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Effect of different types of anticoagulants and storage period on the erythrocyte sedimentation rate in healthy and unhealthy people.

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Abstract

Objective: This study was designed to investigate the effect of different types of anticoagulants; (Ethylene Di-Amine Tetra-Acetic Acid (EDTA), Tri-sodium citrate salt (TSS) and heparin), and period of blood storage on the erythrocyte sedimentation rate in both healthy and unhealthy contrasting genders.

Methods: The samples were divided into two main groups: males and females. Each major group was further divided into two subgroups. For each secondary group, two tests have been performed, immediately and after 24 hours using three different types of anticoagulants. These tests were done on sample hundred and twenty (120) subjects and their ages were 35-45 years. Westergren method was used to measure Erythrocyte Sedimentation Rate.

Results: There was a variation in levels of erythrocyte sedimentation rate. The differences were significant ($p<0.05$) in the case of healthy and unhealthy males, and also were significant ($p<0.05$) for healthy and unhealthy females. This study revealed that, there was a decrease in ESR level in case of TSS compared to EDTA and heparin, where their changes were significantly increasing in both healthy and unhealthy people. The results showed decreased levels of ESR after 24 hours of sample storage for all types of anticoagulants used in this study.

Conclusions: The results of erythrocyte sedimentation rate test are different in regard to the types of anticoagulants used. The ESR levels also affected by the time of the examination.

Keywords: Anticoagulants, EDTA, TSS, Heparin, ESR, Westergren method.

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Introduction

The main aim of medical laboratories is the correct performance of analytic procedures that yield accurate and precise results that help correct diagnosis and treatment. An increasing effort has been made by international committees and working groups to support and develop the standard quality for the pre-analytical period involving, the best use of anticoagulants in sample collection [1]. Sera from coagulated blood are the common specimen for clinical biochemistry analysis. So, the plasma should be mixed with an anticoagulant material might be an equally valid specimen and, in certain conditions preferable to serum. In addition, whole blood sample collected with an appropriate anticoagulant is the specimen of choice for blood pH and specimen for serum harvesting but this is not always possible for all patients. Thus, analysis must be performed on plasma with different types of anticoagulants; the most common is EDTA [1-3]. Although ESR increases with age, an ESR value up to 15 mm/h in males and up to 20 mm/h in females is considered normal. The formula of age/2 in males and+10/2 in females evaluates the ESR based on the age is accepted in general [4-6]. ESR is based on *in vitro* sedimentation of erythrocytes. Since most plasma proteins are positively charged, they reduce their drop rate in the plasma by increasing the formation of aggregation and roll and reducing the impulse of erythrocytes. Cholesterol is one of important factors that inhibit the ESR as it alters electrostatic load of erythrocytes [7]. Pathologically and physiologically, the ESR increases with age and usually is a diagnosis criterion for many inflammation diseases [8-10]. If the sick people with unexplained symptoms or impaired condition are suggestive of inflammatory, neoplastic or infectious disease and the physical examinations are not helpful, the ESR levels should be considered [11-13]. The modern methods allowed using blood containing EDTA instead of citrated whole blood and measure both hematologic parameters and the ESR in the same tube and the same time. However, studies are currently being performed to realize which anticoagulant should be used to obtain the optimum results in ESR measurement. Using citrated whole blood in the

method to measure ESR means that the blood must be collected into separate tubes for ESR [14-17].

Materials and Methods

One hundred and twenty samples (120) of healthy and unhealthy people at 35-45 years of age shared in this the study. The healthy and sick volunteers have been diagnosed by specialist physician. In addition, the unhealthy subjects had the type 2 diabetes mellitus. The samples were collected in Baghdad city during February 2019. The groups were divided into two collections, the first one was healthy with various genders and, the second one was unhealthy volunteers. The samples (10 ml from each participant) were collected in plain tubes. Samples were immediately separated into anticoagulant tubes aliquots for two testes (0 and 24 hours) and until analyzed. The level of ESR was determined by using Westergren method. For determining erythrocyte sedimentation rate (ESR), anti-coagulated blood is diluted with 0.85% saline and aspirated into a calibrated tube. The cells are allowed to settle for a period of one hour [18,19].

Statistical analysis

The statistical analysis of data was done by using a one way of analysis ANOVA table. The value of ($p<0.05$) was considered significant for all analyses tests. A statistical analysis was performed by statistical Package for Social Science (SPSS) V22.

Results

Changes in ESR levels using three different types of anticoagulants are given in Tables 1-8. Significantly, both of male and female specimens differed ($p<0.05$) (immediately and after 24 hours from storage).

Table 1. *The means and standard error values of erythrocyte sedimentation rate (mm/h) at different types of anticoagulants in healthy male.*

		N	Mean Std. Error ±	F	p-value
ESR	EDTA	30	7.3333* 1.20185	11.121	0.01
	TSS	30	4.3333* 0.88192		
	Heparin	30	11.6667* 1.20185		

Note: Means there is significant difference in the means at the 0.05 level

Table 2. *The means and standard error values of erythrocyte sedimentation rate (mm/h) at different types of anticoagulants in healthy male after 24 hours.*

		N	Mean Std. Error ±	F	p-value
ESR	EDTA	30	6.3333* 0.33333	54.2	0
	TSS	30	3.0000* 0.57735		
	Heparin	30	9.3333* 0.33333		

Note: Means there is significant difference in the means at the 0.05 level

Table 3. *The means and standard error values of erythrocyte sedimentation rate (mm/h) at different types of anticoagulants in healthy female.*

		N	Mean Std. Erro r±	F	p-value
ESR	EDTA	30	10.6667 1.45297	10.179	0.012
	TSS	30	6.6667* 0.88192		
	Heparin	30	14.3333* 1.20185		

Note: Means there is significant difference in the means at the 0.05 level

Table 4. *The means and standard error values of erythrocyte sedimentation rate (mm/h) at different types of anticoagulants in healthy female after 24 hours.*

		N	Mean Std. Error ±	F	p-value
ESR	EDTA	30	7.6667* 0.33333	27.375	0.001
	TSS	30	4.3333* 0.66667		
	Heparin	30	10.0000* 0.57735		

Note: Means there is significant difference in the means at the 0.05 level

Table 5. *The means and standard error values of erythrocyte sedimentation rate (mm/h) at different types of anticoagulants in unhealthy male.*

		N	Mean Std. Error ±	F	p-value
ESR	EDTA	30	29.3333* 0.88192	41.743	0
	TSS	30	23.0000* 1.000		
	Heparin	30	37.6667* 1.45297		

Note: Means there is significant difference in the means at the 0.05 level

Table 6. *The means and standard error values of erythrocyte sedimentation rate (mm/h) at different types of anticoagulants in unhealthy male after 24 hours.*

		N	Mean Std. Error ±	F	p-value
ESR	EDTA	30	25.0000 0.57735	10.125	0.12
	TSS	30	28.000 1.1547		
	Heparin	30	31.0000* 1.0000		

Note: Means there is significant difference in the means at the 0.05 level

Table 7. *The means and standard error values of erythrocyte sedimentation rate (mm/h) at different types of anticoagulants in unhealthy female.*

		N	Mean Std. Error ±	F	p-value
ESR	EDTA	30	41.6667* 0.88192	16.636	0.004
	TSS	30	37.6667* 0.88192		
	Heparin	30	46.6667* 1.45297		

Note: Means there is significant difference in the means at the 0.05 level

Table 8. *The means and standard error values of erythrocyte sedimentation rate (mm/h) at different types of anticoagulants in unhealthy female after 24 hours.*

		N	Mean Std. Error ±	F	p-value
ESR	EDTA	30	35.6667* 0.88192	37.136	0
	TSS	30	30.0000* 1.1547		
	Heparin	30	41.0000* 0.57735		

Note: Means there is significant difference in the means at the 0.05 level

Discussion

The effect of anticoagulants was assessed via blood analysis of Erythrocyte Sedimentation Rate test. This study was aimed to assess the impact of different coagulants on ESR levels. Our findings display that, there is an elevated level of ESR in EDTA and heparin-treated samples, while the levels of ESR decreased in TSS-treated samples. In the current study, the mean sedimentation of healthy male for TSS was 4.3333 mm/h, whereas it was 7.3333 mm/h, 11.6667 mm/h EDTA and heparin respectively (Table 1). Statistical analysis demonstrated that the variation between values were significant. For healthy male, after 24 hours the values dropped for EDTA, TSS, and heparin with 6.3333 mm/h, 3.0000 mm/h, and 9.3333 mm/h, respectively, as given in Table 2. Statistically, there were significant differences ($p<0.05$) (Table 3). The means values of erythrocyte sedimentation rate were 10.6667 mm/h, 6.6667 mm/h and 14.3333 mm/h for EDTA, TSS, and Heparin respectively. The results obtained from this study showed a decrease in the means values of ESR for healthy female group after 24 hours from drawing the blood for all types of anticoagulant that involved in our study, the changes were 7.6667 mm/h, 4.3333 mm/h, 10.0000 mm/h for EDTA, TSS, and Heparin, respectively, as shown in Table 4. The mean proportion of ESR for unhealthy male group was 23.0000 mm/h for TSS while mean values of the EDTA and heparin were 29.3333 mm/h, 37.6667 mm/h, respectively as given in the Table 5. According to unhealthy male group after 24 hours, this study reported that different anticoagulant causes changing in levels of ESR, the mean value of EDTA became 25.0000 mm/h and that of Heparin became 31.0000 mm/h compared to the TSS level of ESR with using TSS which was 28.0000 mm/h (Table 6). The result of unhealthy female group for EDTA was 41.6667 mm/h while their values for TSS and Heparin were 37.6667 mm/h and 46.6667 mm/h respectively (Table 7). In comparison to the values of heparin after 24 hours from drawing the blood which was 41.0000 mm/h (Table 8), the mean values for EDTA and TSS were 35.6667 mm/h and 30.00001 mm/h, respectively. Blood clots within minutes after collecting. The process can be accelerated by environmental factors, such as temperature, which creates logistical challenges for researchers using the blood ex vivo to support pre-clinical safety studies of novel drug products, as well as for clinical blood tests [20]. The differences in some analysts that induced by anticoagulants have been described in man, dog, cattle, camel, horse, and sheep [21-23]. In the present study, citrate produced lower levels of most analysts than serum. However, it seems that citrate has a more negative effect on ionized than on total calcium concentration. In previous studies, citrate inhibits aminotransferase activity and because it complexes molybdate, it decreases the color yield in phosphate measurements and thus produces low results [24].

Conclusion

In conclusion, this study explains that, the type of anticoagulant and period of storage of blood samples give ESR results for healthy and unhealthy persons with various genders where the results of heparin was the highest one while the TSS was lowest. On the other hand, the results of EDTA were in between.

Conflict of Interest

No conflict of interest was declared by the authors.

Data Availability

All data created during this research is openly available from the University of Raparin/Medical Laboratory Science Department/Research Data Archive at [insert DOI here]. All data supporting this study is provided as supplementary information accompanying this paper. All data is provided in full in the results section of this paper.

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