	12	44	0

Table 8: Significant Correlation table

	Total Protein	Albumin	Hemoglobin
ESR(r)	**-0.239**	**-0.199**	**-0.26**
Significance	**p < 0.05 Significant**	**p< 0.05 Significant**	**p <0.01 Highly significant**
r^2	**0.057**	**0.039**	

r^2= Coefficient of Determination

Table 9: Correlation between ESR and hemoglobin

	ESR and hemoglobin	
	r	**p**
Pawlotsky et al [17]	**-0.407**	**<0.01**
Present study	**-0.26**	**<0.01**

DISCUSSION

ESR is a non-specific test widely used in the clinical practice [7]. The Westergren method of ESR detection is a manual method which has served the medical community for more than 70 years and remains the "gold standard" reference method [8].

The ESR does not measure an analyte but rather a physical phenomenon that depends on a larger number of variables. In this regard, the Westergren method may be affected by sources of variability, such as dilution of blood with anticoagulants (e.g.Citrate), hematocrit values, the internal diameter and the length of the column, column material, temperature and the time from venipuncture [9].

Many clinical variables may affect the ESR, including age, sex etc. studies done by Happe et al[10]& KH Costenbader[11], also showed close comparative mean age of 56.4years and 55.5 years respectively (Table 3)

It is worth to emphasize that in the present study, 32% of female patients showed elevated ESRs among the 100 patients studied as against the 54.6% & 76% in the other studies. (Table 3). In our study, the highest value of elevated ESR among females, obtained was 105mmat the end of first hour.

Clinically the test is often used to assess the activity of disease and to aid in the interpretation of functional and non-specific symptoms. An abnormally increased ESR is suggestive of organic disease, is a non-specific reaction. Although as an analogous to pulse rate, body temperature and leukocyte count as an index of disease, ESR may be abnormal when these are normal. Using an ESR of more than 30mm at the end of first hour for both males and females [18,19], we found that out of 100 patients, 60% had infection or inflammatory conditions, 18% had tumors, 10% had alcoholic liver disease, 5% had rheumatic diseases, 5% had psychiatric disorders & 2% showed diagnosis of renal diseases. It was in concordance with the study by Happe et al[10] and cha et al[12], in which they pointed out that majority of cases with elevated ESRs at the end of first hour were related to infections and other inflammatory conditions.(Table 4).

There are various diseases which can cause an ESR of 100mm or greater at the end of first hour. In this study, out of 100 patients with elevated ESRs, 15 patients showed extremely elevated ESRs of >100mm/at the end of first hour. A close comparison is obtained between the present study and the study done by Kirekby & Leren [13], showing that majority of the patients with significantly elevated ESR (>100mm/at the end of first hour) have been diagnosed to have inflammatory condition or infection 60% & 25.86% respectively (Table 5), while malignancy accounts for 20% & 18.68% respectively. Malignancy comprised of 20% among these cases.

The serum protein electrophoresis was done for all the 100 patients. It was noticed in most of the cases, that the levels of total proteins, serum albumin, serum globulin, alpha 1-globulin, alpha 2-globulin, beta globulin, gamma globulin A:G ratio were affected. Correlation was calculated between ESR and all these affected parameters. (Table 6).

It is known that albumin normally disperses rouleaux and thus retards red cell aggregation and hence tends to reduce the ESR. Harris et al. [16]showed that in old aged population the raised ESR was due to reduced albumin, because of which the RBC rouleaux formation is enhanced thus increasing red cell aggregation.

In the present study, serum albumin is consistently reduced without showing even one case with elevated levels. This finding is well comparable with Zacharski& Kyle [15] (Table 7). Serum albumin and total protein fractions showed significantly reduced values with rise in ESR values. This negative correlation between serum albumin and total protein with elevated ESRs were statistically analyzed by using Karl Pearson Coefficient (r). The value obtained were r= -0.1999 & r=-0.239 and the probability test (p) less than 0.05 was a significant finding. (Table 6,7). Using the coefficient of determination(r^2), the proportion of varience of total protein, albumin and globulin were predicted from the ESRs obtained. Our study showed an increased percentage of correlation between total protein and serum albumin levels with the elevated ESRs. This was in close comparison with study done by Cha et al[12].

Studies have shown that in cases of anemia with decreased hemoglobin concentration, there is alteration of ratio of red cells to plasma which encourages rouleaux formation and accelerates sedimentation. In our study, it was noted that, even in the absence of anemia, there was a very high significant correlation between hemoglobin and ESR exists(Table 8). Pawlotsky et al. [17] have showed the correlation between ESRs and hemoglobin levels, and have shown a highly significant correlation between ESR and hemoglobin levels (Table 9).

Thus, ESR is an important laboratory test which can be used as an aid for diagnosing various conditions. This holds good especially, in condition where the ESR is elevated, more than 100mm/first hour. The erythrocyte sedimentation rate is affected by reduction in the hemoglobin concentration, and in this study, we have proved a very significant inverse correlation between ESR and hemoglobin.

1`qdfs Changes in ESR in acute or chronic conditions leads to changes in the serum concentration pf acute phase proteins, with albumin acting as a negative phase protein. Thus, this study gives an overview that with an elevation in ESR, there is a significant decrease in the total serum protein and serum albumin

concentrations or vice versa. Along with this, there is subsequent increase or decrease in all the individual globin fractions, depending on the underlying diseased conditions.

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