Deanship of Graduate Studies

AL-Quds University

Comparison Between EDTA and Citrated Blood Using Manual and Automated ESR

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MSc. Thesis

Jerusalem-Palestine

1433 Hijri / 2012 AD

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A thesis submitted in partial fulfillment of requirements for the Degree of Master in Medical Laboratory Sciences/Hematology Track, Faculty of Health Professions – AL-Quds University

1433 Hijri / 2012 AD

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Thesis Approval

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Master thesis submitted and accepted, Date: 15/5/2012.

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Dedication

To my beloved, wonderful parents *Emil & Paulette*

To my beloved sister *Rania* and her wonderful husband *Fadi*

To my beloved little angels *Mireille & Maria*

To my family, friends and colleagues

To all my teachers who have taught me

Rasha Emil George Ghanem

Declaration:

I Certify this thesis submitted for the degree of Master of Medical Laboratory Sciences, Hematology Track is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for higher degree to any other university or institution.

Signed

Rasha Emil George Ghanem

Date: ……../……./…….

Acknowledgement:

First and foremost of all, I would like to express my gratitude to my great God, Jesus and virgin Mary for the power and endurance they gave me throughout my life and studying period.

I want to express my deeply-felt and gratitude to Al-Quds University, Department of Medical Laboratory Sciences represented by all teachers and colleagues, who believed in me, for giving me the opportunity to study my Master degree by joining this program. I am deeply indebted to Dr. Akram Kharroubi, Dr. Mahmoud Srour, Dr. Sameer Barghouthi, Assistant Professor Khalid Younis, and Dr. Sameera Barghouthi for their support, for guiding me and treating me as a daughter.

My deepest gratitude and appreciation is extended to my supervisor Assistant Professor Khalid Younis and my co-supervisor Dr. Mahmoud Srour for their enthusiasm, inspiration and their great efforts for guidance and encouragement

I want to show my appreciation to all my colleagues, Ola, Suhair, Ashraf, Mohammad, Ibtehal, Rozan, Taghreed and all the students in this program who believed in me, also to Mr. Ibrahim Ghannam for his help in statistical work. Also I show my deep thank for Dr. Hussain Hallak and Miss. Suzy Attalh for editing my thesis.

My gratitude is extended to all my colleagues in Bethlehem Arab Society for Rehabilitation “Special Surgical Hospital”, Beit-Jala Governmental hospital for their encourage and help, also I would like to thank all my friends who believed in me and supported me during my study period.

Lastly, and most importantly, my deep thank will be to my beloved family for their love, caring, support, encouragement and for their believe and trust. To them I dedicate this thesis.

Abstract

Erythrocyte sedimentation rate (ESR) is a simple, inexpensive, non-specific test that is used to differentiate diseases with similar symptoms such as myocardial infarction versus angina pectoris or acute from chronic infection or inflammation. International committee for standardization in Hematology (ICSH) considered Westergren manual method (citrated blood) as a reference method. Different manual and automated systems are commercially available and all are claiming correlation/agreement with classic Westergren method. This study aimed to investigate the agreement or consensus between laboratories in performing ESR using manual and automated methods, as well as investigating the effect of the type of anticoagulants (EDTA and citrate) on the ESR results using these methods.

Three ESR methods widely used in Palestine were chosen for this study. These methods were: the Sediplast® tubes (LP Italiana S.P.A./ Italy); the Kima Sed® tubes or Quick method (Vacutest Kima S.r.l. / Italy); and the Monosed® tubes and MICROsed-System® (Vital Diagnostics S.r.l./ Italy). The study population consisted of 105 patients suffering from different diseases and 95 apparently healthy persons (control).

The results obtained from the patients' and control groups showed that there was a large variation in results using different methods (two manual and one automated) and different anticoagulants. Statistical analysis showed that there was no agreement among the different methods or anticoagulants used (r was <0.95 for all methods compared). The mean results of ESR for Quick citrate, Automated citrate and Westregren citrate were 35.3, 29.0 and 39.4 mm/hr respectively while they were for EDTA 50.3, 46.5 and 47.7 mm/hr respectively. In general, the ESR results obtained using EDTA as anticoagulant was higher than those obtained with citrated blood.

The patients group which consists of 105 patients suffering from different diseases, 69 females (65.7%) and 36 males (34.3%). Results obtained from this group showed that there was a variation and inconsistency in results using the different methods and different anticoagulants. EDTA and Na-citrate scatter plots showed that there was a positive correlation among these different systems, but it was worthless as the correlation coefficient was always <0.95 and the slope of the regression line was < 0.9. This means, that there was no agreement at all between the different methods. However, there was a large variation in the results as most of the results were above and below the lines that represent 95% CI for the regression line. Also, many extreme results were present.

For control group, it consists of 95 apparently healthy individuals. It included 26 females (27.4%) and 69 males (72.6%). Results obtained showed that there was a variation and inconsistency in results also. Scatter plots of different methods showed that there was a positive correlation but not to the level of agreement among different methods. Despite this correlation, there was a large variation in the results as most of the results were above and below the lines that represent 95% CI for the regression line. Also, many extreme results were present.

In conclusion, there was a large variation in the ESR results and no agreement among the three ESR measuring methods used in this study. Therefore, we recommend unifying the procedures and methods used by laboratories for performing ESR test at the level of the country or at least, the laboratory should maintain using the same system consistently and the patient is advised to use the same laboratory to monitor his/her status.

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List of Abbreviations:

ACE: Angiotensin-Converting Enzyme.

ACR: American College of Rheumatology.

ASO: Antistreptolysin Reaction.

CAD: Coronary Artery Disease.

CHD: Coronary Heart Disease.

CHF: Chronic Heart Failure.

CI: Confidence Interval.

CRP: C-reactive Protein.

DAS: Disease Activity Score.

ECG: Electrocardiography.

ESR: Erythrocyte Sedimentation Rate.

EULAR: European League Against Rheumatism.

GCA: Giant Cell Arteritis.

HIV: Human Immunodeficiency Virus.

ICSH: International Committee for Standardization in Hematology.

K_3EDTA: Tri-potassium Ethylene Demine Tetra Acetic Acid.

MI: Myocardial Infarction.

MOH: Ministry of Health.

NSAID: Non Steroidal Anti-inflammatory Drugs.

OM: Osteomyelitis.

PMR: Polymyalgia Rheumatica.

r: Pearson Correaltion Coefficient.

RA: Rheumatiod Arthritis.

RBCs: Red Blood Cells.

RCC: Renal Cell Carcinoma.

SA: Septic Arthritis.

SCD: Sickle Cell Disease.

SD: Standard Deviation.

SPF: Serum Protein Fractions.

SPSS: Statistical Package for Social Sciences.

TB: Tuberculosis.

TKA: Total Knee Arthroplasty.

UTI: Urinary Tract Infection.

WBCs: White Blood Cells.

Chapter One: Introduction

1.1. Introduction

Blood is a living tissue which consists of a straw colored fluid medium called plasma in which red blood cells (RBCs) “erythrocytes”, white blood cells (WBCs) “leukocytes” and platelets “thrombocytes” are suspended. The main function of circulating blood is to transport oxygen and nutrients to the tissues and to remove carbon dioxide and waste products, so blood is considered as an inner mirror for our bodies. For blood to perform its function, there are many physiochemical properties that influence this fluid medium such as isotonocity, colloid osmotic pressure and viscosity. All these characteristics may influence rouleaux formation of erythrocytes (stacks of red blood cells). Some factors are for while others are against sedimentation. Those that are for include, changing in erythrocyte plasma ratio, while those that are against include, the negative charge on the RBCs which lead them to repulse (zeta potential) (Emelike *et al*., 2010; McPherson and Pincus, 2007).

Erythrocyte sedimentation rate (ESR), also called “Sed rate determination” (Emelike *et al*., 2010) is a simple, inexpensive, non specific test that is used to differentiate diseases with similar symptoms such as myocardial infarction versus angina pectoris. Also this test may be used to diagnose gout in conjunction with hyperuricemia (classic feature of gout). However, both WBCs and ESR may be elevated due to gout in the absence of infection, while nearly half of the gout cases occur without hyperuricemia. ESR can also be used to differentiate acute from chronic infection and inflammation. Traditionally, this test was used for the diagnosis of rheumatoid arthritis and other collagen diseases such as polymyalgia rheumatica or giant cell arthritis and rheumatic fever. ESR was also shown to be of value in the early diagnosis of infective hepatitis (Wood, 1945; Deshpande *et al*., 1971; Epperly *et al*., 2000). The importance of this test

includes using it as a guide for diagnosis, management and follow-up of specific clinical situations for example increased ESR results over 100 mm/hr in cancer patients usually means that cancer metastasis is present (Koepke, 2002). In addition it has a clinical significance in sickle cell disease (SCD). ESR is usually low in the absence of painful crisis, due to an intrinsic property of sickle red blood cell morphology (Saadeh, 1998), as sickle cells are unable to participate in rouleaux formation, ESR increase during painful sickling crisis and infections. The elevation of ESR during painful crisis is partially related to elevated plasma fibrinogen level, while during infection elevated serum globulin level contributes to high ESR level (Ahmed *et al*., 2000).

In general, ESR test serves as a "sickness index" in conjunction with the patient's clinical history and physical examination findings (Koepke, 2002). Rouleaux formation and RBC clumping are greatly enhanced by acute phase reactants in plasma: fibrinogen, haptoglobin, ceruloplasmin, α1-acid-glycoprotein and C-reactive protein. ESR is increased by immunoglobulins, but decreased by albumin (Abbag and Al Qahtani, 2007).

ESR is a test that measures red cell aggregation of different hydrophilic protein fractions. The sedimentation rate depends directly on the size of aggregates formed (the rate of sedimentation being directly proportional to the square of the radius of the aggregate-Stoke's Law), and the rate at which these aggregates or pseudo-agglutinations are formed (Eastham *et al*., 1958). Correction of sedimentation rate for variations of specific gravity and plasma proteins should be done because according to "Stoke's Law", the speed of fall of spherical body in a liquid medium varies directly with the difference in specific gravity between the body and the liquid. Mean corpuscular hemoglobin concentration was used as an index for cell specific gravity. Due to this, ESR measures the distance that RBCs need to settle under the influence of specific gravity during one hour (Mayers *et al*., 1953). Opposite opinions were present since "corrected" ESR may often be misleading to the clinician as many correction charts were present. Stoke's Law applies only to the velocity of settling of a particle in a fluid

of infinite extension. It does not take into account the effect of the simultaneous presence of other falling particles in close proximity (Rouke and Ernstene, 1929).

The sedimentation of red cells in plasma is complex and consists of three phases, lag, decantation, and packing, which vary greatly and are interrelated in a complicated manner. So the sedimentation time for each phase may vary from patient to another (Eastham *et al.*, 1958; Koepke, 2002). The kinetic process that occurs in erythrocyte sedimentation can be characterized by an S-shaped curve with time. Three clear phases can be described: in *phase 1*, there is a delay in sedimentation during this phase (sedimentation is zero) and this is due to the formation of spheres in equal size. In *phase 2*, the sedimentation continues in a constant rate (linear). This is due to the steady rate of sedimentation in the spheres. While in *phase 3*, sedimentation is complete (Fabry, 1987; Pawlotsky *et al.*, 2004).

Marked rouleaux formation of RBCs in peripheral blood film was responsible for increased ESR results (Hoffbrand *et al.*, 2006). As have been mentioned previously, infections and inflammations increase ESR values. Also, some drugs may cause elevated ESR these include: dextran, methyldopa, oral contraceptives, penicillamine, theophylline and vitamin A. Other medications may cause lower ESR and these include: aspirin, cortisone and quinine (Pagana and Pagana, 2002). As the incidence of mild respiratory infections increase in seasonal changes, this will lead to increased ESR. Smoking also leads to increased ESR. Also, there is a positive correlation between ESR and obesity and independently between ESR and hypercholesterolemia (Pincherle and Shanks, 1967; Sirhindi and Ali, 2007).

Lower ESR can occur when the specimen stands for longer than three hours before the test was performed. Lower than expected results occur in polycythemia vera because of the high RBCs concentration, while higher than expected results can occur in severe anemia because of the low RBCs concentration (Hoffbrand *et al.*, 2006). Also the effects of toxicology had been studied on rats, and it was found that mercury toxicity

decreased total erythrocyte count, so ESR values in these rats increased. This can be explained by the fact that rouleaux formation depends on the total erythrocyte count and the density of the mass increases along with reduced erythrocyte count, so, ESR increases in low erythrocyte count (Agrawal and Chaurasia, 1989).

On the other hand some scientists believed that ESR should not be ordered as it does not measure an analyte but rather a physical phenomenon that depend on large number of variables, its sensitivity and specificity are unsatisfactory, and this could lead to variation in results obtained (Piva *et al.*,. 2001; Jou *et al.*, 2011).

1.2. Biography: History of ESR

Erythrocyte sedimentation rate is the test that is used for the diagnosis, monitoring and follow up of many diseases. Ancient Greek and Roman doctors noticed changes in the sedimentation of blood which occur in various diseases. Medical practitioners described that the red blood clot are covered with a layer of whitish fluid in some diseases, calling it *'crusta inflammatoria'* or *'phlegma'*. William Hewson (1772) and Herman Nasse (many years later) noticed that if blood is without fibrinogen, the sedimentation of red blood cells is slower than in whole blood, but they attached no clinical significance to these observations as reported by Kowalczyk (2006).

In 1893, Professor Edmund Faustyn Biernacki, the polish physician who was born in 1866 in the Kingdom of Poland, observed that blood has different properties in various disorders. By studying red blood cell volume he was able to find out *"spontaneous blood sedimentation"* as he called it.

Professor Edmund Biernacki.

In 1894, he presented his paper *"On the ratio of erythrocytes to plasma and the value of various methods of estimation of total erythrocytes volume"* that led to the discovery of the ESR. Then he proved that the ESR is accelerated in inflammatory diseases such as rheumatoid fever, chronic nephritis, tuberculosis, pneumonia, anemia and cancer. He was also able to prove that the ESR was faster in blood with increased plasma fibrinogen and decreased in de-fibrinated blood (Kowalczyk, 2006).

In 1896, he invented a sedimentation cylinder and described a method for performing the ESR test. In his experiments, the amount of blood used was (1, 5, 25 and 100 mL) and varying the time of reading, starting from 30 minutes, through 60, 90 and 120 minutes up to 24 hours, and using different cylinders. He recommended that the blood should be taken from the median cubital vein with a syringe. The procedure that he used to perform this test was that a sample of 1 mL blood was mixed with 2 mg of sodium oxalate to prevent coagulation and placed in a 20 mm calibrated cylinder at a room temperature of 17 – 19°C. The test should be read at 30 and 60 minutes. In 1897, he published his observations as "Spontaneous blood sedimentation as a method for scientific, practical and clinical investigation" (Kowalczyk, 2006). Subsequently Fahraeus, the Swedish physician, re-discovered the ESR test in 1921 (Koepke, 2002). Fahraeus and his scholar Westergren popularized this test (Arkin and Aklin, 2007). So it is often referred to as Biernacki or Fahraeus– Westergren test.

1.3. Principle of ESR

The ESR test is the distance in millimeters that RBCs need to settle in a vertical tube during a specific period of time which is usually one hour, under the influence of specific gravity between RBCs and plasma (Mayers *et al.*, 1953; Koepke, 2002).

Some literature recommended that the RBCs–plasma meniscus must be read every 5 minutes and the data read within one hour must be recorded (Koepke, 2002). Luckily for laboratory technologists, this method is not very popular and is no longer used.

1.4. Reference Ranges for ESR

As ESR is sex, age and blood storage dependent test, the reference range for this test for males is up to 20 mm/hr and for females up to 25 mm/hr "Westergren method" (Pagana and Pagana, 2002; AlFahdi and Al-Awadhi, 2005; Arkin and Aklin, 2007). According to Hilder and Gunz (1964), the mean results for ESR in males rose from 3.1 mm/hr at ages 18-30 years to 5.3 mm/hr at ages over 60 years, and in females from 5.1 to 9.4 mm/hr. So, they concluded that ESR results of normal individual increases with age. Their results came to support previous studies that demonstrated that ESR increases normally after age of 60 years. In 1960, Dawson considered the reference range for ESR to be 0-10 mm/hr for males and 0-15 mm/hr for females while Ansell and Bywaters (1958) considered results that did not exceed 20 mm/hr to be normal. Also, they came to the same conclusion that people older than 60 years old have higher ESR results than younger age grouped people, all these results had been repoted by Hilder and Gunz in 1964.

1.5. Methods Used to Measure ESR

Manual ESR test is done on blood samples that are on standing positions (Wiwanitkit, 2001). Disposable ESR tubes are made from clear plastic tubes that have plastic lid and contains 0.5 mL of 3.8% sodium citrate with preservatives added to prevent mold. The preservatives are a mixture of 0.2 gm propyl-hydroxybenzoate and 1.5 gm of methyl hydroxybenzoate in every liter of citrate solution. When comparing the ESR results (Westergen method) using plastic containers with and without preservative with glass tubes with and without preservative, there was a recordable difference between using

citrate with and without preservative. The use of the disposable tubes produces a slight inhibitory effect on ESR results when comparing with glass tubes irrespective to the type of citrate solution, this is due to the variation in pH (Sterndale, 1963). The effect of the materials used in manufacturing the ESR tubes had been studied. The glass VACUETTE® tubes versus the plastic VACUETTE® tubes and the anticoagulant used in both was trisodium citrate; the results obtained from both tubes show no significant statistical difference (Evaluation of VACUETTE® Plastic ESR Tube with the manual closed ESR measurement, 2011).

1.5.1. Westergen Method

Westergren manual method is considered *as the reference method*. According to the International Committee for Standardization in Hematology (ICSH), Westergren manual method was recommended in 1977 to be adapted worldwide (Alexy *et al.*, 2009). The original ICSH reference method for measuring ESR was based on using diluted blood samples (4 volumes of blood plus 1 volume of 3.8% sodium citrate) in open ended glass tubing of 300 mm in length, mounted vertically in a rack or stand. However, ICSH introduced a standardized method as an alternative and potential replacement for the reference method (ICSH, 1993).

Figure 1.1: Westergen manual method.

The traditional Westergren method was designed in such a way that included mechanical/mouth suction to fill the pipette with the sample. Due to the increased

awareness to biohazard risk inherent in performing the traditional Westergren-based method, the ICSH had proposed a closed system which is now recommended as an ICSH standardized method (ICSH, 1993).

In 1988, the ICSH recommended to perform ESR on undiluted blood samples (hematocrit 0.35 or less) under standardized conditions in a Westergren open ended glass pipette. The modified method includes using EDTA blood instead to citrated samples (dilution less than 1%). This method was recognized by ICSH as ***a new standardized method***, because some test systems do not employ the traditional 60 minutes for reading the results. Test systems may incorporate a mathematical adjustment for initial height of the blood column, hematocrit of the sample, temperature, or duration of sedimentation. The results from both methods should be expressed as ESR = (undiluted) x mm. While for comparison of the results obtained from the traditional method using diluted blood, a correction formula can be applied: [diluted blood ESR mm = (undiluted blood ESR mm x 0.86) –12] (ICSH, 1993).

The Westergren method used blood that was obtained from clean vein puncture; the 4 volumes of blood were mixed with 1 volume of filtered solution of sodium citrate. At room temperature, the test must be set up within 4 hours of vein puncture (Lewis, 1973). Blood samples can be stored for more than 4 hours at 4 °C, but any such longer period of storage must be validated. Blood samples obtained from hyperlipidemia or hyperbilirubinemia, were unsuitable for testing. As the ICSH standardized method was performed on undiluted blood compared with most routine methods which use diluted blood, it was necessary to apply a correction to the standardized method for lack of dilution. Absence of dilution in the standardized method permits the detection of errors in the volume or quantity of diluents used in routine methods. So that, the ICSH standardized method could be used as a quality control procedure. For verification, it was important to select an EDTA sample that had a hematocrit of 0.35 or less. If the blood samples show evidence of agglutination they should be discarded (ICSH, 1993).

Citrated blood samples should be mixed thoroughly by gentle repeated inversion and a clean dry standard Westergren tube is filled and adjusted to the *zero* mark. The tube is then placed in a strictly vertical position under room temperature conditions (18-25°C), not exposed to direct sunlight and free from vibrations and draughts. The tube that was used in this method is called Westergren tube which is a straight pipette that is 300 ± 1.5 mm, the tube bore 2.55 ± 0.15 mm and the uniformity of tube bore ± 0.05 mm (Lewis, 1973; ICSH, 1993).

The tube must possess the following markings: the inscription *'Westergren'*, the scale graduated in mm, extending over the lower 200 ± 0.35 mm. Maximal tolerated error between two subsequent (mm) markings is 0.2 mm. Graduations should be fine, clearly marked lines of uniform thickness (0.2 mm) and numbered from 200 at the bottom up to 0 in steps of 10 or less (Lewis, 1973). The reagent used in these tubes as anticoagulant and diluents solution is 0.105 molar solution (range 0.10-0.136) of sodium citrate, which is added to one liter of distilled water in a sterile glass bottle then it is filtered and kept refrigerated without preservatives (McPherson and Pincus, 2007). Variables that affect Westergren method are: specimen collection, time and temperature of specimen storage, sedimentation equipment, and methodological variables (Clinical and Laboratory Standards Institute, 2000).

1.5.2. Wintrobe Method

Wintrobe method was one of the techniques used to perform ESR. Its tubes were 3 by 1/2 inches (7.5 by 1.25cm.) that were graduated with a mark at 2 mL and were coated with 4 mg of Heller and Paul's anticoagulant mixture (three parts ammonium oxalate and two parts potassium oxalate) and stopped with rubber stoppers. The results obtained when comparing Westergen and Wintrobe methods on female patients suffering from pulmonary tuberculosis, indicated that Westergen method was more reliable than the Wintrobe method (Gilmour and Sykes, 1951). In Wintrobe method venous blood was collected in 2.5 mL tubes that were anticoagulated. The mixture was then stirred

slightly and placed in the Wintrobe test tube that were filled to the zero graduation mark without any air bubbles and left for one hour at 25°C and the results were expressed in mm/hr. (Miao, 2002). This method is no longer used.

1.5.3. Automated ESR Devices

ESR is a commonly ordered laboratory test that has limitations which include: slow turnaround time since it requires one hour for getting the results; is the use of an open tube system (biohazard risk) and inaccurate results occur if the tube is not in a vertical position or if there is a delay or inaccurate reading for the results (Alexy *et al.,* 2009).

Many automated systems have been developed but these also require 60 minutes to obtain the results and are expensive. On the other hand there is a device that utilizes a closed U-shaped disposable test kit *(Rheolog™, Rheologics, Inc., Exton, PA)* and it is able to monitor the position of RBCs columns in two vertical tubes. To predict one hour ESR values, data collected during the third and final minute of viscosity testing are used and this new parameter is known as Sedimentation Index (SI). The calculation of SI is based on determining the position of the RBC-plasma interface in both vertical tubes. Thus zero flow through the small-bore tube is not absolutely essential. Performing this test is relatively inexpensive, requires no special training and has minimal biohazard risk (Alexy *et al.,* 2009).

Another automated technique used for ESR involves photography of the ESR tests using “Polaroid MP-4 camera with Tominon f4-5, 135 mm lens and Copal shutter” that measures ESR exactly 1 hour after being set up. In such a way that the photographs are easily and rapidly obtainable when required and the results can be easily read. The timing device of this technique consists of a shutter-activating system, which uses a simple main-driven timer (ORMON-Type STPNH, 72 min) which switches on an electric motor after a pre-set time. The motor has a large reduction gear box which gives a shaft speed of one revolution per minute. So, the adequate torque generated from the

low-power motor activates the camera shutter. The electrical circuit of this technique consists of two parts: one that set up the procedure and the other that control the timer and motor operation (King *et al.,* 1980).

ESR analyzers manufactured by Diesse Diagnostica Senese/Italy were the Ves-matic Senior (60 samples) and the Ves-matic Junior (20 samples). Diesse Ves-matic ESR System (DVM) provides ESR results within 20 minutes. This system improves efficiency in determining the ESR by standardization of dilution of blood with anticoagulant diluents, standardization of the degree of mixing and therefore red cell aggregation at the start of the test, and adjustment for temperature. Small volume of blood is required, approximately one mL of blood (samples can be 3 mm over or 12 mm under the reference line marked on the tube). The tubes are then placed in the analyzer, which holds the tubes at an angle of 18°. After 20 minutes, a photoelectric cell automatically records the ESR value in millimeters per hour. Faster sedimentation is possible because of the Boycott phenomenon (streaming down the wall of an angled tube so that red cells sedimentation occur more quickly than standing in a vertical position). The technical advancements of this system include the specially molded plastic collection tubes (Vacu-tec) that contain 0.25 mL of a 0.105-mol/L sodium citrate solution and are directly filled through vacuum vein puncture technique (Caswell and Stuart, 1991).

The newer system is the 4-sample Diesse Mini-Ves (DMV) ESR analyzer (Diesse Diagnostica Senese/Italy) that was designed to provide more flexibility in clinical laboratories. Also, it can simultaneously evaluate up to 4 samples, even when started at different times (Koepke *et al.,* 1990). The 4-sample DMV analyzer is precise and provides results comparable to those of the Westergren reference ESR method (Happe *et al.,* 2002).

Test 1 from (Alifax, Padova, Italy) is one of the automated closed techniques that are used to determine the length of sedimentation reaction in blood in a standard-size

primary tube with a perforating stopper. This technique used tubes that are placed in specific rack and by using a closed aspiration needle, the blood is directly drawn, centrifuged at about 20g for 2 minutes and the sensing area temperature is maintained at 37°C. This system uses an infrared ray microphotometer with a light wavelength of 950 nm and performs 1,000 readings during 20 seconds (Cha CH *et al.,* 2009). Also, it provides precise results at mid and high ESR values, at low ESR results precision decreases (Plebani *et al.,* 1998).

StaRRsed (Vital Diagnostics/Italy) is another automated system for ESR based on the Westergren sedimentation technique. Although the method is slightly modified, it uses 3 mL of EDTA blood. The instrument uses a vacuum pump to aspirate 1.6 mL of sample and dilute it with 0.4 mL of 3.8% Na-Citrate solution. After that, the diluted sample is aspirated to Westergren pipette and it uses the optical density at 950 nm for measurement exactly within 30 min. There is a need for a correlation curve to transform the results into 60 min and for the correction of temperature to 18ºC. The instrument gives the results after performing all the corrections (Horsti *et al.,* 2010).

Centrifugation method is one of the procedures that are done using EDTA-anticoagulated blood to obtain ESR results. The blood should be mixed by inversion at least 15 times before 25 µL is drawn (by capillary action) into a heparin-coated, self-seal capillary analysis tube (according to the manufacturer, *ESR STAT PLUS, HemaTechnologies, Lebanon, NJ*). The tubes are placed in ESR STAT PLUS and are spun at 1,500 to 2,000 rpm for 3 minutes. According to the manufacturer's operator manual, an infrared laser tracks the erythrocyte-plasma interface and makes multiple measurements, from which the linear portion of the sedimentation curve is identified and used by the software algorithm to determine the ESR result (mm/hr). This method requires a minimum of 15 mixing intervals, followed by a 5-minute limit (maximum) before drawing into the capillary tube. If there is a failure to follow the procedure strictly, the process must be repeated with another capillary tube, failure to follow the procedure results in higher intra-assay variation. When comparing this method with the Westergren method, the ESR results obtained from the centrifugation method exceeded

the Westergren by a small but statistically significant amount in the lower range (0-20 mm/hr), where most normal ranges fall (Shelat *et al.*, 2008).

Finally, some automated systems used electrical impedance technique to measure ESR which is primarily determined by plasma resistance (Rp), cell interior fluid resistance (Ri) and cell membrane capacitance (Cm). The membrane capacitance from patients with high ESR levels had been found significantly higher than that of blood with low ESR levels. This indicates that the components of plasma elevate the capacitance (Zhao and Lockner, 1993).

1.6. Factors That Influence ESR

ESR is influenced by many factors and their influences on this test have been understood in the early decades of the last century. These factors include forces that are either for or against sedimentation. Those that are against sedimentation include the negative charge on the RBCs surface which causes repulsion between the RBCs (zeta potential). The decrease in zeta potential which occurs due to asymmetrical composition of protein molecules (fibrinogen, β-globulin, α- globulin and γ- globulin) promotes the formation of rouleaux (McPherson and Pincus, 2007). Fibrinogen and α-2 globulin are associated with tissue repair (Smith, 1936), while γ-globulin is an antibody containing fraction of protein (Mayers *et al.*, 1953).

These proteins vary in their capacity to reduce zeta potential, on a scale of 1-10: fibrinogen 10 “acute phase reactant”, β-globulin 5, α-globulin 2, γ-globulin 2, and albumin 1 (Hameed and Wagas, 2006). Albumin gets a value of one because it normally disperses rouleaux and thus retards red cell aggregation and hence tends to reduce the ESR (Hutchinson and Eastham, 1977).

The zeta potential results from negatively charged sialic acid groups of the red cell membrane. So, the repulsive effect of these negatively charged red blood cells is attenuated by the presence of ions and dielectric effect of the surrounding proteins which are affected by the asymmetric macromolecules that are oriented on the field and may have a disproportionate large effect. The zeta potential of suspended red cell decreases as fibrinogen and γ-globulin increases, so allowing rouleaux formation and more rapid sedimentation rate. This is the mechanism that is most likely to explain elevated ESR results in disease (Bull and Brailsford, 1972).

Another factor that influences ESR is erythrocyte plasma ratio which favors rouleaux formation independently of changes in plasma protein concentration. This occurs because ESR is directly proportional to the weight of cell aggregates and inversely proportional to the surface area. So in anemia, ESR increased as microcytes sediment more than macrocytes (decreased surface area/volume ratio, mimic rouleaux), while RBCs with abnormal shape and rigidity may hinder rouleaux and decrease ESR such as sickle cells and spherocytes (McPherson and Pincus, 2007).

It has been demonstrated that hyperlipoproteinemia by itself cannot be the cause of a significant high ESR results, this is because the required lipoprotein concentrations are too high to occur even in severe disorders of fat metabolism. So, this possibility can be ruled out in cases where the ESR is already raised due to some other pathological processes even if the lipoproteins may possibly contribute to the increase in ESR results (Schier *et al.*, 1976; Speed and Haslock, 1995).

The influence of hemodialysis on ESR results was evaluated in dialysis patients. According to Al-Homrany (2002), mean ESR results before dialysis do not significantly differ from the mean ESR results after dialysis. Also, he found that these patients (200 patients in outpatient dialysis center in Abha, Saudi Arabia) had a tendency for elevated ESR and almost one third of them (32%) had ESR >100 mm/hr in the absence of malignancy or other clinical factors known to cause such elevation of ESR results.

There was a significant correlation between elevated ESR and fibrinogen level. Thus, an ESR of $\geq$ 100 mm/hr does not necessarily need extensive investigations for causes other than the renal failure/hemodialysis state unless other indicators exist (Al-Homrany, 2002). However according to Brouillard and his colleagues (1996), it was found that there was a high degree of positive correlation between ESR and fibrinogen concentration as well as a similar repartition of normal and elevated values. They came to a conclusion that ESR can be used in patients on hemodialysis as in general population (Brouillard *et al.*, 1996). The relationship between fibrinogen and ESR is not likely to be one of cause and effect (Ropes *et al.*, 1939), since the incidence of diseases that may increase ESR is a priori higher in hemodialysis patients than in the general population, thus increasing the positive predictive value of the test (Sox, 1986).

1.6.1. Variables Responsible for Abnormal ESR Results

There are many variables that affect ESR results, some of them are responsible for elevated ESR results and others are responsible for lower results.

Variables that spuriously elevate ESR include (Jurado, 2001):

1. Anemia with normal RBC morphology which are affected by the ratio of erythrocytes to plasma, which favors rouleaux formation, independent of the changes in fibrinogen concentration (less friction to keep the RBCs suspended caused by changes in the ratio).
2. Elevated serum concentrations of non-fibrinogen proteins: M proteins, macroglobulins, and RBC agglutinins.
3. Renal failure.
4. Hypercholesterolemia.
5. Extreme obesity.
6. Pregnancy (as the ESR test was first used for).
7. Females (as sex has an influence on the ESR results).

8. Advanced age (as age has an influence on the ESR results), the formula for calculating the maximum normal ESR level at a given age: in men, age in years ÷ 2; in women, (age in years + 10) ÷ 2 (Jurado, 2001; Miller *et al.*,1983).
9. Artificial factors causing increased ESR results include vibration of the ESR tube; the tube being non-vertical, and increased temperature (Hameed and Wagas, 2006). An angle of even 3 from the vertical may accelerate the ESR by as much as 30 points (Jurado, 2001).

Variables that spuriously decrease ESR include (Jurado, 2001):

1. Morphological abnormalities of the RBCs which can interfere with RBCs pellet formation, thus affecting the ESR. The abnormal or irregular shape of the RBCs such as sickle cells, hinder rouleaux formation, so a decrease in ESR results occur.
2. Spherocytes, anisocytosis and poikilocytosis also interfere with the stacking of erythrocytes, and they also decrease ESR results.
3. Polycythemia which has an opposite effect than anemia has on RBC pellet formation.
4. Extremely elevated WBC count.
5. Diffuse intravascular coagulation (due to hypofibrinogenemia).
6. Dysfibrinogenemia and afibrinogenemia.
7. Extremely high serum bile salt levels which altered the RBCs membrane properties.
8. Congestive heart failure.
9. Low-molecular-weight dextran.
10. Artificial factors lead to decreased ESR results include: refrigeration of blood samples (if the sample must be kept in the refrigeration it should be allowed to reach room temperature before performing the test). Inadequate

anticoagulation with clotting of blood sample which will consume fibrinogen and may lower the ESR. The age of the sample also lower ESR results (Hameed and Wagas, 2006), so, it is favorable that the test is performed on blood samples that are obtained within 2 hours of testing because standing blood samples tend to become spherical, and shape of the RBCs will interferes with rouleaux formation (Jurado, 2001).

1.7. The Effect of Age, Sex and Gender on ESR

Age has an effect on ESR results: ESR results increase with age, and this rise is associated with lower hemoglobin values as a function of age. A formula for calculating the maximum normal ESR values at any age have been proposed. The probability of disease at any age increases by increasing ESR values, and becoming clinically significant if its results exceeded 50 mm/hr (Saadeh, 1998). It has been reported that ESR results are increased in over 40% of patients who are 50 years of age or more. The reason for the rise is unclear. Although, it may be related to some primary changes in the elements of the blood with age (Hayes and Stinson, 1976). In a study done by Eckerstrom (1949), concluded that it is not easy to verify the theory that ESR values increase by age, however, in 1951 another study done by Tillisch found that the mean ESR value increases with age. In 1955, Dahlberg and Josephson found that the mean ESR for men and women under 50 to be 4 and 7 mm/hr respectively and for men and women between 70 and 80 to be 12 and 20 mm/hr. In 1965, Borchgrevink and his colleagues found that 18% of all people in age group 51-66 had an ESR above 15-20 mm/hr. All these results had been reported by Bottiger and Svedberg (1967).

Normal values of ESR results in blacks are higher 2mm/hr to 13mm/hr even after correction of age, hemoglobin concentration and certain chronic diseases (Saadeh, 1998). This is due to the fact that blacks have higher serum total protein while the serum albumin is slightly lower than in whites, they also have higher γ- globulin levels (Gillum, 1993).

The rapid increase in ESR values in women after the age of 50 are influenced by hormones and this influence acts indirectly by altering the amount of specific plasma proteins. Stuinitz and Nyman in 1957 demonstrated that females that are treated with androgens had increased serum level of α2-globulins. In 1959, Nyman found that there was a small increase in serum haptoglobin values in aged people. The effect of sex hormones on the serum glycoprotein content in rats had been studied by Houssay and Blumenkrantz in 1964 and they came to a conclusion that estradiol increased the content of serum glycoproteins in ovarictomized female rats while testosterone decreased the values in castrated male animals, all these results gad been reported by Bottiger and Svedberg in1967.

Other Factors

The effect of iodine value in hypothyroidism was studied by Larsson in 1962. He found a negative correlation between the ESR values and protein-bound iodine values in hypothyroidism. Also, he found that an increase of the β2-globulin fraction, which contains the β-lipoprotein that increases in hypothyroidism. So, he came to a conclusion that the ESR depends not only on proteins but also perhaps on lipids, this study had been reported by Bottiger and Svedberg (1967).

1.8. Applications of ESR

1.8.1. ESR and C - Reactive Protein

Inflammatory response indicators include: ESR, C-reactive protein (CRP) and plasma viscosity (Saadeh, 1998). ESR and plasma viscosity are the most satisfactory monitors of acute phase response to disease after the first 24 hrs (Katz *et al*., 1990). During the first 24 hrs of the inflammatory process, CRP may be a better indicator of the acute phase response (Miettinen *et al.*, 1993). However, this test is less widely available than ESR because quantitative CRP is more expensive than ESR (Katz *et al*., 1990). In

contrast to ESR test, CRP is not directly affected by the changes in neither hematocrit nor storage of the plasma for up to 48 hours (Harkness, 1971).

The advantages and disadvantages of these three indicators are the following: for ESR, advantages include inexpensive cost, quick and simple to be performed, while for CRP its rapid response to inflammation, for plasma viscosity it is not affected by anemia or RBCs size. On the other hand, the disadvantages for ESR indicator are the sensitivity and factors affecting its result (anemia and RBCs size), while for CRP its wide reference range, cost and batch processing that may delay the results, for plasma viscosity it includes its cost, availability and technical performance (Alao, 2010).

CRP and ESR, which were used as minor criteria for infective endocarditis, were found not to correlate to the diagnosis of endocarditis. Gouriet and his colleagues in 2006 concluded that positive results of rheumatoid factor are the only inflammatory marker that helps in the diagnosis of the patients that are suspected to have infective endocarditis.

CRP and ESR have been widely used to determine the presence of infection after total knee arthroplasty (TKA). Park and his colleagues in 2008 assessed that postoperative levels of CRP and ESR could be an important adjunct to make a correct diagnosis of infection after TKA. On the other hand, they found that interpreting the postoperative data of CRP and ESR was not straightforward because of their nonspecific nature and wide variations in preoperative levels (Park *et al.*, 2008).

The temporal changes of CRP values were faster and greater than those of ESR. CRP level increased rapidly, reaching peak on the second day after the operation. Then CRP levels decreased biphasically, the CRP levels decreased to less than the normal reference on the day 42nd but returned to preoperative levels on day 19th. On the opposite direction ESR levels decreased slightly on the first postoperative day and increased to peak on the fifth day. The peaked level gradually decreased and remained

elevated above the normal reference level on the forty-second postoperative day, so they came to a conclusion that in comparison to ESR, CRP had faster temporal changes, greater folder changes, less frequent atypical patterns, and weaker associations between preoperative and postoperative levels. In practice, it may not be rare to have abnormal levels of CRP or ESR while there are no clinical symptoms and signs suggesting the presence of infection (Park *et al.*, 2008).

The usefulness of CRP and ESR in acute osteoarticular infections of childhood which comprise essentially three entities, septic arthritis (SA), osteomyelitis (OM), and their combination (OM + SA), is that it helps clinicians to evaluate the disease, as ESR is still the main yardstick in monitoring the course of illness, it increases arbitrarily and normalizes so slowly in active infection. CRP is faster than ESR and WBC count in predicting the effectiveness of therapy in SA, OM, and OM + SA, as its value increases within 6 to 8 hours and if the infection subsides, CRP levels decline by approximately 50% a day. ESR and CRP values normalized fastest in patients with OM, whereas in OM + SA patients ESR and CRP values normalized slowly. In OM + SA patients ESR, CRP, and WBC count are higher. Lower values of ESR, CRP, and WBC count are a character for OM (Paakkonen *et al.*, 2010).

1.8.2. The Effect of ESR and CRP on Urinary Tract Diseases

The usefulness of peripheral leukocyte count, differential leukocyte count, and ESR and CRP level in febrile urinary tract infection (UTI) was studied by Naseri in 2008. As these tests were considered simple noninvasive tests that were used for diagnosis of invasive bacterial infections and determining the UTI level. According to this study, children admitted to emergency department of Dr Sheikh Children's Hospital (Iran) during a period of 5 years (2002 to 2006) and aged between 1 month and 10 years with documented febrile UTI and not suffering from other sites of infection and inflammation, having axillary temperature $\geq$ 38ºC, the data for leukocyte count and differential leukocyte count were available for all 61 patients that were studied and

they were divided into 4 groups: (1) normal leukocyte count, (2) neutrophilic leukocytosis or leukocytosis with an absolute increase in neutrophil count, (3) relative neutrophilia (an increase in the percentage of neutrophils without increased absolute leukocyte count), and (4) lymphocytic leukocytosis (an absolute increase in lymphocytes count). However, data for ESR and CRP were only available for 41 and 36 patients respectively and they were divided into two groups: CRP positives levels range from (1+ to 3+) or CRP negatives and those with low and high ESR (< 30 mm/hr and ESR ≥ 30 mm/hr). The CRP positive results depend on the presence or absence of agglutination and size of agglutinated droplets on microscopic examination (no agglutination was considered negative; small-sized agglutinated droplets, 1+; medium-sized agglutinated droplets, 2+; and large-sized agglutinated droplets, 3+) (Naseri, 2008).

In general, children were more sensitive to neutrophilic response than adults and febrile UTI pyelonephritis main response was an increase in the percentage of neutrophils rather than absolute leukocyte count. In addition, most patients with pyelonephritis did not show alterations in CRP, ESR and leukocyte count, so no direct correlation between systemic inflammatory responses (leukocyte count, ESR, CRP, and severity of fever) and urinary tract inflammatory response (pyuria) was identified. This conclusion was due to the results that had been obtained from 33 children with measured values of both CRP and ESR. Seventeen patients had high ESR and positive CRP and the remaining patients did not. No significant difference in ESR result was observed between CRP positives and CRP negatives patients. In 10 patients, relative neutrophilia, positive CRP, and high ESR were found together. In 6 patients, neutrophilic leukocytosis was present along with positive CRP and high ESR (Naseri, 2008).

Rasamoelisoa and colleagues in 1999 found that there was a direct correlation between CRP values and leukocytes level in presumed bacterial infections. Karmer and colleagues (1993) assessed the relation between the clinical course of UTI, interleukin levels, inflammatory markers and findings on renal scintigraphy. A positive correlation was found between urinary interleukin-6 and interleukin-8 levels and ESR, CRP, and

leukocyte count, but no relation was found between urinary cytokine levels and the presence of inflammatory changes detected by renal scintigraphy. Most of the children in this study did not have leukocytosis. They confirmed that ESR results > 30 mm/hr had valuable effect in diagnosis of febrile UTI, but CRP measurement by qualitative methods (latex method) was not useful for diagnosis of pyelonephritis in children. Another finding was that peripheral leukocyte count and CRP measurement by qualitative methods were not valuable tests for ruling out pyelonephritis and defining the UTI level. (Naseri, 2008).

Both CRP and ESR had high diagnostic values when measured before making decision of surgical exploration in acute scrotum. The predictive values of both tests can differentiate epididymitis from non-inflammatory causes of acute scrotum including spermatic cord torsion (the definition of acute scrotum is the painful inflammation of the scrotum or its components presenting with local signs and systemic symptoms). As the most common cause of acute scrotum is epididymitis, differentiation of these two conditions is sometimes challenging. Diagnosis of spermatic cord torsion must be made within the first 4 to 6 hours; otherwise it could lead to an irreversible ischemic injury to the testis and loss of gonads, especially if complete spermatic cord torsion is present. Spermatic cord torsion usually occurs during adolescence, often in men younger than 20 years old, whereas epididymitis was mostly seen in men older than 35 years. (Asgari *et al.*, 2006).

1.8.3. ESR and Cancers

ESR is used as an indicator for the diagnosis of many disease conditions and for monitoring disease activity (Fernando and Isenberg, 2005). In patients having cancer, if the ESR results exceeded 100 mm/hr it means that metastases is usually present (Sox, 1986), in prostate cancer if ESR ≥37mm/hr it had a higher incidence of disease progression and death (Johansson *et al.*, 1992; Saadeh, 1998). Patients with renal cell carcinoma (RCC) had elevated ESR results even with other tumors in the kidney

meaning that the elevation of ESR was frequent and occasionally dramatically high. Sengupta and his colleagues (2005) evaluated heparinized ESR samples that were drawn freshly from RCC patients**.** The results were obtained after 1 hour at room temperature. The abnormal ESR results were defined as having ESR results that were more than 22 mm/hr for males and more than 29 mm/hr for females. The great proportion of patients (the study included 3008 patients) had elevated ESR values. The patients also had anemia, leukocytosis, hematuria, pyuria, elevated serum creatinine, hypercalcemia and hyperglycemia compared with patients who had normal ESR. As the elevation of ESR is associated with increasing risk of mortality for RCC, patients who had elevated ESR were more likely to have tumors with adverse pathologic features (Sengupta *et al*., 2006).

The mechanism of elevation of the ESR remains poorly understood. Previous studies suggested a possible correlation with the inflammatory cytokine interleukin-6 perhaps mediated through hepatic synthesis of fibrinogen and α-globulins. Additionally, low hematocrit levels were found in patients suffering from RCC. So, this alone may cause elevation of the ESR. However, 29 % of the patients that had normal or high hematocrit level had elevated ESR values. This suggests that this could not be the only factor for the elevation of ESR (Sengupta *et al*., 2006).

Due to the limitation of ESR results, the ability to differentiate between “health” and “sick” status depending on ESR results alone is difficult because unlike other laboratory tests, ESR reflects the interaction of numerous blood components, not all of them have been fully recognized. The two major determinants of ESR are erythrocyte aggregation and hematocrit. So, the measurement of ESR is not a valuable screening test to establish the presence or absence of malignant diseases. If ESR results are elevated it provokes anxiety, while if it is normal it implies good health, neither of these results are necessarily in the patient’s interest (Monig *et al*., 2002).

1.8.4. ESR in Coronary Artery Disease and Stroke

Traditionally, risk factors for coronary heart disease (CHD) includes: lipid abnormalities, high blood pressure, diabetes, and smoking. These factors explain only a part of the risk associated with CHD; while recently, the role of inflammatory nature of atherosclerosis had been identified (Andresdottir *et al*., 2003).

Atherosclerosis is a chronic inflammatory disease that remains asymptomatic for decades (Ross, 1993). So, the interpretation of the association between ESR in the normal range and CHD can be viewed as a result of persistent low- level inflammation. The preventive effect of aspirin and statin therapy among persons with high CRP levels independent of the lipid-lowering effect was demonstrated by Andresdottir and his colleagues (2003) as evidence to the role of inflammation in atherosclerosis. So, this study supported evidence of the inflammatory process that occurs in atherosclerosis. Therfore, the ESR is an independent, long-term predictor of CHD in both men and women (Andresdottir *et al*., 2003). Also, the ESR emerged as a short and long term predictor of CHD mortality among health, positive electrocardiography (ECG) test and those with angina pectoris. This is due to increased fibrinogen levels as age, obesity, elevated blood pressure, elevated cholesterol and triglyceride levels, diabetes, season (winter) and infections are responsible for this, while exercise, alcohol consumption and hepatitis B surface antigen had the opposite effect (Erikssen *et al.*, 2000).

Patients having ischemic stroke, elevated D-dimer levels, suggested ongoing thrombosis and fibrinolysis, were more likely to have laboratory evidence of inflammation as manifested by an elevated ESR than those with normal D-dimer levels. An elevated ESR in the presence of raised D-dimers was most common in young and black patients, and was seldom associated with obvious infection or autoimmune disease. Ischemic strokes complicate chronic meningeal infections that cause inflammation and secondary thrombosis, so this will lead to increase ESR results (Swartz *et al*., 2005).

HIV virus can cause peri-arterial inflammation. The virus by itself may precipitate stroke via a vasculopathy or indirectly secondary to opportunistic infections or coagulopathy. Although in the absence of HIV infection, recent or current systemic infection may increase stroke risk by inducing inflammation and creating a prothrombotic state. A recent study focuses on the effect of *Chlamydia pneumoniae* and *Helicobacter pylori* in the pathogenesis of atherosclerosis. In a study performed by Swartz and colleagues (2005), found that no correlation between leucocytosis and ESR, the elevated ESR levels with raised D-dimer levels, in the absence of an elevated WBC count or clinical evidence of infection, was probably the result of ongoing thrombosis and fibrinolysis or occult inflammation (Swartz *et al*., 2005).

In stroke, if ESR results are ≥ 28 mm/hr, it indicates poor prognosis. In white men having coronary artery disease (CAD), if ESR exceeds 22 mm/hr it indicates high risk of CAD (Saadeh, 1998). Patients suffering from CAD had increased ESR results with age, ESR values increased with age by 2.5 percent per year for men and 1.9 percent per year for women **(**Andresdottir *et al*., 2003). Also it had been noticed that cardiac failure has a definite retarding influence on the ESR and tends to cause normalization of its results (Sanghvi, 1960), whereas ESR values increased in syphilitic aortitis and in certain cases of angina (Whitby and Britton, 1957; Park *et al.*, 2011).

In frequent chronic disease, the parameters that were previously thought to be related to better prognosis can (within 10 years) become a predictor of impaired survival. In chronic heart failure (CHF), ESR values decreased as heart failure worsened and rose again when condition improved (Wood, 1936; Shahzad *et al.*, 2009). Treatment of CHF patients with angiotensin-converting enzyme (ACE) inhibitor may improve their responsiveness to metabolic and immunological abnormalities, and ESR was then increased proportionally. ACE inhibitor dose was not related to ESR. The mechanism whereby ACE inhibitors effect the changing in ESR is still unknown (Sharma *et al*., 2000).

ESR correlates independently to coronary atherosclerosis and it was considered as a predictor of cardiac death in patients with probable ischemic heart disease. Elevation of ESR should direct physician's attention to coronary atherosclerosis and its consequences. This correlate with the indices of acute phase response that is elevated in type 2 diabetic patients. The three indices of inflammation (CRP, WBCs count and fibrinogen), correlate with obesity, central distribution of body fat, higher blood pressure, hyperglycemia, lower HDL cholesterol, and hyperinsulinemia (risk factors for atherosclerosis). So, CRP predicted myocardial infarction (MI) in hospital-based study whereas ESR-predicted future ischemic heart disease events in a population-based study. ESR was significantly higher in non-smokers than in past- or current-smokers (Natali *et al.*, 2003).

ESR was related to the severity of heart failure. So, it correlates with the mean right atrial pressure, in patients who had a diuresis after effective therapy was due to fibrinogen. Patients with chronic heart failure, have chronically elevated levels of fibrinogen (>4 g per liter). This may be due to their advanced age or to the high prevalence of coronary artery disease, systemic hypertension and diabetes mellitus. However, during periods of acute decompensation or when the clinical syndrome of right-sided heart failure occur in patients with longstanding left ventricular failure, right atrial pressure rises and hepatic congestion develops, so the expansion in volume that occur may dilute the concentration of fibrinogen and thus reduce the sedimentation rate. Increased right atrial pressure impairs the formation or accelerates the degradation of fibrinogen. If this occurs both the plasma fibrinogen level and the sedimentation rate would decline (possibly into the normal or low range) (Haber *et al.*, 1991).

1.8.5. ESR in Polymyalgia Rheumatica and Giant Cell Arteritis

ESR is the simplest test that can be performed for the diagnosis of: 1- polymyalgia rheumatica (PMR) (which is an inflammatory disorder involving pain and stiffness in the hip or shoulder area, and it occur mostly in patients over 50 years old), 2- giant cell

arteritis (GCA) (inflammation and damage to blood vessels that supply the head area, particularly the large or medium arteries that branch from the neck and supply the temporal area) which are related diseases that can present in a nonspecific manner. Although the muscle pain, constitutional disturbance, and headaches may be attributed to depression, elevation of ESR in patients suffering from these diseases aids in the diagnosis. As ESR results return to normal with corticosteroid therapy, it can be used as a guide for the adjustment of treatment. However, clinical relapse may occur while the ESR is normal (Park *et al.*, 1981; Mandella, 2004).

The clinical activity of both the PMR and GCA components of the disease could be assessed on a scale from 0 to 4 (inactive to highly active). As there is a positive correlation between ESR, CRP, α1-antitrypsin, orosomucoid, and haptoglobin in treated and untreated patients with PMR and/or GCA. Some discrepancy between ESR and CRP was reported in patients with inactive disease (grade 0) or slightly active disease (grade 1 or 2). However, these measurements were invariably in agreement in patients with active disease (grade 3 or 4) (Park *et al.*, 1981).

ESR is also used for the diagnosis GCA which is a common vasculitic syndrome that affects older people and it affects medium- and large-sized arteries and is characterized by an intense acute phase response. The hallmark of this disease is the markedly elevated ESR results. According to the American College of Rheumatology (ACR) criteria include ESR $\geq$ 50 mm/hour as 1 of the 5 criteria found to be useful in the classification of GCA (Hunder *et al.*, 1990). The occurrence of GCA with normal ESR is not rare, and because the ESR results are considered a hallmark in the diagnosis, treatment may be delayed when the ESR is normal in a patient suspected of having GCA (Salvarani and Hander, 2001).

1.8.6. ESR, Rheumatoid Arthritis and Temporal Arteritis

Rheumatiod arthritis (RA) which is a chronic inflammatory disease that affects body systems, tissues and organs, its cause is still unknown, but it is considered as a systemic autoimmune disease. It attacks synovial joints (it is an inflammatory response secondary to hyperplasia of synovial cells, excess synovial fluid, and the development of pannus in the synovial. The pathology of this disease leads to the destruction of articular cartilage and ankylosis of the joints. Also it can produce diffuse inflammation in lungs, pericardium, pleura, sclera and nodular lesions in subcutaneous tissues.

ESR test was traditionally used for the diagnosis of RA. Also, it is used as a mean for the staging of this disease rather than diagnostic criteria. Most rheumatologists still believe that ESR is useful in the diagnosis of questionable and definite evidence of inflammation that might affect therapeutic decisions (Weinstein, 1994; Mandella, 2004). Patients suffering from temporal arteritis (inflammation and damage to blood vessels that supply the head area, particularly the large or medium arteries that branch from the neck) will have high ESR values, but occasional patients may have normal ESR values (Wise *et al.*, 1991; Mandella, 2004). The ESR average results of these patients exceeds 30 mm/hr (in 99% of the patients) while it exceeded 90 mm/hr according to one study (Huston *et al*., 1978). If the patient had a solid clinical evidence of temporal arteritis, the patient should undergo temporal artery biopsy as the normal ESR results should be disregarded (Wise *et al.*, 1991).

In health, the contribution of the γ-globulins to the serum viscosity is small, while in rheumatoid arthritis, there is a gross increase in the serum γ-globulins that have a significant effect. This increase in γ-globulins is associated with an increase in fibrinogen and it may be that these increases account for the correlation of total γ-globulins with thrombin clottable protein. ESR and plasma viscosity represents both a direct relation by way of the serum viscosity and an indirect effect due to fibrinogen. So, in RA, the ESR is affected by changes in both the fibrinogen and the total γ-

globulins and these probably reflect changes in the acute-phase protein and rheumatoid factor respectively (Crockson and Crockson, 1974). The relationship between ESR and CRP in RA patients is roughly linear. This relationship, however, is altered under the influence of different drugs; patients receiving non-steroidal anti-inflammatory drugs (NSAID) which do not affect either measurement of CRP nor ESR. Drugs like penicillamine, and prednisone, which are known to lower both measurements, had a greater effect on CRP than ESR (Walsh *et al.*, 1979).

The risk of developing heart failure is twice in patients suffering from RA than those without RA. The mechanism is still unknown, but it seems to be independent of the traditional risk factors, suggesting that the persistent inflammatory state in RA may have an important pathological role. ESR is affected by many factors other than acute-phase response, including the patient's age, sex, the size, shape and number of erythrocytes, other plasma constituents, such as serum immunoglobulins, rheumatoid factor and various drugs such as salicylates, and even smoking. Also NSAID which are used commonly by RA patients, are associated with increased risk of heart failure. The proportion with significantly raised ESR was highest during the 6-month period immediately before diagnosis of new-onset heart failure, as compared with any other period over their entire follow-up time (including both before and after heart failure). So, it is important for clinicians to carefully evaluate cardiovascular status in RA patients with raised ESR (Maradit-Kremers *et al.*, 2007).

American College of Rheumatology (ACR) criteria and the Disease Activity Score (DAS) are widely used in RA. The DAS index combines information relating to the number of swollen and tender joints, general health of the patient and acute phase response. The DAS28 is based on a count of 28 swollen and tender joints, with a score ranging from 0 to 9.4. It can be used to evaluate the patient response to treatment. Value of 3.2 defined as the threshold for a low disease activity state and 2.6 as the threshold for remission. The European League Against Rheumatism (EULAR) response criteria combines the DAS28 score at the time of evaluation with the change in DAS28 score between two time points and this enables the user to define improvement or response to

treatment. The DAS28 based on ESR (DAS28 ESR) and on CRP (DAS28 CRP) can be used as benchmarks to assess patient improvement and treatment effect, and can aid in the description and interpretation of changes in disease activity in patients with RA (Wells *et al.*,2009).

1.8.7. ESR and Rheumatic Fever

Rheumatic fever which is an inflammatory disease that follows *Streptococcus pyogenes* infection, is believed to be the cause of antibody cross-reactivity that can involve the heart, joints, skin, and brain. The illness typically develops two to three weeks after infection.

The acute form of the disease commonly appears in children between the ages of 6 and 15 years. Only 20% of first-time attacks occur in adults. The name (Rheumatic Fever) is due to its similarity in presentation to rheumatism, and laboratory tests that are used to diagnose this disease include: ESR, CRP, serum protein fractions (SPF), plasma fibrinogen level and antistreptolysin reaction (ASO). In children suffering from the first attack and receiving steroids, the ESR remains elevated for an average of 25 days. Sometimes the results remained high until thirtieth, forty-fifth days. If the patient suffered from tonsillitis during rheumatic infection the results remained elevated for 60 days (Popov and Stanisheva, 1958; Naher *et al.*, 2002).

In rheumatic fever, CRP test remained positive for about three months as did the ESR and SPF; this test is the first to appear positive and the first to become negative with the exception of fibrinogen level which was often the first to become negative in children with a first attack receiving steroid therapy. CRP results usually range from +1 to +3. Conversely, albumin appears to be depressed during the acute phase of the attack. The severity of the disease parallels the degree of protein alteration and duration of the abnormal pattern. ESR and CRP tend to return to normal levels during the first week of the disease, but the level of fibrinogen remains high for a long time if the condition

becomes protracted particularly in repeated attacks. The level of ASO in children with previous attacks and treated with steroids remained high for 40 days (Popov and Stanisheva, 1958; Naher *et al.*, 2002).

Serum electrophoresis in rheumatic fever patients shows that albumin falls, while the concentrations of α-1, α-2 and γ-globulin rise. Ernstene in 1930 showed that when plasma fibrinogen concentration increased, hematocrit decreased, so these changes were responsible for the rise in ESR. CRP increased early in the course of the acute inflammation. Yocum and Doerner in 1957 claimed that CRP migrated with the β-globulin fraction, while Hedlund and Brattsten in 1955 reported that it migrated with γ-globulin. However, in acute rheumatic fever, CRP more probably migrates with α-1 globulin fraction. CRP level could here rise to more than 33 mg/100 mL, that was still only a fraction of the total α-globulin (Easthman *et al.*,1958).

Serum diphenylamine reaction which reflects changes in α-globulin, particularly in the α-1 fraction and protein-bound polysaccharide present in the γ- globulin fraction have an effect on ESR. There was also a correlation between serum tetrammonium turbidity and ESR in rheumatic fever. Diphenylamine reaction which depends on sialic acid, is derived from serum mucoprotein that is mainly associated with the serum α-globulin fraction, while ESR results are affected by the plasma fibrinogen and globulin concentrations (particularly α and γ-globulin fractions) and packed cell volume. So, serum diphenylamine reaction and tetrammonium turbidity reaction are not sensitive enough as ESR and CRP to determine the presence or absence of rheumatic activity. As ESR is more frequently abnormal in negative cases than the CRP, and is influenced by both anemia and polycythemia. Westergren method is influenced by the reduction in packed cell volume and blood samples are diluted with sodium citrate solution, the use of 200-mm, columns delays the onset of packing, so, CRP may be more useful than ESR (Easthman *et al.*, 1958).

When normal human serum comes in contact with Dische's diphenylamine reagent, it produces a reddish-purple color. In rheumatic fever, diphenylamine reaction is much more intense in the acute phase, it decreases with the lessening of the symptoms, and it returns to normal in subsidence. This effect parallels ESR and it is inhibited by "antiphlogistic" drugs, such as salicylates, cortisone, and hydrocortisone. The substance in the serum which reacts with diphenylamine has not been identified, it might be a mucoprotein found in α- globulin fraction, with a very low iso-electric point (less than 2). The reddish-purple color is believed to be due to the carbohydrate portion of the mucoprotein. Also this reaction may be used in rheumatoid arthritis; the reaction increases constantly during the active phases of the disease and follows the clinical variations more faithfully and promptly than ESR (Cecchi and Ferraris, 1955).

1.8.8. ESR as an Indicator for Occult Diseases

As the ESR test can be used to detect occult diseases (Pagana and Pagana, 2002; Arkin and Aklin, 2007) also it provides the same information as an acute phase reactant protein (Piva *et al.*, 2001; Pagana and Pagana, 2002). It is useful for the diagnosis of polymyalgia rheumatica (PMR) and for monitoring patients with Hodgkin's disease. High values (>100 mm/hr) have a 90% predictive value for serious diseases or malignancy (particularly myeloma) (Hoffbrand *et al.*, 2006). Also it is used commonly in diagnosis of Kawasaki's disease (Emelike *et al.*, 2010) (which is an autoimmune disease that is characterized as a systemic necrotizing medium-sized vessel vasculitis and it affects commonly children under five years of age). This is because these diseases are mirrored by changes in ESR results (AlFahdi and Al-Awadhi, 2005).

Grossly elevated ESR results (≥ 100 mm/hr) are an alarming situation that needs more diagnosis and treatment. It has a 90% predictive value for serious underlying diseases, and there are a minimal number of tests that are available in developing countries that reveal the cause. According to Haque and his colleagues (2009) who studied the causes of grossly elevated ESR in Bangladesh, they found that hematological disorders appear

to be the most common cause (41%) followed by infectious diseases (36%) as they are prevalent in developing countries like Bangladesh in which tuberculosis (pulmonary and extra pulmonary) are considered the most common causes of infection followed by kala-azar also known as Visceral leishmaniasis (is a deadly disease caused by the parasitic protozoa ***Leishmania donovani*** and transmitted to humans by the bite of infected female sand fly, ***Phlebotomus argentipes***) that causes elevated ESR results. Connective tissue disorders (17%) are the third cause. In 2% of the cases, the causes are chronic liver diseases while in 4% the cause cannot be elucidated. This study had been done on 100 patients (Haque *el al*., 2009).

The usefulness of ESR in HIV is still neglected in developing countries, despite that it is considered as an indicator of disease progression. Some studies disagreed or demonstrated only a negligible fall in CD4 count with rising ESR, this due to the fact that not all HIV positive patients have elevated ESR. However, Lowe in 2011 believed that scientists should start measuring ESR value again; perhaps they would find that it is a more useful test than CD4 and CRP as chronically elevated ESR is very informative because it reflects immune activation, correlating well with cytokines including interleukin-6 and tumor necrosis factor-alpha (Lowe, 2011).

ESR values ≥ 100 mm/hr are associated with tuberculosis (TB). In a study done in Qatar by Ukpe and Southern (2005) reported several studies on patients suffering from TB with HIV co-infection, one-third of the TB cases in this study had normal ESR values. There would be little value in using ESR as a diagnostic aid in childhood TB. In another study done in India, most of the TB patients with HIV co-infection had much lower ESR values than those for the TB cases without HIV infection. Therefore it's probably true that ESR values are lower in TB cases associated with HIV infection in developing countries such as India. On the other hand, high ESR values lower the chance for TB patients to be associated with HIV infection. In contrast to this study, a study performed in South Africa suggested that active TB is associated mostly with very high ESR values irrespective to HIV status, and that concomitant HIV infection tended to increase the proportion of TB cases with these very high values. This may be due to factors such

as anemia and hypoalbuminemia that cause false ESR results, as anemia was present in 95% and hypoalbuminemia in 91% of the 110 active TB cases in this study, irrespective to HIV status (Ukpe and Southern, 2005).

In a retrospective study performed in South Africa, all patients having ESR results ≥100 mm/hr were divided into one of six groups: (*i*) infection; (*ii*) malignancy; (*iii*) inflammatory/connective tissue disease; (*iv*) renal disease; (*v*) miscellaneous diseases; and (*vi*) idiopathic causes. Infection was the most common disease associated with extreme elevations in the ESR (39.0%), malignancy (23.0%), inflammatory/collagen disease (22.1%), miscellaneous diseases (12.6%), renal disease (6.0%) and idiopathic causes (2.4%). The most common infections were: pneumonia (70.67%), gastroenteritis (6.77%), meningitis (6.27%), urinary tract infections (5.26%), orthopaedic sepsis (3.76%), obstetric and gynaecological infections (1.50%) and general sepsis (0.50%). Other infections (2.76%) included: hepatitis, encephalitis, malaria, pericarditis, infective endocarditis and rickettsial infections. The most common single infective organism was *Mycobacterium tuberculosis* (36.88%). The correlation between ESR and plasma constituents could be ranked from strongest to weakest as: increased fibrinogen, α-globulins, γ-globulins, reduced albumin levels, and high cholesterol levels. False increase in ESR results occurs due to Stomatocytosis and anemia (Levay and Retief, 2005).

Even though infections lead to increased ESR values, patients suffering from dengue have normal ESR values. Dengue disease which is an arbovirosis disease 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 (mosquito *Aedes aegypti*). The lack of ESR response is likely because dengue causes plasma leakage that leads to hemoconcentration of blood and 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 (José de Souza *et al.*, 2008).

1.9. Problem Statement

ESR serves as a "sickness index" (Koepke, 2002) is a simple, inexpensive, non-specific test that is used to differentiate diseases with similar symptoms and to differentiate acute from chronic infection, inflammation and tissue necrosis or infarction (Wood, 1945; Deshpande *et al.*, 1971; Epperly *et al.*, 2000). The importance of this test includes using it as a guide for diagnosis, management and follow-up of specific clinical situations.

Different methods from different suppliers and different anticoagulants are used to perform this test. All manufacturers claim that their methods correlate well with classic Westergren method.

This study is done in order to evaluate the harmony and agreement between laboratories using different anticoagulants and different methods in performing the ESR test in Palestine. It is the first study performed in Palestine to compare the ESR results obtained from manual and automated techniques using EDTA and citrate as anticoagulant.

1.10. Justification

In order to get the maximum validation of the ESR tests, the ESR results obtained from different laboratories using different manual and automated methods should show an acceptable degree of intra- and inter-laboratory harmony. However, studies that compare the results of ESR are scarce. In Palestine, the ESR is performed using different manual and automated methods and studies to compare or investigate the correlation between the results are lacking. So, this study is performed to compare ESR results using different methods and different anticoagulants.

1.11. Goals

ESR, which is a simple, non-specific, inexpensive test that is frequently ordered in clinical laboratories. To differentiate different disease conditions. Also it is used to monitor and manage many diseases. The aim of this study is to investigate the agreement or consensus between laboratories using manual and automated ESR methods, as well as to investigate the effect of the type of anticoagulants (EDTA and citrate) on the ESR results.

One of the most important goals is to reach a recommendation on what method to use and the problems of performing such a test in Palestine.

Chapter Two: Materials and Methods

2.1. Materials Used in This Study

Materials used in this study are listed in Table (2.1). They include those used to draw fresh blood from the two study groups (patients' and apparently healthy controls) to perform ESR.

Table (2.1): Materials used in this study.

Number	Item	Manufacturing Company/ Catalog Number
1	Needles	_____
2	Tourniquet and holder	_____
3	3.5 ml K_3EDTA tubes	_____
4	70% isopropyl alcohol swabs	_____
5	Sterile gauze swabs and adhesive dressings	_____
6	Automatic pipette	_____
7	ESR tubes / Sediplast® and rack	LP Italiana SPA, Italy/ 244177
8	ESR tubes/ Monosed®	Vital Diagnostic, Italy/ 11B-1145
9	Microsed Automated System	Vital Diagnostic, Italy
10	ESR tubes/ Kima Sed® and rack	Kima Sed, Italy/ 14250

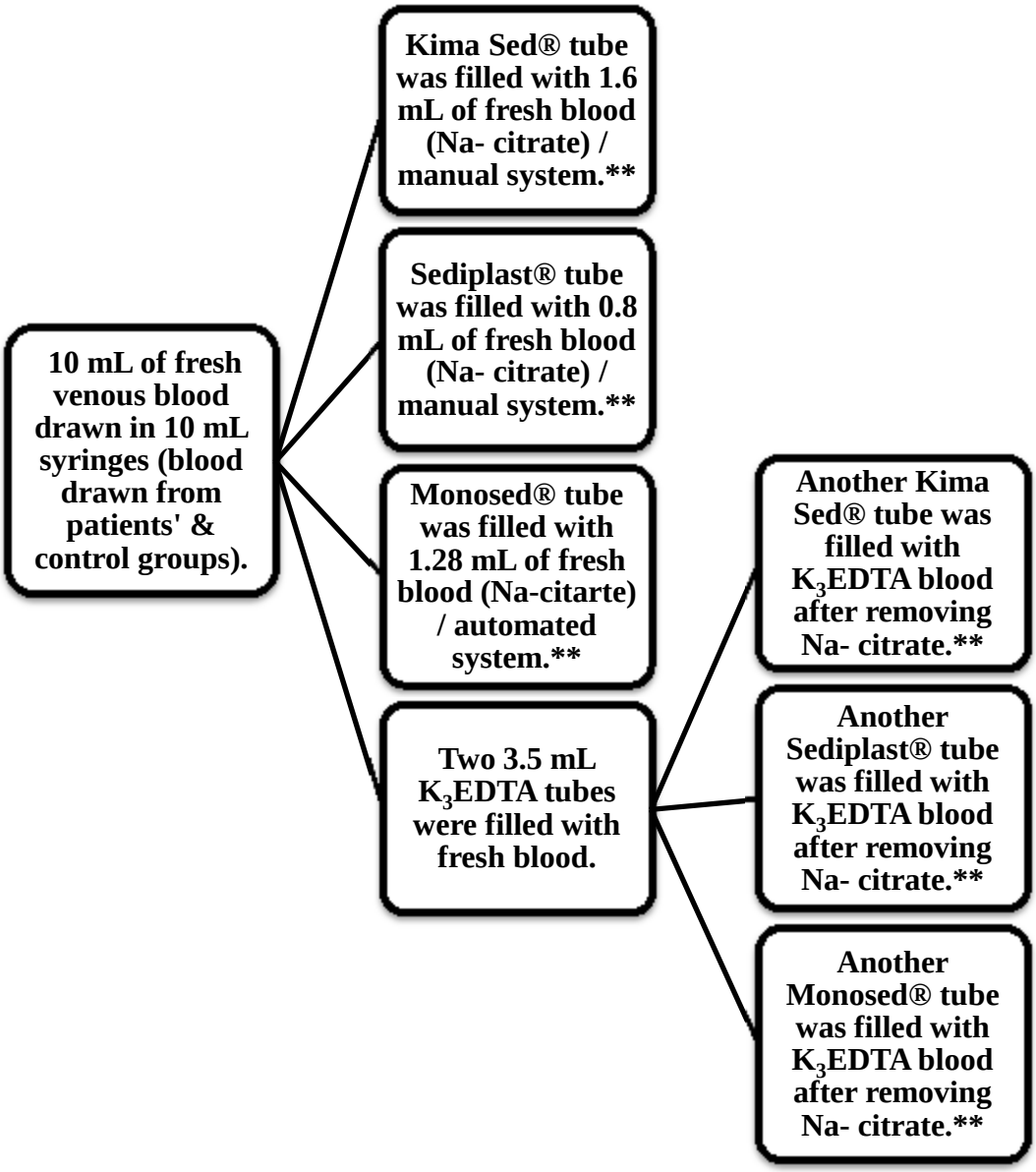
2.2. Preparation and Distribution of the Questionnaire

Two questionnaires have been prepared: one for the patients' group and the other for apparently healthy persons that we considered as the control group. The questionnaire included the name, age, sex, diagnosis, address, and the medications that he/she received (if any). The two groups that participated in this study had filled the study questionnaires and signed a consent form.

The study included 105 patients (36 males and 69 females) suffering from different diseases. Also pregnant women were included in this group. The ages of this group ranged from 10-83 years. The patients were those admitted or visited outpatient clinics of the Bethlehem Arab Society for Rehabilitation "Special Surgical Hospital" and Beit-Jala Governmental Hospital, Beit-Jala. The control group included 95 apparently healthy volunteers working in Bethlehem Arab Society for Rehabilitation "Special Surgical Hospital" and Beit-Jala Governmental Hospital, Beit-Jala. Additionally, some of the control group volunteers brought some of their healthy family members for participation in the control group. This group included 69 males and 26 females. There ages ranged from 11-80 years.

2.3. Preparation of the Study Samples

The main purpose of this study is to compare the ESR results obtained from two manual and one automated technique using EDTA and citrate as anticoagulant. The following chart describes the sample preparation process.



**ESR test was performed at the same time for EDTA & citrated blood, for the same person.

Results obtained from different systems using different anticoagulants were recorded.

All blood samples were analyzed within two hours of blood collection.

Figure 2.1: Chart for sample preparation process.

2.4. Kima Sed® Tubes (Quick Method)

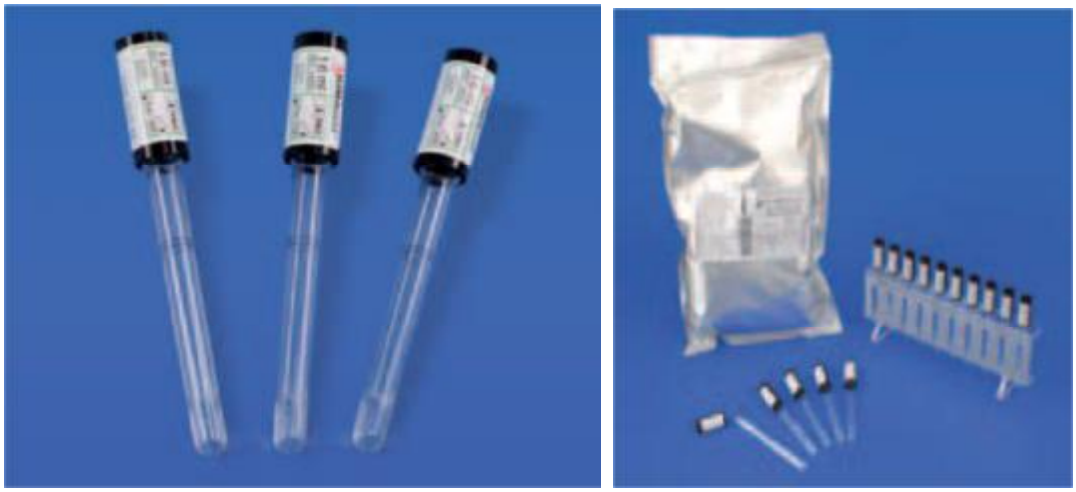


Figure 2.2: Kima Sed® tubes and rack.

The Quick method is used by all laboratory services in the Ministry of Health (MOH) in the West Bank. It is called Kima Sed® tubes, and manufactured by Vacutest Kima S.r.l./Italy. According to the manufacturer instructions, the sterile plastic tube (13x75 mm) with predetermined vacuum contains 0.4 mL sodium citrate that needs 1.6 mL of blood to be filled. Blood collection must be performed according to the standard procedures; the blood must be filled correctly in the tubes. After collection of blood, the sample has to be gently shaken by slowly inverting the tube to allow the correct mixing of the blood with the anticoagulant, before starting the ESR.

Bubbles in the sample, insufficient sample mixing after collection and before the assay, vibration during the assay and insufficient volume of the sample should be avoided. For EDTA-ESR, the sodium citrate was removed and the tubes were filled with EDTA blood then placed on its special rack. After 30 minutes, the results were read according to manufacturer instructions. The results are supposed to reflect the one hour Westergren test.

2.5. Sediplast® Tubes (Westergren Method)

Sediplast® tubes are manufactured by LP Italiana S.P.A./ Italy. These tubes contain two parts: the conical shape plastic tubes with a stopper and the graduated pipette. The tubes contain 0.2 mL of 3.8% sodium citrate that needs 0.8 mL of blood to be filled. The graduated pipette are made from a transparent plastic that receives blood from the test tube leaving an air space in such a way that, by adjusting the volume of the air space, it is possible to bring the blood level to zero. The pipette technical data includes: overall length 200 mm, graduations 0-150 ± 0.35, bore size 2.5 ± 0.15 mm, uniformity of bore ± 0.05 mm.

Figure 2.3: Sediplast® tubes.

The plastic tubes which have a conical shape with a stopper allow blood to go up to the graduation mark. After adding the blood, the tube was plugged and mixed immediately by gently inverting several times. The tube was placed in a vertical rack and the graduated pipette was gently inserted through the pierceable stopper without creating air bubbles. The graduated pipette must touch the bottom of the tube. For EDTA-ESR, sodium citrate was removed and the tubes were filled with EDTA blood then placed in the provided rack. After 60 minutes, results were read according to the manufacture procedure. This method is used by many laboratories in the West Bank, 57 out of 68 laboratories in Bethlehem and Ramallah (83%) use this method.

This method is the closest to classic Westergren method and the LP ESR pipette is manufactured in accordance with ICSH recommendations. Thus, in this study it was considered as the reference/comparative method either using EDTA or citrated blood. Since the classic Westergren method is no longer used due to its unsafe mouth pipetting

and its glass non-disposable tubes. The results of all other methods were compared with it. Henceforth, we refer to this method as Westergren method.

2.6. Monosed® Tubes and MICROsed-System® (Automated Method)

The Monosed® tubes and MICROsed-System® are manufactured by Vital Diagnostics S.r.l./Italy. MICROsed-System® is the automated machine that is used to measure ESR. It contains 10 channels for analysis. It gives a pre-indication of the result, on the display after only 10 minutes and the test is finished at 30 minutes by printing the result that are supposed to reflect the 1 hour Westergren test. The operating conditions for this system include: that the temperature should be 15ºC-32°C, the measuring method of this machine is the infrared barrier.

Monosed® tubes are glass tubes that are 8 mm in diameter and have a vacuum rubber stopper. Each tube contains 3.2% sodium citrate that needs 1.28 mL of blood to be filled.

For EDTA-ESR, the sodium citrate solution was removed and tubes were filled with EDTA blood, then placed in the machine and the results were automatically recorded according to manufacturer instructions.

Figure 2.4: Monosed® tubes and MICROsed- System®.

2.7. Statistical Analysis for Population Study

The ESR tests using different methods with EDTA and citrated blood were done at the same time for all samples included in this study. The ESR results for both patients' group and control group are shown in Appendix I and II. ESR results obtained were analyzed using the statistical package for social sciences program (SPSS) (version 19) (SPSS, 2011). The following statistical parameters were obtained per group and per method of ESR analysis: mean, standard deviation, Pearson correlation, Paired samples test and scatter plots.

Comparison studies were done to evaluate the ESR test methods (manual and automated methods). In addition, ESR results using EDTA versus citrated blood were compared. For citrated blood, the Westergren Na-citrate was chosen to be the recognized comparative method as it is the closest method to Westergren classic method. As for EDTA blood, the Westergren EDTA is the recognized comparative method for the same reason mentioned earlier. In addition, Westergren Na-citrate and Westergren EDTA were also compared.

Chapter Three: Results

3.1 Questionnaire Results and Population Study

The problem statement can be stated as such: Is there harmony in the results among different methods used to measure ESR? Can we find accepted correlation between ESR results using manual and automated methods when EDTA and citrated blood samples are used? In order to address this problem statement, a comparison study was done to evaluate the different test methods. For methods to match in comparison studies, the value of Pearson correlation coefficient "r" should be greater than 0.95, the slope of the regression line should be 0.9-1.1, and the y-intercept is close to zero. In addition to that, all results should lie always within the lines of the 95% CI (Confidence Interval) (Snyder and Larsen, 1983). Two questionnaires were prepared; one for patients' group and the other for the apparently healthy persons that we considered as control group.

Our patients' group included those suffering from different disease conditions of either hospitalized patients or patients presenting at the outpatient clinics in Bethlehem Arab Society for Rehabilitation "Special Surgical Hospital" and Beit-Jala Governmental Hospital. The 105 patients included in our study were 69 females (65.7%), the range of the age was 19-83 and the average of the age was 42.3, while males included 36 patients (34.3%), the range of the age was 10-75 and the average of the age was 47.9 (Table 3.1).

The control group was introduced in this study to evaluate if the three different methods correlate well at the level of normal values for ESR. However, most manufactures, if not all, claim that their methods correlate well with classic Westergren method (LP

Italiana S.P.A., Vital Diagnostic, Kima Sed). Hence, the control group was used also to assess their claim if the correlation obtained is accepted or worthless.

The control group included 95 samples obtained from apparently healthy volunteers working in Bethlehem Arab Society for Rehabilitation "Special Surgical Hospital" and Beit-Jala Governmental Hospital. Additionally, some of the control group volunteers brought some of their healthy family members for participation in the control group. They were 26 females (27.4%), the range age was 11-80 and the average of the age was 33.9. While males included 69 (72.6%), the range of the age was 18-75 and the average of the age was 32.6 (Table 3.1).

Table 3.1: The frequency of patients' and control group depending on gender.

Frequency of patients' group			
	Frequency	**Percent**	**Average age / Age range**
Female	69	65.7	42.3/19-83
Male	36	34.3	47.9/10-75
Total	105	100.0	-----
Frequency of control group			
	Frequency	**Percent**	**Average age / Age range**
Female	26	27.4	33.9/11-80
Male	69	72.6	32.6/18-75
Total	95	100.0	-----

The control group was chosen according to the following criteria: any subject being in good health, not suffering from any chronic diseases and not taking any medications at the time of sample collection and they are volunteers for this study.

On the other hand patients' group included different diseases that were distributed according to Table 3.2.

Table 3.2: The distribution of different diseases in patients' group.

Disease	**Number of Patients**	**Age Range (Years)**
Cancers	16	51-64
Renal Failure	11	40-50
Hypertension	9	54-63
Leukemia	10	30-57
Lymphoma	4	32-38
Chronic Heart Failure	10	45-52
Infections	12	10-25
Pregnancy**	5	21-27
Anemia	7	37-83
Rheumatoid Arthritis	5	65-74
Disseminated Intravascular Coagulopathy	4	35-39
Renal Transplantation	2	30-32
Diabetes	4	65-73
Discectomy	6	52-57
Total	105	------

**Pregnancy is not a disease, but included here because it is associated with an increase in ESR.

3.2 Statistical Analysis of the Data for Patients' Group

The descriptive statistics of ESR values for each method (Westergren, Automated and Manual) using EDTA and sodium citrate as anticoagulant for patients' group are listed in Table 3.3.

Table 3.3: The descriptive statistics of patients' group, n=105 patients.

Descriptive statistics for patients' group						
	Quick Na Citrate	**Automated Na Citrate**	**Westergren Na Citrate**	**Quick EDTA**	**Automated EDTA**	**Westergren EDTA**
Mean	35.3	29.0	39.4	50.3	46.5	47.7
Standard Deviation (SD)	24.43	18.41	24.64	24.71	18.94	25.18

Table 3.4: Paired samples test for patients group, n = 105 patients.

Paired samples test for patients' group		
		***p* - Value**
Pair 1	Westergren Na Citrate - Westergren EDTA	<0.001
Pair 2	Automated Na Citrate - Westergren Na Citrate	<0.001
Pair 3	Quick Na Citrate - Westergren Na Citrate	0.014
Pair 4	Automated EDTA - Westergren EDTA	0.455
Pair 5	Quick EDTA - Westergren EDTA	0.171

The descriptive statistical results for patients' group showed that there was a large variation in the mean values when compared using different anticoagulants for the same method. For Quick citrate and Quick EDTA, the results showed that the mean ESR values were 35.3 and 50.3, respectively. Automated citrate and Automated EDTA showed that mean ESR values were 29.0 and 46.5, respectively. Finally, results for Westergren citrate and Westergren EDTA showed that mean ESR values were 39.4 and 47.7, respectively, as shown in Table 3.3

Furthermore, when comparing the different methods used, results obtained showed that Quick method and Westergren method appeared to have higher mean values than Automated method for the same anticoagulant used. For citrated blood, mean ESR values were 35.3 for Quick method and 39.4 for Westergren method and 29.0 for Automated method. While for EDTA blood mean ESR values were 50.3, 47.7, 46.5, for Quick method, Westergren method and for Automated method, respectively, as shown in Table 3.3.

Table 3.3 showed high variations in mean ESR values for different methods when EDTA and citrated blood were used; these variations were reflected by the high standard deviation obtained for each method. For Quick citrate, Automated citrate and Westergren citrate the standard deviations were 24.43, 18.41 and 24.65 respectively. While for Quick EDTA, Automated EDTA and Westergren EDTA the standard deviations were 24.71, 18.94 and 25.18 respectively.

Table 3.4 showed the results of paired t-test among the different methods. It showed that there was a significant difference results between Westergren citrate and Westergren EDTA ($p<0.001$), Automated citrate and Westergren citrate ($p<0.001$), Quick citrate and Westergren citrate ($p= 0.014$). While there was no significant difference between Automated EDTA and Westergren EDTA ($p= 0.455$) and Quick EDTA and Westergren EDTA ($p= 0.171$) .

3.3. Pearson Correlation for Patients' Group

To investigate the correlation between the different ESR measurement methods and different anticoagulants used in this study, Pearson correlation test was done to analyze the results obtained from patients' group as shown in Table 3.4.

Table 3.5: Pearson Correlation for patients' group, n= 105 patients.

Pearson Correlation for patients' group							
		Westergren Na Citrate	**Westergren EDTA**	**Quick Na Citrate**	**Automated Na Citrate**	**Quick EDTA**	**Automated EDTA**
Westergren Na Citrate	Pearson Correlation (r)	1.00	0.834**	0.774**	0.870**	0.814**	0.819**
	p - value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Westergren EDTA	Pearson Correlation (r)		1.00	0.575**	0.714**	0.718**	0.754**
	p - value			< 0.001	< 0.001	< 0.001	< 0.001
Quick Na Citrate	Pearson Correlation (r)			1.00	0.802**	0.778**	0.664**
	p - value				< 0.001	< 0.001	< 0.001
Automated Na Citrate	Pearson Correlation (r)				1.00	0.747**	0.745**
	p - value					< 0.001	< 0.001
Quick EDTA	Pearson Correlation (r)					1.00	0.688**
	p - value						< 0.001
Automated EDTA	Pearson Correlation (r)						1.00
****. Correlation is significant at the 0.05 level.**							

A positive correlation was obtained for Westergren citrate versus other methods. The "r" values for Westergren EDTA, Quick citrate, Automated citrate, Quick EDTA and Automated EDTA when compared to Westregren citrate were (r = 0.834, $p < 0.001$), (r = 0.774, $p < 0.001$), (r = 0.870, $p < 0.001$), (r = 0.814, $p < 0.001$) and (r = 0.819, $p < 0.001$), respectively.

A positive correlation was also obtained for the relationship between Westergren EDTA and Westergren citrate (r = 0.834, $p < 0.001$). While the "r" values were as follows for Quick citrate (r = 0.575, $p < 0.001$), for Automated citrate (r = 0.714, $p < 0.001$), for Quick EDTA (r = 0.718, $p < 0.001$) and for Automated EDTA (r = 0.754, $p < 0.001$) when compared with Westergren EDTA.

In comparison studies, however, a correlation coefficient greater than 0.95 is considered good (Snyder and Larsen, 1983). In correlation analysis shown in Table 3.4, none of the "r" values exceeded 0.9.

3.4. Scatter Plots for Patients' Group

3.4.1. Correlation Between ESR Values Using Westergren Citrate and Westergren EDTA

The Westergren citrate and Westergren EDTA are the two recognized comparative methods for citrated blood and EDTA blood. However, in this analysis, the Westergren citrate method was chosen to be the comparative method and the Westergren EDTA as the test method.

Figure 3.1 plots the correlation between Westergren citrate and Westergren EDTA (r = 0.8335, $p < 0.001$). This means that there was a significant positive correlation. Despite this correlation, there was a variation in the results as most of the results are either above or below the 95% CI of the regression line. Only 22 values fall within the 95% CI lines. Also Figure 3.1 showed extreme results, such as those rounded by circles. For example, one had a value of 8 for Westergren citrate and zero for Westergren EDTA, while the other had a result of 118 for Westergren citrate and 58 for Westergren EDTA. In general, Westergren EDTA blood gives higher ESR values than Westergren citrated blood, and as the ESR values increases, the deviation increases.

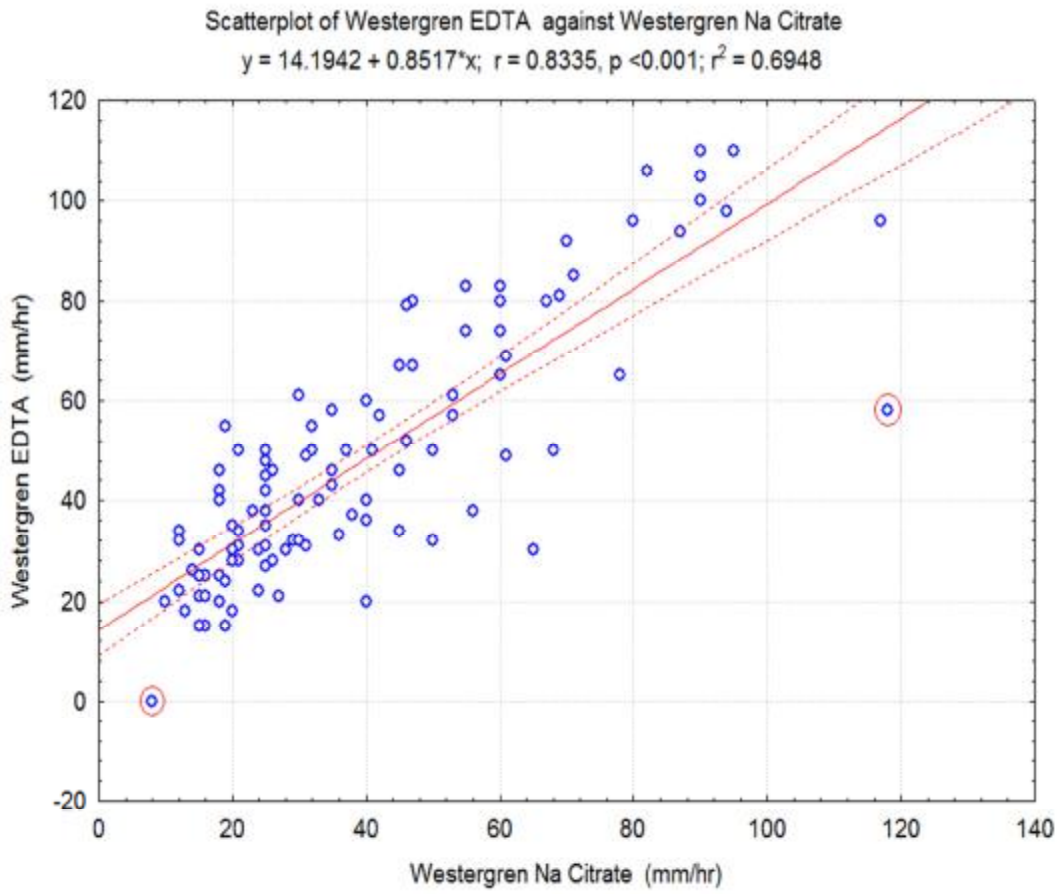


Figure 3.1: The correlation between ESR values using Westergren citrate and Westergren EDTA in patients' group. The dotted lines around the regression line represent the 95% CI, n = 105 patients.

The harmony or consistency of results among the two methods is not clear since r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

3.4.2. Correlation Between ESR Values Using Automated Citrate and Automated EDTA

The correlation between ESR values measured by Automated citrate and Automated EDTA methods is shown in Figure 3.2. The value for r = 0.7446 and $p < 0.001$, which means that there was a significant positive correlation.

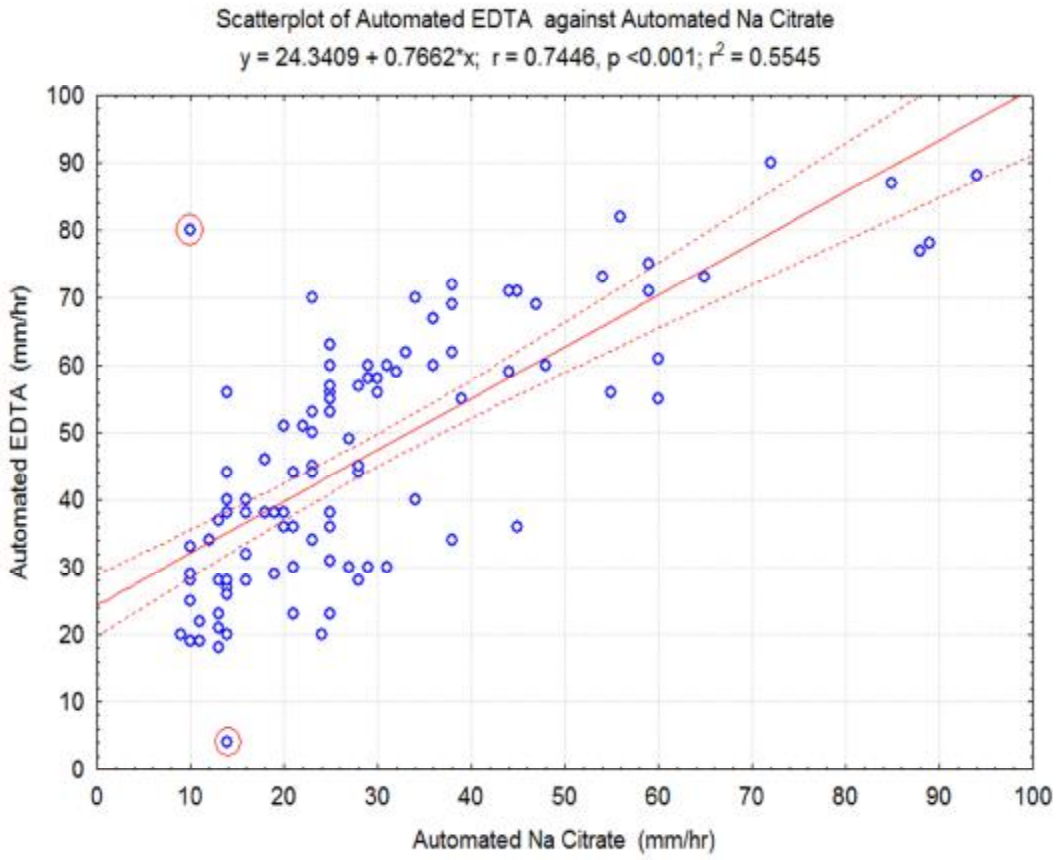


Figure 3.2: The correlation between ESR values using Automated citrate and Automated EDTA in patients' group. The dotted lines around the regression line represent the 95% CI, n= 105 patients.

Despite this correlation, there was a variation in the values as most of the values fall either above or below the lines that represent 95% CI for the regression line. Only 19 values fall within the 95% CI lines. Extreme results also exist such as those rounded by

circles. One had a result of 10 for Automated citrate and 80 for Automated EDTA, the other had a result of 14 for Automated citrate and 4 for Automated EDTA. ESR values close to the lower limit of the range (< 30 mm/hr for both variables), had small deviation from each other, while ESR values close to the upper limit of the range (> 30 mm/hr for both variables), had higher deviation. In general, Automated EDTA gave higher values.

The harmony or consistency of results among the two methods is not clear since r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

3.4.3. Correlation Between ESR Values Using Automated Citrate and Westergren citrate

Figure 3.3 shows the correlation between ESR values measured by Automated citrate and Westergren citrate methods. There was a positive correlation as r = 0.8700, p < 0.001.

Despite this correlation, there was a variation in the values as most of the values fall either above or below the lines that represent 95% CI for the regression line. Only 28 values fall within the 95% CI lines. Extreme results also exist such as those rounded by circles. One had a result of 10 for Westergren citrate and 45 for Automated citrate, the other had a result of 82 for Westergen citrate and 89 for Automated citrate. ESR values close to the lower limit of the range (< 30 mm/hr for Automated citrate and, 40 mm/hr for Westergren citrate), were comparable in both methods. However, the deviation of these results increases as results increase. In general, values obtained from Automted citrate methods gave lower values than those of Westregren citrate.

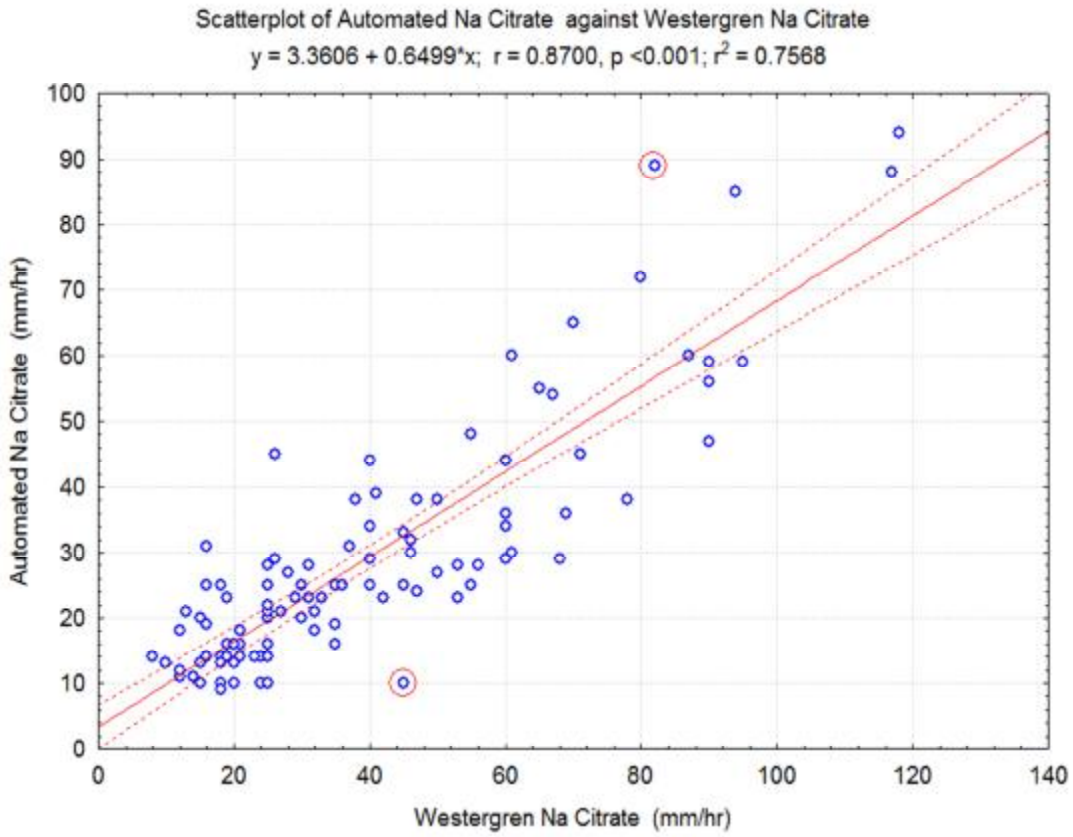


Figure 3.3: The correlation between ESR values using Automated citrate and Westergren citrate in patients' group. The dotted lines around the regression line represent the 95% CI, n= 105 patients.

The harmony or consistency of results among the two methods is not clear since r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

3.4.4. Correlation Between ESR Values Using Quick EDTA and Quick Citrate

The correlation between ESR values measured by Quick EDTA and Quick citrate methods is shown in Figure 3.4. The r = 0.7781, $p < 0.001$, which means that there was a significant positive correlation. Despite this correlation, there was a variation in the

values as most of the values fall either above or below the lines that represent 95% CI for the regression line. Only 15 values fall within the 95% CI lines. Extreme results also exist such as those rounded by circles. One had a result of zero for Quick EDTA and 30 for Quick citrate, the other had a result of 75 for Quick EDTA and 15 for Quick citrate.

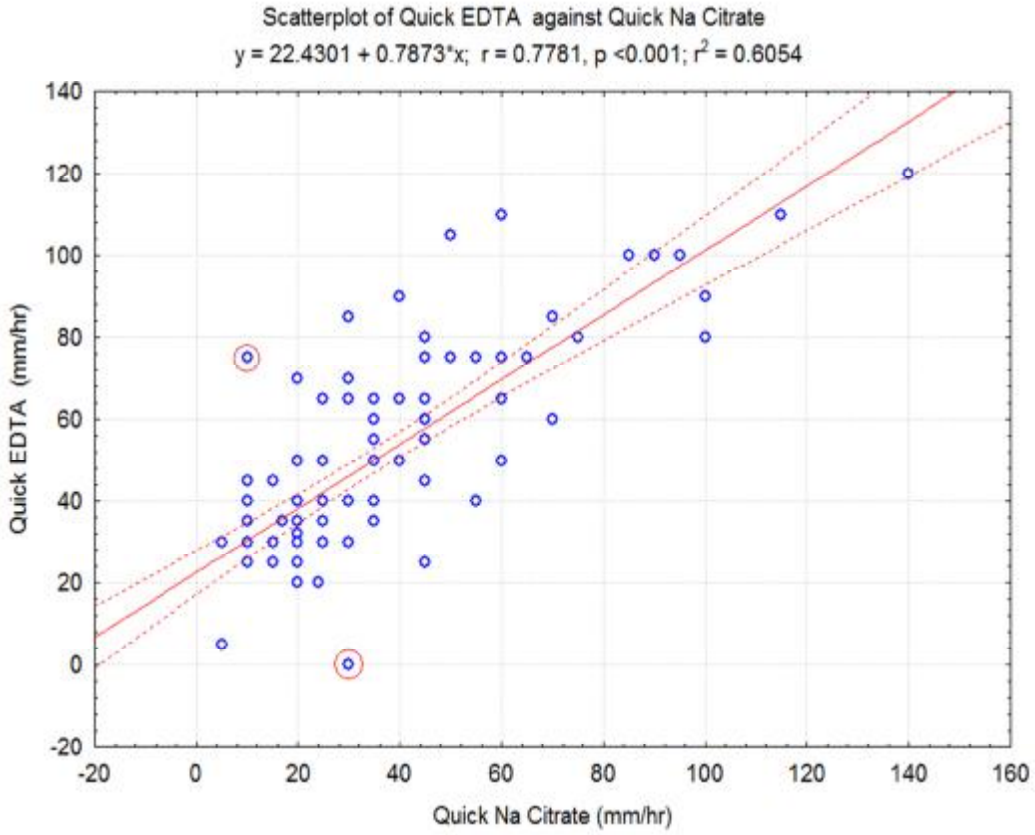


Figure 3.4: The correlation between ESR values using Quick citrate and Quick EDTA in patients' group. The dotted lines around the regression line represent the 95% CI, n = 105 patients.

The harmony or consistency of results among the two methods is not clear because r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

3.4.5. Correlation Between ESR Values Using Quick Citrate and Westergren Citrate

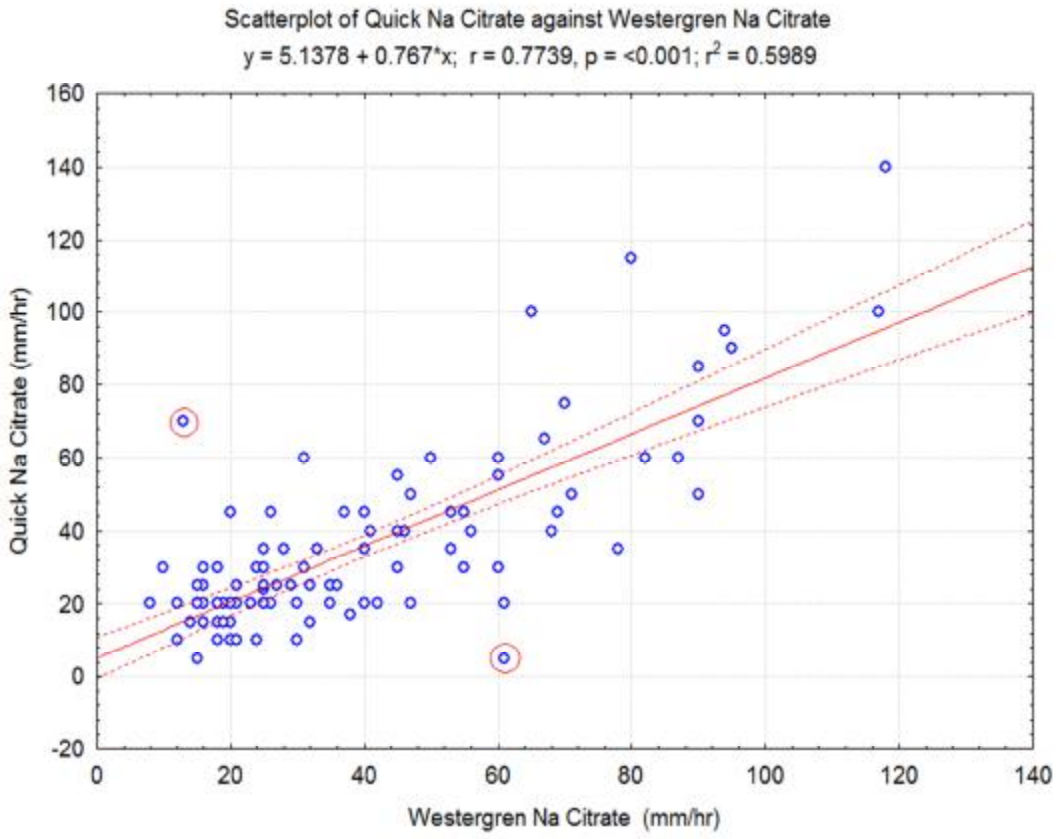


Figure 3.5: The correlation between ESR values using Quick citrate and Westergren citrate in patients' group. The dotted lines around the regression line represent the 95% CI, n = 105 patients.

There was a significant positive correlation between Westergren citrate and Quick citrate as r = 0.7739 and $p < 0.001$, as shown in Figure 3.5. Also, most of the values fall either above or below the lines that represent the 95% CI for the regression line. Only, 22 values fall within 95% CI lines. Despite that extreme results exist as those rounded by circle. One of them had a result of 61 for Westergren citrate and 5 for Quick citrate, while the other had 18 for Westergren citrate and 70 for Quick citrate. The deviation of

these values increases as they increase. In general, Quick citrate gave lower values than Westergren citrate.

The harmony or consistency of results among the two methods is lost as r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

3.4.6. Correlation Between ESR Values Using Quick EDTA and Westergren EDTA

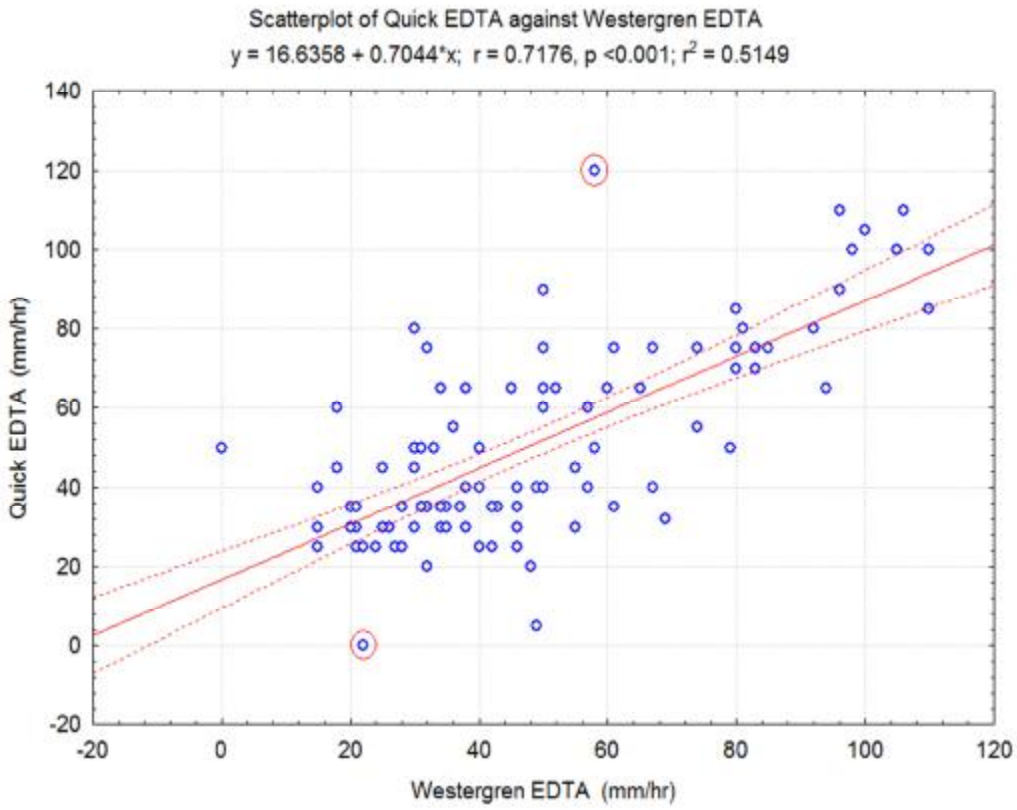


Figure 3.6: The correlation between ESR values using Quick EDTA and Westergren EDTA in the patients' group. The dotted lines around the regression line represent the 95% CI, n = 105 patients.

The correlation between ESR values measured by Westergren EDTA and Quick EDTA methods showed that there was a significant positive correlation between these two variables, r = 0.7176 and $p < 0.001$ as shown in Figure 3.6. Despite this correlation, there was a variation in the values as most of the values fall either above or below the lines that represent 95% CI for the regression line. Only 19 values fall within the 95% CI lines. Extreme results also exist such as those rounded by circles. One had a result of 120 for Quick EDTA and 58 for Westergren EDTA; the other had a result of zero for Quick EDTA and 22 for Westregren EDTA.

The harmony or consistency of results among the two methods is lost as r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

3.4.7. Correlation Between ESR Values Using Automated EDTA and Westergren EDTA

Figure 3.7 showed that therewas a significant positive correlation between ESR values measured by Westergren EDTA and Automated EDTA methods, as r = 0.7540 and $p < 0.001$. Despite this correlation, there was a variation in the values as most of the values fall either above or below the lines that represent 95% CI for the regression line. Only 27 values are within the 95% CI lines. Extreme results also exist such as those rounded by circles. One had a result of 80 for Westergren EDTA and 20 for Automated EDTA; the other had a result of 22 for Westergen EDTA and 80 for Automated EDTA.

The harmony or consistency of results among the two methods is lost as r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

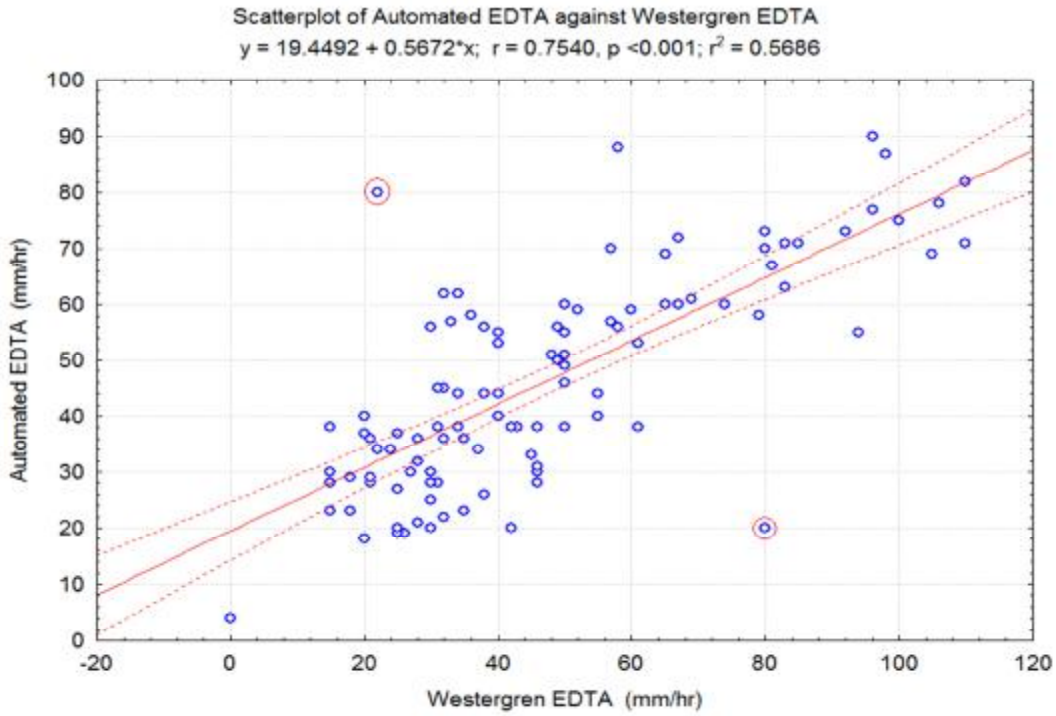


Figure 3.7: The correlation between ESR values using Automated EDTA and Westergren EDTA in patients' group. The dotted lines around the regression line represent the 95% CI, n=105 patients.

3.5. Statistical Analysis of the Data for Control Group

The descriptive statistics for each method (Westergren, Automated, Quick) using EDTA and citrated blood for apparently healthy control group are listed in Table 3.5.

Table 3.5: The descriptive statistics of control group, n = 95 person.

Descriptive statistics for control group						
	Quick Na Citrate	**Automated Na Citrate**	**Westergren Na Citrate**	**Quick EDTA**	**Automated EDTA**	**Westergren EDTA**
Mean	6.5	6.2	5.9	8.0	1.0	8.6
Standard Deviation (SD)	6.10	4.40	5.38	6.37	7.96	8.27

These results showed that there was variation in the mean of values using different anticoagulants for the same method. For Quick citrate and Quick EDTA, the mean was 6.5 and 8.0, respectively. For Automated citrate and Automated EDTA show that it was 6.2 and 1.0, rspectively. Finally, for Westergren citrate and Westergren EDTA their means were 5.9 and 8.6 respectively. When comparing different methods, the mean for Quick citrated blood was 6.5, while for Automated citrate was 6.2, for Westergren citrated blood it was 5.9. For EDTA blood it was 1.0 for Automted method, 8.6 for Westergren method and 8.0 for Quick method.

For standard deviation (Table 3.5) showed that the results for Quick citrate, Automated citrate and Westergren citrate it was 6.10, 4.40 and 5.38 respectively. While for Quick EDTA, Automated EDTA and Westergren EDTA it was 6.37, 7.96 and 8.27 respectively. So, these results express the fact that there was huge variation in the different methods used in this study for both anticoagulants used.

Table 3.7: Paired samples test for control group, n = 95 person.

Paired samples test for control group		
		***p*- value**
Pair 1	Westergren Na Citrate - Westergren EDTA	< 0.001
Pair 2	Automated Na Citrate - Westergren Na Citrate	0.497
Pair 3	Quick Na Citrate - Westergren Na Citrate	0.368
Pair 4	Automated EDTA - Westergren EDTA	0.016
Pair 5	Quick EDTA - Westergren EDTA	0.416

Table 3.7 showed the results of paired t-test among the different methods. It showed that there was a significant difference in the results between Westergren citrate and Westergren EDTA (p<0.001), Automated EDTA and Westergren EDTA (p= 0.016). While there was no significant difference between Automated citrate and Westergren citrate (p= 0.497), Quick citrate and Westergren citrate (p= 0.368), Quick EDTA and Westergren EDTA (p= 0.416) .

3.6. Pearson Correlation for Control Group

To identify the correlation between different methods and different anticoagulants used in this study, Pearson correlation test was done to analyze the results obtained from control group as shown in Table (3.8).

A positive correlation was obtained for Westergren citrate versus other methods. The "r" values for Westergren EDTA, Quick citrate, Automated citrate, Quick EDTA and Automated EDTA when compared to Westregren citrate were (r = 0.863, p < 0.001), (r = 0.436, p < 0.001), (r = 0.767, p < 0.001), (r = 0.651, p < 0.001) and (r = 0.758, p < 0.001), respectively.

A positive correlation was also obtained for the relationship between Westergren EDTA and Westergren citrate (r = 0.863, p < 0.001). While the "r" values were as follows for Quick citrate (r = 0.348, p < 0.001), for Automated citrate (r = 0.670, p < 0.001), for Quick EDTA (r = 0.548, p < 0.001) and for Automated EDTA (r = 0.755, p < 0.001) when compared with Westergren EDTA.

In comparison studies, however, a correlation coefficient greater than 0.95 is considered good (Snyder and Larsen, 1983). In correlation analysis shown in Table 3.4, none of the "r" values exceeded 0.9.

Table 3.8: Pearson Correlation for control group, n = 95 person.

Pearson Correlation for control group							
		Westergren Na Citrate	**Westergren EDTA**	**Quick Na Citrate**	**Automated Na Citrate**	**Quick EDTA**	**Automated EDTA**
Westergren Na Citrate	Pearson Correlation (r)	1.00	0.863**	0.436**	0.767**	0.651**	0.758**
	p - value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Westergren EDTA	Pearson Correlation (r)		1.00	0.348**	0.670**	0.548**	0.755**
	p- value			< 0.001	< 0.001	< 0.001	< 0.001
Quick Na Citrate	Pearson Correlation (r)			1.00	0.499**	0.659**	0.308**
	p - value				< 0.001	< 0.001	0.002
Automated Na Citrate	Pearson Correlation (r)				1.00	0.578**	0.686**
	p- value					< 0.001	< 0.001
Quick EDTA	Pearson Correlation (r)					1.00	0.619**
	p - value						< 0.001
Automated EDTA	Pearson Correlation (r)						1.00
	p - value						
****. Correlation is significant at the 0.05 level.**							

3.7. Scatter Plots for Control Group

3.7.1. Correlation Between ESR Values Using Westergren EDTA and Westergren Citrate

The following scatter plots show the correlation between Westergren citrate and Westergren EDTA, Automated citrate and Automated EDTA, Westergren citrate and Automated citrate, Quick citrate and Quick EDTA, Westergren citrate and Quick citrate, Westergren EDTA and Quick EDTA, Westergren EDTA and Automated EDTA for the control group.

Figure 3.8 plots that the correlation between ESR values measured by Westergren citrate and Westergren EDTA ($r = 0.8627$, $p < 0.001$), which showed that a positive correlation exist. Despite this correlation, there was a variation in the values as most of the values lie either above or below the lines that represents 95% CI for the regression line. Only, 13 results fall within the 95% CI lines. Also extreme results exist as those rounded by circles. One had a result of 10 for Westergren citrate and 30 for Westergren EDTA; the other had a result of 15 for Westergren citrate and 11 for Westergren EDTA.

The harmony or consistency of results among the two methods is lost as $r < 0.95$, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

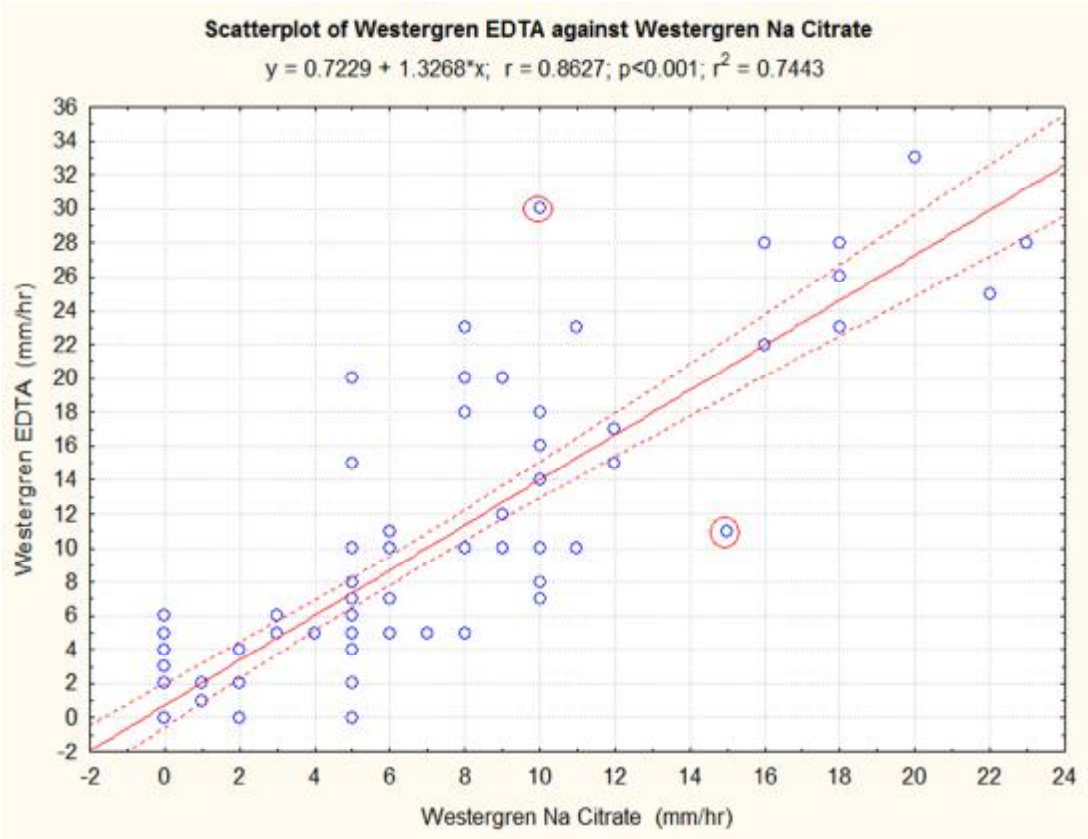


Figure 3.8: The correlation between ESR values using Westergren EDTA and Westergren citrate in control group. The dotted lines around the regression line represent the 95% CI, n = 95 person.

3.7.2. Correlation Between ESR Values Using Automated EDTA and Automated Citrate

The correlation between ESR values measured by Automated EDTA and Automated citrate are shown in Figure 3.9, r = 0.6859, $p < 0.001$, it showed that there was a positive correlation. Despite this correlation most of the values fall either above or below the lines that represent 95% CI for the regression line. Only, 14 results fall within the 95% CI lines. Also, most of the values fall below 7 mm/ hr for Automated citrate and below 15 mm/hr for Automated EDTA. Extreme results also exist as those rounded

by circle. One had a result of 34 for Automated EDTA and 8 for Automated citrate, and the other had a results of 13 for Automated citrate and 4 for Automated EDTA.

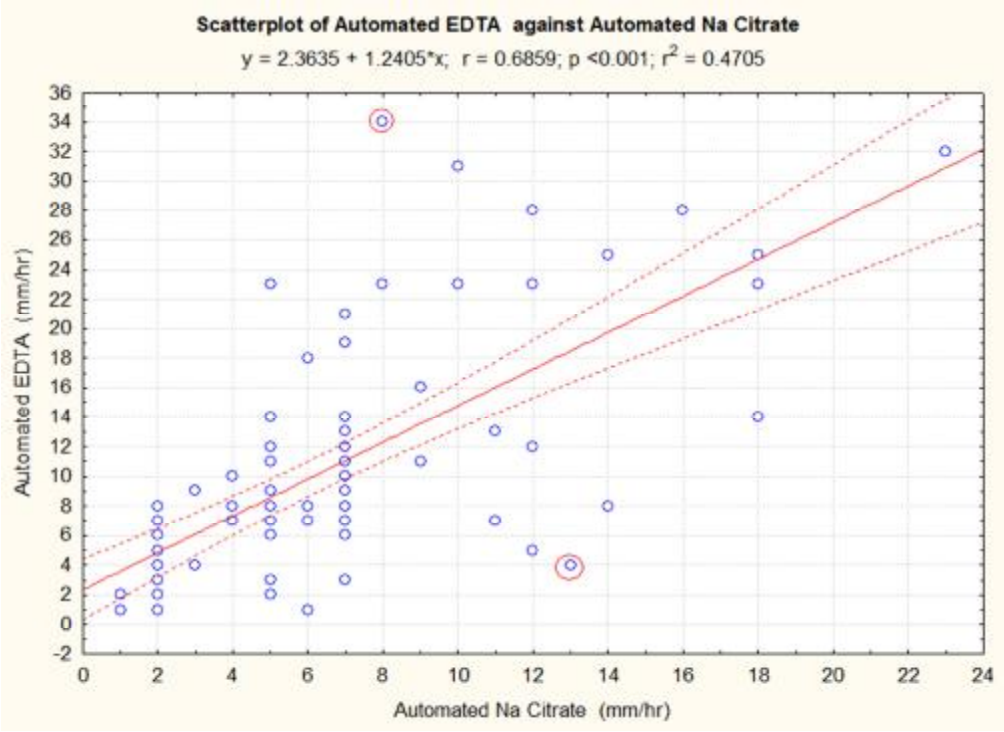


Figure 3.9: The correlation between ESR values using Automated EDTA and Automated citrate in control group. The dotted lines around the regression line represent the 95% CI, n = 95 person.

The harmony or consistency of results among the two methods is lost as r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

3.7.3. Correlation Between ESR Values Using Automated Citrate and Westergren Citrate

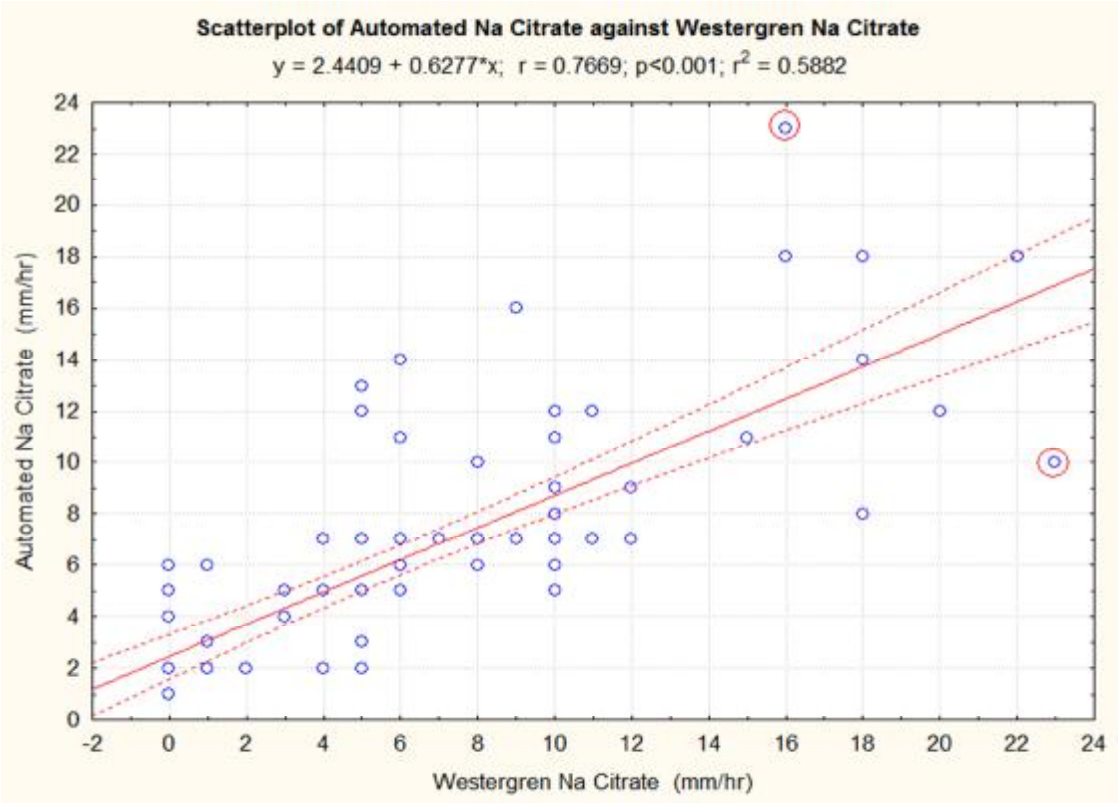


Figure 3.10: The correlation between ESR values using Automated citrate and Westergren citrate in control group. The dotted lines around the regression line represent the 95% CI, n = 95 person.

Figure 3.10 showed the correlation between ESR values measured by Automated citrate and Westergren citrate, it also showed that there was a positive correlation as r = 0.7669, $p < 0.001$. The values fall either above or below the lines that represent the 95% CI for regression line despite this positive correlation. Only, 12 results fall within the 95% CI lines. Also, most of the results are ≤ 10 mm/hr for Westergren citrate and ≤ 12 mm/hr for automated citrate. Extreme results also exist, those that are rounded by circle are examples, one of them had a result of 16 for Westergren citrate and 23 for

Automated citrate, while the other had a result of 23 for Westergren citrate and 10 for Automated citrate.

The harmony or consistency of results among the two methods is lost as r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

3.7.4. Correlation Between ESR Values Using Quick EDTA and Quick Citrate

The correlation between ESR values measured by Quick EDTA and Quick citrate are shown in Figure 3.11, r = 0.6595, $p < 0.001$, this means that a positive correlation exist. Despite this correlation, the values fall both above or below the lines that represent 95% CI for the regression line. Only 2 values fall within the 95% CI lines. Also, we extreme results exist; those values rounded by circle are examples. One had a result of 20 for Quick EDTA and 5 for Quick citrate, while the other had a result of 15 for Quick citrate and zero for Quick EDTA. Repeated results existed and are responsible for the low number of plots in this figure.

The harmony or consistency of results among the two methods is lost as r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

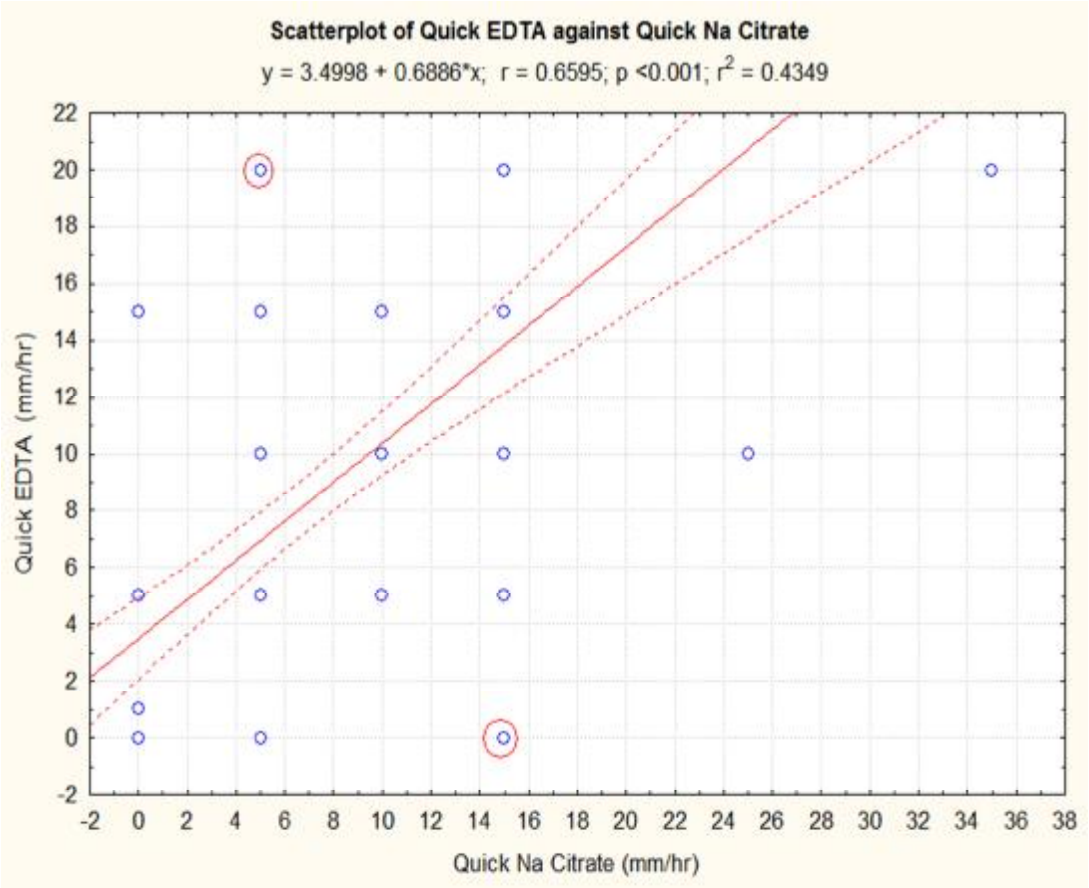


Figure 3.11: The correlation between ESR values using Quick EDTA and Quick citrate in control group. The dotted lines around the regression line represent the 95% CI, n = 95 person.

3.7.5. Correlation Between ESR Values Using Quick Citrate and Westergren Citrate

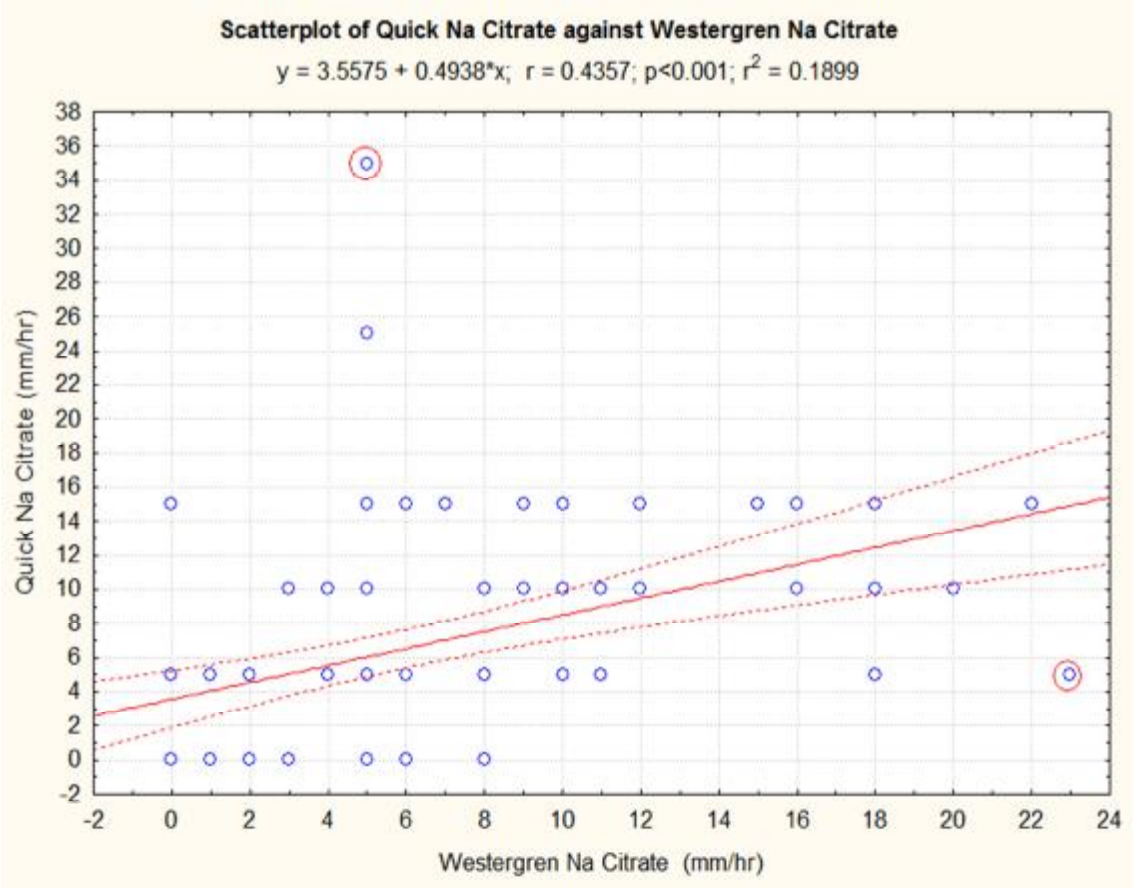


Figure 3.12: The correlation between ESR values using Quick citrate and Westergren citrate and in control group. The dotted lines around the regression line represent the 95% CI, n = 95 person.

Figure 3.12 showed the correlation between ESR values measured by Quick citrate and Westergren citrate; r = 0.4357 and $p < 0.001$, which means that a positive correlation exist. Despite this correlation most of the values were distributed above and below the lines that represent 95% CI for the regression line. Only, 10 values fall within the 95% CI lines. Also, extreme results exist; as those rounded by circle, one of them had a result of 23 for Westergren citrate and 5 for Quick citrate, the other had a result of 5 for Westergren citrate and 35 for Quick citrate.

The harmony or consistency of results among the two methods is lost as r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

3.7.6 Correlation Between ESR Values Using Quick EDTA and Westergren EDTA

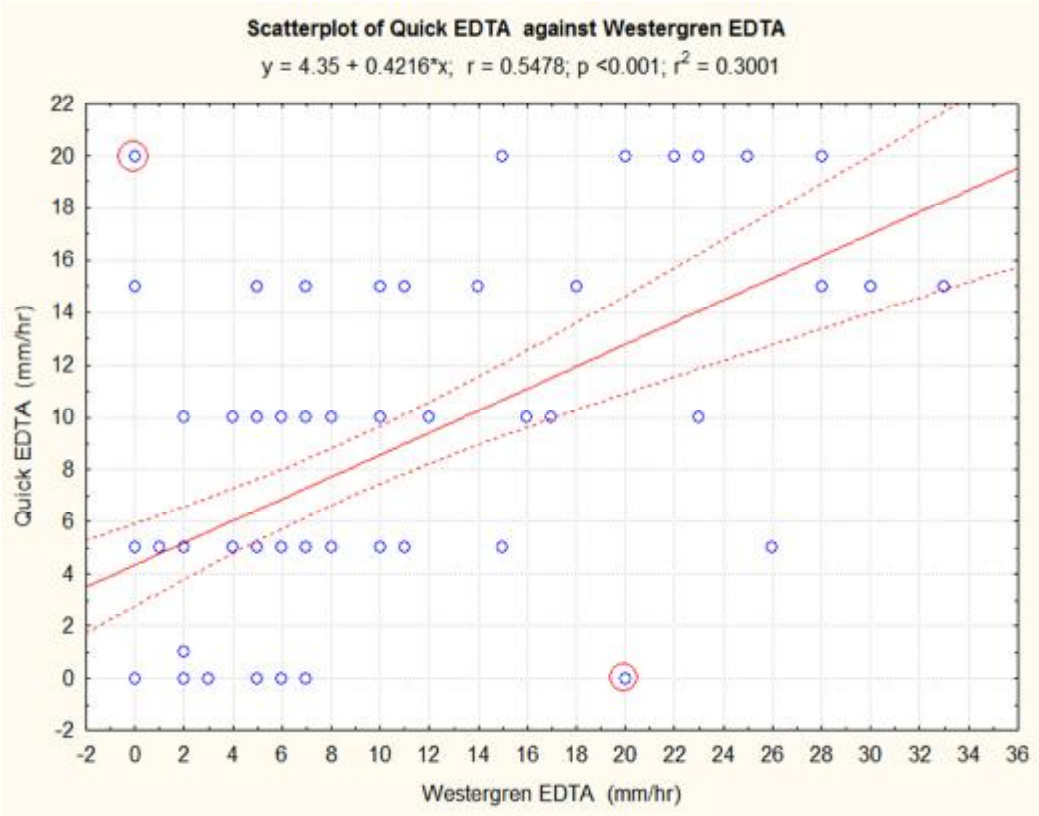


Figure 3.13: The correlation between ESR values using Quick EDTA and Westergren EDTA in control group. The dotted lines around the regression line represent the 95% CI, n = 95 person.

There was a positive correlation between ESR values measured by Westergren EDTA and Quick EDTA, r = 0.5478, $p < 0.001$ (Figure 3.13). Despite that most of the values fall above or below the lines that represent the 95% CI for the regression line. Only 8 values fall within the 95% CI lines. Extreme values also exist; those that are rounded by circle are examples. One had a result of zero for Westergren EDTA and 20 for Quick EDTA, while another one had a result of 20 for Westergren EDTA and zero for Quick EDTA.

The harmony or consistency of results among the two methods is lost as r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

3.7.7. Correlation Between ESR Values Using Automated EDTA and Westergren EDTA

A positive correlation between ESR values measured by Automated EDTA and Westergren EDTA exist, as 0.7547, $p < 0.001$, as shown in Figure 3.14. However, most of the values fall above or below the lines that represent 95% CI for the regression line. Only 18 values fall within the 95% CI lines. Also, most of the values are ≤ 10 mm/hr for Westergren EDTA and below 15 mm/hr for Automated EDTA. Extreme values also exist, those that are rounded by circle. One of the them had a value of 20 for Westergren EDTA and 2 for Automated EDTA; the other had result of 34 for Automated EDTA and 14 for Westergren EDTA.

The harmony or consistency of results among the two methods is lost as r < 0.95, slope of the regression line < 0.9, and the y-intercept is not close to zero (Snyder and Larsen, 1983).

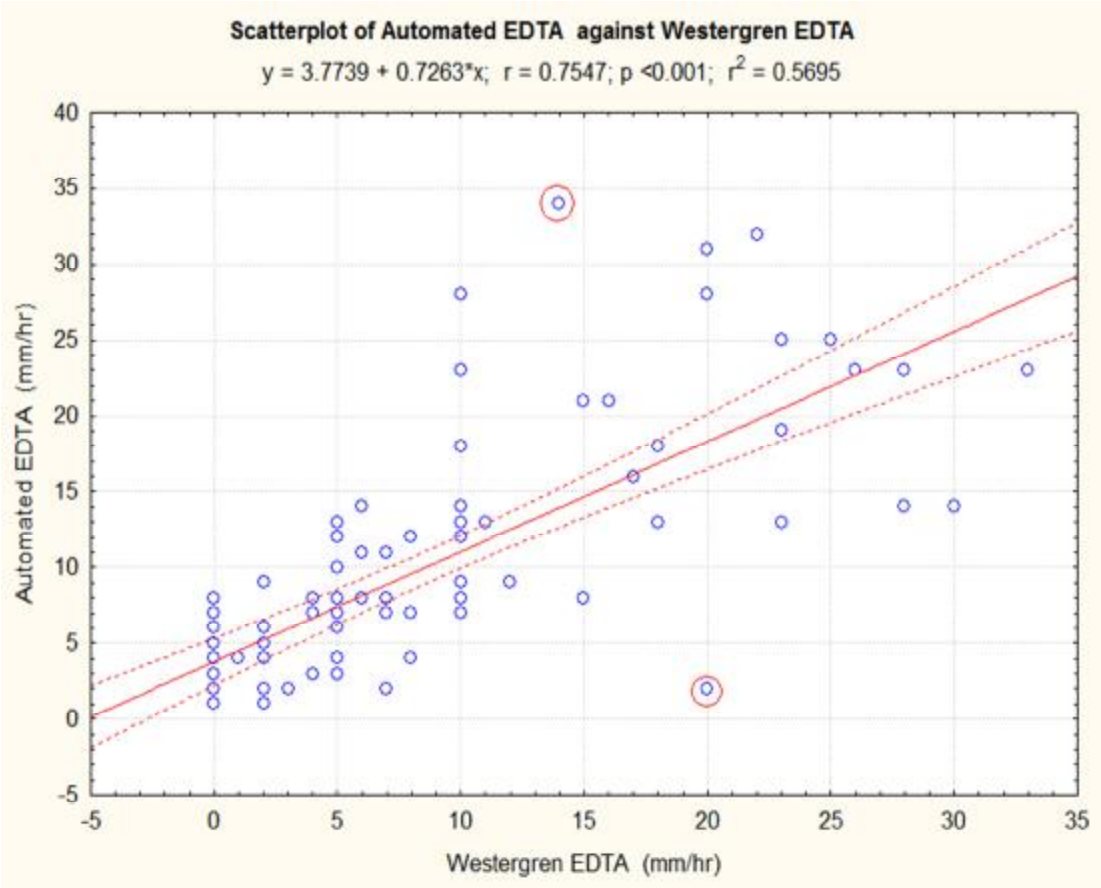


Figure 3.14: The correlation between ESR values using Westergren EDTA and Automated EDTA in control group. The dotted lines around the regression line represent the 95% CI, n = 95 person.

Chapter Four: Discussion

4.1. Discussion

Erythrocyte sedimentation rate (ESR) is a simple, inexpensive, non specific test that is used to differentiate diseases with similar symptoms such as myocardial infarction versus angina pectoris. It is also used to differentiate between acute from chronic infection and inflammation. Traditionally this test was used for the diagnosis of rheumatoid arthritis and other collagen diseases such as polymyalgia rheumatica or giant cell arthritis and rheumatic fever. ESR was also shown to be of value in the early diagnosis of infective hepatitis (Wood, 1945; Deshpande *et al.*, 1971; Epperly *et al.*, 2000).

ESR test serves as a "sickness index" in conjunction with the patient's clinical history and physical examination findings (Koepke, 2002). Rouleaux formation and RBC clumping are greatly enhanced by acute phase reactants in plasma: fibrinogen, haptoglobin, ceruloplasmin, α1 acid-glycoprotein and C- reactive protein. ESR is increased by immunoglobulins, but decreased by albumin (Abbag and Al Qahtani, 2007).

The sedimentation of red cells in plasma is complex and consists of three phases: lag, decantation, and packing, which vary greatly and are interrelated in a complicated manner. So the sedimentation time for each phase may vary from patient to another (Eastham *et al.*, 1958; Koepke, 2002).

ICSH considered the Westergren method as a reference method for ESR (Alexy *et al.*, 2009). This method is based on using diluted blood samples (4 volumes of blood plus 1

volume of 3.8% sodium citrate) in open ended glass tubing which is a straight pipette that is 300 ± 1.5 mm long, the tube bore 2.55 ± 0.15 mm; uniformity of tube bore ± 0.05 mm (Lewis , 1973; ICSH, 1993). They should be mounted vertically in a rack or stand.

Because of the biohazard risk inherent in performing the Westergren-based method, as the traditional Westergren method was designed that mechanical/mouth suction is used to fill the pipette with the sample, the ICSH introduced a standardized method as an alternative and potential replacement for the reference method. The modified method includes using EDTA blood instead of citrated samples (ICSH, 1993).

Nowadays, the closest method to the classical Westergren method is the Sediplast® tubes that are manufactured by LP Italiana S.P.A./ Italy. The tube contains 0.2 mL of 3.8% Na-Citrate anticoagulant and needs 0.8 mL of blood (free of anticoagulants or EDTA-blood) to fill it. The graduated pipette that is a transparent plastic receives blood from the test tube leaving an air space in such a way that, by adjusting the volume of the air space, it is possible to bring the blood level to zero. The pipette has a length of 200 mm that is graduated from 0-150 ± 0.35; the bore size is 2.5 ± 0.15 mm and the uniformity of bore ± 0.05 mm. In this study we considered it as a reference/comparative method and we called it “Westergren“.

The effect of the materials used in manufacturing the ESR tubes had been studied. The glass VACUETTE® tubes versus the plastic VACUETTE® tubes and the anticoagulant used in both was trisodium citrate; the results obtained from both tubes showed no significant statistical difference (Evaluation of VACUETTE® Plastic ESR Tube with the manual closed ESR measurement, 2011).

In Palestine and up to my knowledge, all ESR tubes used in the clinical laboratories are plastic, exept those used by the Automated systems.

Our study, which is the first study of its kind performed in Palestine, compares different methods available for measuring ESR using different anticoagulants. In this study, Quick, Automated and Westergen methods are compared using sodium citrated and EDTA as anticogulant.

The ESR results obtained for patients' group in appendix I showed inconsistency and lack of harmony among the three methods used for the same patient. It is rare enough to have similar or close results for the same patient by the three different methods using the same anticoagulant. Even when the results of the same method using citrate and EDTA were compared, there was high variability and inconsistency in the results. Sometimes it gave similar results but very often it gave either higher or lower results. This indicates the presence of an unkown intrinsic factor influenced by the type of anticoagulant and eventually affect the rheology of RBCs.

The results of this study also showed that there was a positive correlation between the three different methods using either EDTA or citrate as anticoagulant for both groups (patients' and control) as shown in Tables 3.4 and 3.6. However, none of the correlation coefficients was > 0.95 in order to accept the harmony of results among methods (Snyder and Larsen, 1983). This implies that the correlation observed among the different methods is worthless. This finding negates the claim that all ESR methods in the markets correlate with classic Westergren method and the claim that there is no significant difference in using EDTA or citrate for ESR.

There was a high variability in the mean of ESR values using different anticoagulants for the same method (Table 3.3). For Quick citrate and Quick EDTA the results showed that mean ESR values were 35.3 and 50.3 respectively. Automated citrate and Automated EDTA showed that mean ESR values were 29.0 and 46.5 reaspectively. Finally the results for Westergren citrate and Westergren EDTA showed that mean ESR values were 39.4 and 47.7 respectively. These results indicated that ESR tests using EDTA blood had higher means and in general higher ESR values than citrated blood for

the different methods used in this study. Also when comparing the different methods evaluated, the results obtained showed that Quick method and Westergren method had higher mean ESR values than Automated method for the same anticoagulant used. For citrated blood, it was 35.3 for Quick method and 39.4 for Westergren method and 29.0 for Automated method. While for EDTA blood it was 50.3 for Quick method and 47.7 for Westergren method and 46.5 for automated method. In general, Westergren EDTA or citrate gave higher results. This observation agreed with the results reported by an earlier report that compared tri-sodium citrate and EDTA as diluents for ESR testing. According to the study that was performed only on apparently healthy control, the reason for the higher ESR values observed with EDTA was not known, but it was hypothesized that tri-sodium citrate reduced rouleaux formation that lead to decreased ESR or due to a difference in viscosity, where EDTA blood may be less viscous than tri-sodium citrate resulting in higher values (Emelike *et al*., 2010). Moreover, our results disagreed with the claim of Denise Harmening that sodium citrate or EDTA can be used without an effect on ESR (Harmening, 2002). Also, our results disagreed with the claim of most ESR tube manufactures that their method agreed with the standardized Westergren method (Vital Diagnostic, Kima Sed).

The variation in results was reflected by the standard deviation obtained for each method. For Quick citrate, Automated citrate and Westergren citrate it was 24.43, 18.41, and 24.65 respectively. While for Quick EDTA, Automated EDTA and Westergren EDTA it was 24.71, 18.94, and 25.18 respectively. So, these results clearly showed that variability was maintained for the same method irrespective to the anticoagulant used and Automated method had less variability than the other methods. However, the standard deviation for EDTA and citrated blood for different methods is nearly similar, which means that the variability is consistent for the same method irrespective to the anticoagulant used (Table 3.3).

Results obtained from paired t- test among the different methods used in this study showed that there was a significant difference between Westergren citrate and Westergren EDTA (p<0.001), Automated citrate and Westergren citrate (p<0.001),

Quick citrate and Westergren citrate (p= 0.014). While for Automated EDTA and Westergren EDTA, Quick EDTA and Westergren EDTA there was no significant difference in results as p= 0.455 and p= 0.171 respectively as shown in Table 3.4.

For methods to match the correlation coefficient "r" should approximate 1.00 and most of the results should fall within the lines that represent 95% CI for the regression line (Snyder and Larsen, 1983). However, the scatter plots obtained for the correlation between ESR values using Westergren citrate and Westergren EDTA, Automated citrate and Automated EDTA, Westergren citrate and Automated citrate, Quick citrate and Quick EDTA, Westergren citrate and Quick citrate, Westergren EDTA and Quick EDTA, Westergren EDTA and Automated EDTA for patients' group, showed that most of the results fell either above or below the lines that represent 95% CI for the regression line. Also, extreme results were often obtained as shown in Figures 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7.

For the control group, the scatter plots obtained by the correlation between ESR values using Westergren citrate and Westergren EDTA, Automated citrate and Automated EDTA, Westergren citrate and Automated citrate, Quick citrate and Quick EDTA, Westergren citrate and Quick citrate, Westergren EDTA and Quick EDTA, Westergren EDTA and Automated EDTA did not differ from those obtained from the patients' group. Most of the results obtained from the control group were either above or below the lines that represent 95% CI for the regression line and extreme results were also present as shown in Figures 3.8, 3.9, 3.10, 3.11, 3.12, 3.13, 3.14.

In comparative studies and when methods do match, the results are accepted if and only if r >0.95, y- intercept for the regression line is close to zero and the slope of the regression line is between 0.9 and 1.1 (Snyder and Larsen, 1983). However, none of our comparison studies met these criteria, neither for the patients' group nor for the control group.

In conclusion, there was no harmony or consistency in results among the three different methods used in our study to measure ESR for both anticoagulants used (EDTA or citrate). The loss of harmony was in both patients' group and control group. Furthermore, the variability of results was almost the same in both groups as shown by the high standard deviation for each method. It is advised to stick with the standardized method recommended by ICSH using EDTA blood (ICSH, 1993). Otherwise, at least, to use the same method and the same anticoagulant in the same laboratory.

4.2. Recommendations

According to our results, ESR results obtained from different methods available for measuring ESR are different. Also differences in the results can be observed when comparing the same method using EDTA or citrate as anticoagulant for both patients' and control groups.

The lack of harmony in the results obtained using these methods and anticoagulants leads us to recommend that only one method should be adopted at the level of the country to minimize the variation of ESR results between laboratories. Also we recommended using the standardized method recommended by ICSH using EDTA blood (ICSH, 1993). Otherwise, laboratories should maintain using the same procedure and the same method to perform ESR test because such variations confuse both patients and physicians and minimize their trust in the results obtained from different laboratories. This creates mistrust among laboratories and physicians and also among laboratories and patients.

Our recommendation for patients is to perform ESR test in the same laboratory all the time, to minimize the variation of results among different laboratories.

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Appendices

Appendix I

The results obtained from different methods using EDTA and citrate for patients' group

No.	Age	Gender	Quick Na Citrate	Automated Na Citrate	Westergren **Na Citrate**	Quick EDTA	Automated EDTA	Westergren EDTA
1	83	Female	5	30	61	5	56	49
2	57	Female	24	20	25	20	51	48
3	32	Female	20	29	26	25	30	46
4	30	Female	20	21	25	25	30	27
5	46	Female	15	14	18	25	20	42
6	42	Female	15	11	14	30	19	26
7	55	Female	10	14	24	30	20	30
8	35	Female	20	14	21	30	44	34
9	56	Female	30	10	45	30	28	46
10	25	Female	15	16	19	30	40	55
11	28	Female	20	25	25	30	36	35
12	54	Female	20	34	40	35	40	20
13	47	Female	20	28	25	35	28	31
14	45	Female	25	16	21	35	32	28
15	70	Female	25	23	29	35	45	32
16	41	Female	25	19	35	35	38	43
17	13	Female	35	16	25	35	38	42
18	37	Female	10	13	20	35	23	35
19	33	Female	20	16	35	35	38	46
20	28	Female	20	25	18	40	31	46
21	58	Female	35	23	33	40	44	40
22	49	Female	55	25	45	40	60	67
23	37	Female	20	14	25	40	56	38
24	19	Female	15	21	32	45	44	55

25	51	Female	35	27	28	50	30	30
26	70	Female	45	48	55	55	60	74
27	50	Female	35	28	53	60	57	57
28	81	Female	40	39	41	65	55	50
29	62	Female	45	44	40	65	59	60
30	52	Female	40	28	56	65	44	38
31	60	Female	60	60	87	65	55	94
32	46	Female	30	29	60	65	60	65
33	22	Female	20	24	47	70	20	80
34	37	Female	30	25	55	70	63	83
35	43	Female	60	27	50	75	49	50
36	52	Female	50	38	47	75	72	67
37	53	Female	50	45	71	75	71	85
38	45	Female	60	36	60	75	60	74
39	73	Female	55	44	60	75	71	83
40	42	Female	65	54	67	75	73	80
41	42	Female	45	36	69	80	67	81
42	52	Female	75	65	70	80	73	92
43	46	Female	70	56	90	85	82	110
44	48	Female	40	29	68	90	60	50
45	60	Female	100	88	117	90	77	96
46	57	Female	95	85	94	100	87	98
47	69	Female	115	72	80	110	90	96
48	33	Female	60	89	82	110	78	106
49	32	Female	5	10	15	30	25	30
50	24	Female	25	25	16	30	23	15
51	23	Female	30	10	18	30	19	25
52	19	Female	30	13	10	30	18	20
53	19	Female	30	14	16	30	27	25
54	23	Female	20	14	23	30	26	38
55	27	Female	20	60	61	32	61	69

56	33	Female	17	38	38	35	34	37
57	43	Female	20	18	12	35	38	34
58	26	Female	25	13	15	35	28	21
59	42	Female	25	14	21	35	38	31
60	30	Female	25	18	32	40	38	50
61	31	Female	45	16	20	45	28	30
62	26	Female	60	28	31	50	45	31
63	28	Female	35	29	40	55	58	36
64	47	Female	45	31	37	60	60	50
65	29	Female	35	38	78	65	69	65
66	35	Female	40	32	46	65	59	52
67	22	Female	10	20	30	75	36	32
68	48	Female	45	23	53	75	53	61
69	45	Female	60	38	50	75	62	32
70	10	Male	30	10	24	0	80	22
71	76	Male	20	11	12	20	22	32
72	58	Male	45	45	26	25	36	28
73	26	Male	20	13	20	25	21	28
74	58	Male	10	14	18	25	40	40
75	59	Male	20	19	16	25	29	21
76	56	Male	25	21	27	30	36	21
77	75	Male	20	25	30	35	38	61
78	56	Male	20	23	42	40	70	57
79	62	Male	30	23	31	40	50	49
80	50	Male	10	18	21	40	46	50
81	55	Male	35	25	40	40	53	40
82	13	Male	15	10	20	45	29	18
83	58	Male	10	9	18	45	20	25
84	36	Male	25	25	35	50	56	58
85	49	Male	20	14	8	50	4	0
86	19	Male	40	30	46	50	58	79

87	74	Male	25	25	36	50	57	33
88	71	Male	30	10	25	65	33	45
89	60	Male	25	22	25	65	51	50
90	64	Male	100	55	65	80	56	30
91	55	Male	30	34	60	85	70	80
92	60	Male	85	47	90	100	69	105
93	75	Male	90	59	95	100	71	110
94	29	Male	50	59	90	105	75	100
95	60	Male	140	94	118	120	88	58
96	38	Male	15	23	19	25	34	24
97	39	Male	20	14	19	25	28	15
98	36	Male	15	31	16	25	30	15
99	36	Male	10	12	12	25	34	22
100	25	Male	20	13	18	30	37	20
101	43	Male	25	13	15	30	37	25
102	23	Male	20	20	15	40	38	15
103	42	Male	20	25	30	50	55	40
104	37	Male	70	21	13	60	23	18
105	43	Male	40	33	45	65	62	34

Appendix II

The results obtained from different methods using EDTA and citrate for control group

No.	Age	Gender	Quick Na Citrate	Automated Na Citrate	Westergren Na Citrate	Quick EDTA	Automated EDTA	Westergren EDTA
1	29	Female	0	7	5	0	3	5
2	30	Female	0	5	6	0	2	7
3	25	Female	5	2	0	0	2	0
4	20	Female	5	11	6	5	13	11
5	16	Female	5	2	5	5	4	5
6	25	Female	5	5	6	5	11	7
7	80	Female	5	7	6	5	7	7
8	28	Female	15	13	5	10	4	8
9	33	Female	5	5	5	10	12	8
10	31	Female	5	2	5	10	3	4
11	26	Female	10	9	12	10	16	17
12	21	Female	10	5	5	10	7	7
13	26	Female	10	7	9	10	9	12
14	57	Female	5	7	5	10	11	6
15	45	Female	10	7	10	10	21	16
16	29	Female	10	12	11	15	28	10
17	43	Female	15	7	10	15	13	18
18	15	Female	15	7	5	15	8	7
19	11	Female	5	6	8	15	18	18
20	74	Female	5	9	10	15	11	7
21	41	Female	15	11	15	15	13	11
22	28	Female	35	5	5	20	3	0
23	57	Female	15	23	16	20	32	22
24	28	Female	15	14	18	20	25	23
25	35	Female	15	18	22	20	25	25

26	29	Female	15	16	9	20	28	20
27	42	Male	0	1	0	0	1	0
28	27	Male	0	2	0	0	2	0
29	26	Male	0	1	0	0	2	0
30	27	Male	0	2	0	0	2	0
31	24	Male	0	2	0	0	1	0
32	24	Male	5	2	0	0	2	3
33	28	Male	15	2	5	0	2	20
34	21	Male	0	2	0	0	2	0
35	33	Male	0	2	0	0	2	0
36	44	Male	0	2	0	0	4	0
37	48	Male	0	3	5	0	9	2
38	39	Male	0	5	3	0	14	6
39	22	Male	0	2	1	0	2	2
40	26	Male	0	6	1	0	1	2
41	24	Male	0	1	0	0	2	0
42	20	Male	0	2	2	0	4	2
43	47	Male	0	10	8	0	31	20
44	38	Male	0	2	0	0	5	0
45	20	Male	0	4	0	0	7	0
46	39	Male	0	2	5	0	7	5
47	37	Male	0	5	5	1	6	2
48	27	Male	5	8	18	5	23	26
49	33	Male	5	3	1	5	4	1
50	29	Male	5	5	0	5	2	2
51	26	Male	0	5	5	5	7	8
52	46	Male	5	5	0	5	6	5
53	27	Male	0	2	2	5	4	0
54	24	Male	5	2	0	5	2	0
55	24	Male	10	3	5	5	4	5
56	25	Male	5	7	6	5	6	5

57	42	Male	5	4	0	5	8	4
58	27	Male	0	7	6	5	14	10
59	23	Male	5	5	5	5	6	5
60	25	Male	5	2	0	5	8	6
61	32	Male	5	7	5	5	7	7
62	42	Male	5	5	4	5	12	5
63	23	Male	5	2	0	5	1	0
64	20	Male	5	2	5	5	4	5
65	21	Male	15	14	6	5	8	10
66	75	Male	5	5	5	5	8	6
67	43	Male	5	5	5	5	8	15
68	50	Male	5	11	10	5	7	8
69	18	Male	10	12	10	10	12	10
70	20	Male	15	2	0	10	7	5
71	29	Male	5	7	8	10	19	23
72	37	Male	5	2	5	10	7	7
73	30	Male	10	4	3	10	10	5
74	33	Male	5	7	11	10	13	23
75	31	Male	10	7	4	10	8	5
76	28	Male	5	2	2	10	7	4
77	36	Male	25	12	5	10	5	2
78	45	Male	5	5	5	10	9	10
79	39	Male	10	7	8	10	12	10
80	26	Male	0	2	0	15	6	0
81	27	Male	0	6	0	15	8	0
82	25	Male	10	8	10	15	34	14
83	35	Male	10	7	9	15	13	10
84	27	Male	5	5	5	15	23	10
85	25	Male	5	6	6	15	7	10
86	32	Male	10	7	8	15	13	5
87	35	Male	10	5	10	15	14	30

88	46	Male	5	2	4	15	4	5
89	32	Male	10	18	16	15	14	28
90	26	Male	15	7	7	15	10	5
91	46	Male	10	6	10	15	18	10
92	57	Male	10	18	18	15	23	28
93	64	Male	10	12	20	15	23	33
94	34	Male	5	10	23	20	23	28
95	25	Male	15	7	12	20	21	15

استبيان

دراسة مقارنة لفحص ترسيب كريات الدم الحمراء بين الفحص اليدوي و الأوتوماتيكي باستعمال موانع التجلط المختلفة "EDTA & Sodium Citrate"

السيد / السيدة المرضى :

تقوم الباحثة رشا غانم – جامعة القدس بإعداد دراسة حول : دراسة مقارنة لفحص ترسيب كريات الدم الحمراء بين الفحص اليدوي و الأوتوماتيكي باستعمال موانع التجلط المختلفة "EDTA & Sodium Citrate". و يهدف البحث لدراسة نوع المادة المانعة للتجلط و طريقة عمل فحص ترسيب كريات الدم الحمراء.

و لذلك نرجو من حضرتك التعاون مع الباحثة بتعبئة الاستبيان المرفق و كذلك السماح لها باستعمال نتائج التحاليل المخبرية التي سوف يتم عملها على عينة الدم خاصتك بهدف دراسة أفضل مادة للتجلط و أفضل طريقة لعمل هذا الفحص.

سوف يتم التعامل مع العينات و نتائج البحث بسرية و خصوصية كاملة، أما نتائج البحث فسوف تستعمل لأغراض البحث العلمي فقط. و سيتم إعلامك بنتائج التحاليل المخبرية.

و أقدم لك شكري و امتناني لك على تعاونك لإنجاح هذا البحث،،،،،

الباحثة،

رشا غانم

برنامج الماجستير

دائرة العلوم الطبية المخبرية

جامعة القدس

أبو ديس – القدس.

دراسة مقارنة لفحص ترسيب كريات الدم الحمراء بين الفحص اليدوي و الأوتوماتيكي باستعمال موانع التجلط المختلفة "EDTA & Sodium Citrate"

§ اسم المركز الصحي:.....

§ تاريخ سحب الدم:.....

لاستعمال الباحثة فقط.

رقم الاستبيان :.....

البيانات الشخصية

ü الاسم المريض: .....

ü الجنس: .....

ü العمر: ..... سنة.

ü هل هذه هي المرة الأولى التي تزور/ين فيها الطبيب؟

.....

ü ما هو تشخيص المرض لديك؟

.....

.....

ü متى بدء المرض عندك؟

.....

.....

ü هل تتناول أي من الأدوية؟ اجب بنعم أو لا؟ إذا كانت إجابتك نعم الرجاء ذكر الأدوية؟

.....

.....

.....

اقرار:

في حالة موافقتك على المشاركة في الدراسة، يرجى التوقيع هنا:.....

استبيان

دراسة مقارنة لفحص ترسيب كريات الدم الحمراء بين الفحص اليدوي و الأوتوماتيكي باستعمال موانع التجلط المختلفة "EDTA & Sodium Citrate"

السيد / السيدة المتطوعين:

تقوم الباحثة رشا غانم – جامعة القدس بإعداد دراسة حول : دراسة مقارنة لفحص ترسيب كريات الدم الحمراء بين الفحص اليدوي و الأوتوماتيكي باستعمال موانع التجلط المختلفة "EDTA & Sodium Citrate". و يهدف البحث لدراسة نوع المادة المانعة للتجلط و طريقة عمل فحص ترسيب كريات الدم الحمراء.

و لذلك نرجو من حضرتك التعاون مع الباحثة بتعبئة الاستبيان المرفق و كذلك السماح لها باستعمال نتائج التحاليل المخبرية التي سوف يتم عملها على عينة الدم خاصتك بهدف دراسة أفضل مادة للتجلط و أفضل طريقة لعمل هذا الفحص.

سوف يتم التعامل مع العينات و نتائج البحث بسرية و خصوصية كاملة، أما نتائج البحث فسوف تستعمل لأغراض البحث العلمي فقط. و سيتم إعلامك بنتائج التحاليل المخبرية.

و أقدم لك شكري و امتناني لك على تعاونك لإنجاح هذا البحث،،،،،

الباحثة،

رشا غانم

برنامج الماجستير

دائرة العلوم الطبية المخبرية

جامعة القدس

أبو ديس – القدس.

دراسة مقارنة لفحص ترسيب كريات الدم الحمراء بين الفحص اليدوي و الأوتوماتيكي باستعمال موانع التجلط المختلفة "EDTA & Sodium Citrate"

- § المركز الصحي:.....
- § تاريخ سحب الدم:.....

لاستعمال الباحثة فقط.

رقم الاستبيان :.....

البيانات الشخصية

- ü الاسم: .....
- ü الجنس: .....
- ü العمر: ..... سنة
- ü هل أنت مدخن؟.....
- ü هل تتعاطي اي من الادوية؟اذكرها اذا كانت اجابتك نعم؟

.....

.....

.....

.....

- ü هل انت تشعر بانك في حال جيدة هذا اليوم؟

.....

- ü هل تعاني من اي امراض مزمنة؟ أذا كانت اجابتك نعم؛ اذكر المرض؟

.....

.....

اقرار:

في حالة موافقتك على المشاركة في الدراسة، يرجى التوقيع هنا: .....

دراسة مقارنة لفحص ترسيب كريات الدم الحمراء بين الفحص اليدوي و الأوتوماتيكي باستعمال موانع التجلط المختلفة "EDTA و Sodium Citrate"

إعداد: رشا إميل جورج غانم.

المشرف الأول: أستاذ مساعد خالد يونس.

المشرف الثاني: د. محمود سرور.

ملخص

يتواجد حاليا في الاسواق العديد من الآلات والطرق المختلفة لإجراء فحص ترسيب كريات الدم الحمراء الذي يعد من الفحوصات الأكثر شيوعا في المختبر نظرا لتكلفته المنخفضة وسهولة إجراءه، حيث يستخدم هذا الفحص للتمييز بين الأمراض ذات الأعراض المتشابهة، مثلا يستعمل هذا الفحص للتمييز بين احتشاء عضلة القلب والذبحة الصدرية، كما يستعمل للتمييز بين الالتهابات المزمنة والحادة.يعتبر المجلس الدولي لتوحيد المقاييس في علم الدم (ICSH) استخدام طريقة Westergren اليدوية مرجعية لإجراء هذا الفحص. جميع الوسائل المستخدمة حاليا لاجراء هذا الفحص تدعي وجود توافق بينها و بين الطريقة المرجعية (طريقة Westergren اليدوية). للتحقق من هذا الادعاء ولمعرفة إذا كان هناك تباين في النتائج بين الطرق المنتشرة بين المختبرات وكذلك لمعرفة إذا هناك تباين في النتائج عند استعمال موانع التجلط المختلفة قمنا بإجراء هذه الدراسة التي تعد الأولى في فلسطين.

لقد تم اختيار الأساليب الثلاثة الأوسع انتشارا في فلسطين لإجراء هذا الفحص التي هي: (الطرق اليدوية) Sediplast® و Kima Sed® و الطريقة الآلية Monosed® tubes and MICROsed-System®. تم استخدام مجموعتين في هذه الدراسة: الأولى هي مجموعة المرضى الذين يعانون من أمراض مختلفة، كان عددهم 105 مرضى ؛ أما المجموعة الثانية فهي مجموعة الأشخاص الذين يكونون أصحاء (ظاهريا) والتي تم اعتمادها كمجموعة ضابطة للنتائج التي تشمل 95 متطوع.

النتائج التي تم الحصول عليها من مجموعة المرضى والمجموعة الضابطة للنتائج أظهرت أن هناك تباين كبير في النتائج باستخدام الأساليب المختلفة (اليدوي و الآلي) و باستخدام موانع التجلط المختلفة. وأظهر التحليل الإحصائي أنه ليس هناك توافق في النتائج بين الأساليب المختلفة باستخدام موانع التجلط المختلفة. وبشكل عام، النتائج التي تم الحصول عليها باستخدام مانع التجلط EDTA كانت أعلى من تلك التي تم الحصول عليها باستخدام مانع التجلط Na-citrate (اعتمادا على معدل النتائج عند استخدام كل من موانع التجلط الأنفة الذكر).

مجموعة المرضى المشاركين في هذه الدراسة و التي تتكون من 105 مرضى، يعانون من أمراض مختلفة، الإناث عددهم 69 (65.7%) و 36 من الذكور (34.3%). وتبين النتائج التي تم الحصول عليها من هذه المجموعة أن هناك تباين وتناقض في نتائج باستخدام الأساليب المختلفة و موانع التجلط المختلفة المستخدمة اعمل فحص ترسيب كريات الدم الحمراء. استخدام مانع التجلط EDTA و Na-citrate تظهر أن هناك علاقة إيجابية بين هذه النظم المختلفة، إلا أنها لا قيمة لها كما كان معامل الارتباط دائما اقل من 0.95 والميل لخط الانحدار خط اقل من 0.9. وهذا يعني، انه ليس هناك توافق في النتائج على الإطلاق بين الأساليب المختلفة. ومع ذلك، هناك تباين كبير في النتائج كما أن معظم النتائج أعلى أو أسفل الأسطر التي تمثل 95% CI. أيضا، توجد العديد من النتائج المتطرفة.

أما بالنسبة للمجموعة الضابطة للنتائج، والتي تتألف من 95 فردا. و تشمل الإناث 26 (27.4 %) والذكور 69 (72.6%). تظهر النتائج التي تم الحصول عليها أن هناك التباين في النتائج. بالرغم من وجود علاقة إيجابية إلا انه ليس هناك توافق في النتائج على الإطلاق بين الأساليب المختلفة. وعلى الرغم من هذا الارتباط، هناك تباين كبير في النتائج كما أن معظم النتائج تقع أعلى أو أسفل الأسطر التي تمثل 95% CI ، توجد العديد من النتائج المتطرفة.

وفي الختام، بما أن هناك تباين كبير في النتائج ولا يوجد هناك توافق في النتائج باستخدام الأساليب الثلاثة المستخدمة في هذه الدراسة فإننا نوصي بتوحيد الإجراءات والأساليب التي تستعملها المختبرات لإجراء هذا الفحص على مستوى البلد، أو على الأقل على المختبر أن يستخدم دائما نفس الطريقة ونفس الأسلوب لإجراء هذا الفحص، كما إننا ننصح المريض باعتماد مختبر واحد فقط لعمل هذا الفحص لمتابعة حالته المرضية.