t males and 4–5 Liters in adult females. –Its colour is bright scarlet in arterial blood and to a dull brick red in venous blood. Composition of blood: a) Cellular elements: 45% and include RBCs, WBCs and platelets b) Plasma : 55 % – It is formed of water (90%) in which more than 100 different substances are dissolved. – Dissolved substances include nutrients, gases, hormones, various wastes and metabolites, many types of proteins, and electrolytes. Chapter I: Blood Practical 3 a– ABO system Blood experiments: 1– Determination of blood groups. 2– Determination of haemoglobin content. 3– Determination of haematocrite value. 4–Analysis of complete blood picture. 5–Determination of erythrocyte sedimentation rate. 6–Haemostatic function tests. –The blood of human is classified into many groups according to certain antigens present on the surface of RBCs. –There are hundreds of different antigens have been found on the surfaces of human RBCs. –Most of these antigens are weak and are mainly of importance in genetic studies. –They include ABO system, Rh system, MNS system, Lewis system and Kell system –This system includes 2 related antigens (or agglutinogens) A and B. – According to the presence or absence of these 2 antigens, the human blood is normally classified into 4 major groups: Group A Group B Group AB Group O % 41 % 9% 3% 47% Agglutinogen (antigen) A B A&B ---- Agglutinin (antibody) B A ----- A&B Genotype AA or AO BB or BO AB OO Blood groups according to ABO system 1–Determination of blood groups Chapter I: Blood Practical 4 Determination of ABO system: 1. Mix one drop of blood with 1ml of isotonic saline in test tube. 2. Put two separate drops of the diluted blood on a glass slide. 3. Add one drop of anti–A serum (blue coloured) to one blood drop and one drop of anti B serum (yellow coloured) to the other blood drop. 4. Mix the blood with anti–A and anti–B sera gently using the blunt end of two separate pins. 5. After 2 min, examine for agglutination: a. if agglutination occurs with anti–A serum only, the blood group is type A. b. If agglutination occurs with anti–B serum only, the blood group is type B. c.If agglutination occurs with both anti–A and anti –B sera the blood group is type AB. d. If no agglutination with either anti – A or anti– B sera, the blood group is type O. Chapter I: Blood Practical 5 b–Rh system (Rh factor) Anti–A Anti–B Anti–D A +ve B +ve AB +ve O –ve Typing of ABO blood grouping in humans Importance of ABO system 1) Blood transfusion: – blood grouping and cross matching test should be done to choose the compatible groups. 2) Disputed paternity: – It is a good –ve test that excludes paternity rather than prove it. 3) Medicolegal use: to prove or disprove the claim of the victim. 4) Susceptibilty to various diseases: a– O group people are susceptible to peptic ulcer. b– AB group people are susceptible to diabetes mellitus. –There are six common types of Rh antigens named C, D, E, c, d, and e. –The type D–antigen is considerably the most antigenic than the others, so; a. Presence of D–antigen Rh +ve. b. Absence of D–antigen Rh –ve. Chapter I: Blood Practical 6 Rh +ve Rh –ve White races 85% 15% Egyptians 90% – 95% 5%– 10% Importance of Rh factor: In blood transfusion: –When Rh –ve person , receives Rh +ve blood , he will develop agglutinins against Rh–factor. –If this person receives Rh+ ve blood again agglutination & haemolysis occur. Determination of Rh group: –Put 2 drops of diluted blood on a glass slide. –Add one drop of anti–D serum to the blood and mix gently with the blunt end of a pin. –Examine for agglutination after 3min. –If agglutination occurs, the blood group is Rh +ve. –If not, the blood group is Rh –ve. Cross test: – this is a direct test of compatibility of donor cell and recipient) التوافق matching (compatibility serum. Steps: 1) obtain 1 ml blood from the recipient 's vein and allow it to clot, when clot retracts the

serum can be pipetted off. 2) prick the finger of the donor , one drop of blood should be placed in 1 ml saline and mixed. 3- place one drop of the donor 's red cells on a glass slide and over it place one drop of recipients serum. 4- wait 10 minutes and observe agglutination. Chapter I: Blood Practical 7 Indications of blood transfusion:- To restore the whole blood as in haemorrhage. 2- To restore one element of the blood when dificent e .g - RBCs in severe anaemia, - WBCs in severe Leukopenia, - Platelets in severe thrombocytopenia, - Clotting factors in severe haemophilia. Precautions before blood transfusion: 1- The transfused blood should be compatible with recipients blood as regards ABO system & Rh system. 2- HB content of transfused shouldn't be less than 90 %. 3- The transfused blood should be free of infections e.g AIDS, viral hepatitis , & malaria. 4- The transfused blood should be fresh not frozen (stored at 4 oC & less than 21 days). 5- Cross matching test should be done before transfusion. Relation of blood group to blood transfusion: i-AB blood is considered universal recipient as it has no antibodies in its plasma. ii- O blood is considered universal donor as it has no antigens on its RBCs. The possible transfusions between various groups are: Group Gives Takes from AB AB All groups O All groups O A A & AB A & O B B & AB B & O Blood transfusion Chapter I: Blood Practical 8 Effects of incompatible blood transfusion: - If the blood is transfused from a person with certain blood group to a person with unsuitable blood group, transfusion reactions occur. -The cause is that the donor „s RBCs are agglutinated by recipient „s antibodies haemolysis of the transfused RBCs. Effects: 1- Blockage of blood capillaries by the clumped RBCs several pains e.g back pain & joint pain. 2- Hazards of intravascular haemolysis. a- Circulatory shock: -The released histamine arterial blood pressure. b- Hyperkalaemia:due to K+ released from haemolysed RBCs. c- Jaundice. e- Acute renal failure. Dangers of blood transfusion: 1- Incompatible blood transfusion (see before). 2- Allergic reactions. 3- Transmission of diseases e.g AIDS, malaria & viral hepatitis. Chapter I: Blood Practical 9 -Haemoglobin (Hb) is a protein having a M.W of 66.000. It represents 34% of RBC volume. It consists of four heme groups combined with one molecule of globin. Normal value: Adult male 13.5 - 16 gm % (average 15gm %) Adult female 11.5 - 16 gm % (average 14gm %) Newly born infant 18-20 gm % Children 11 gm % Sahli's Method for Hb determination: Principle: -This method depend upon converting Hb into acid hematin (has a dark brown color) by adding dilute HCL. -The intensity of this color depends on the concentration of acid hematin which in turn, depends on the concentration of Hb. -The color of the solution after dilution with water is matched with the colour of standard tubes. -The readings are obtained in gm%. Reagents and equipments: -N/10 solution of hydrochloric acid. -Distilled water. Sahli apparatus (haemoglobinometer): consists of: a- Central graduated tube which has 2 graduations, one indicating the amount of Hb in gm/100 cc, while the other indicating the Hb%. b- 2 standard coloured tubes on its side. 2-Determination of Haemoglobin Content Chapter I: Blood Practical 10 - Special pipette (= 0.02ml or 20 mm3) capacity - A glass rod and a droper. Special pipette Sahli Apparatus Procedure: 1. Place N/10 HCl in the central tube up to mark 20 on the percentage graduation. 2. Draw a blood by the pipette up to 0.02 mark. 3. Wipe the tip of the pipette. 4. Push its contents of blood quickly into the central tube, then mix the blood with HCl several times. 5. Now Hb of the blood is converted to acid haematin (dark brown colour). 6. After 15 minutes add distilled water, drop by drop and mix well with the glass rod. 7. Continue this process until the colour in the central tube will be the same as in the

standard tubes. 8. Record your results. Graduated tube Standard tubes Chapter I: Blood Practical 11 Advantages of Sahli Method: –The method is simple & quick. – It does not require any costly apparatus. Significance of Hb determination: 1. Diagnosis of anaemia (if Hb is less than its normal value for the same sex and age) , and polycythaemia (if Hb is more than its normal value) 2. Calculations of some blood used for diagnosis of the type of anaemia. Questions 1. How to diagnose anemia? 2. Mention the importance of Hb determination .....
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..... Chapter I: Blood Practical 12 – it's the percent ratio of RBC's volume to the total blood volume. Normal values: Haematocrit e = RBC's volume Total blood volume × 100 In adult male 46 % In adult female 42 % In newly born 60 % Methods of determination of haematocrite: –A blood sample from the patient is placed in a special tube (wintrope tube) which contain anticoagulant. –The sample is then centrifuged. – It's noticed that the RBCs are packed at the bottom of the tube, while clear plasma remains above. WBCs platelets form a small layer just above RBCs column. – the haematocrite is then calculated by dividing RBCs column by the total blood column. Then multiply x l00 Factors affecting haematocrite value: –It's affected by changes in plasma & RBCs in that: Increased in Decreased in – Polycythemia. –Dehydration. – Anaemia. – Overhydration. Uses of haematocrite value: 1– Calculation of blood indices. 2– Diagnosis of anemia & polycythemia 3– Haematocrite value = Packed cell volume, PCV Chapter I: Blood Practical 13 – Complete blood count (CBC) is one of the most commonly ordered blood tests. –The complete blood count is the calculation of the cellular elements of blood. –These calculations are generally determined by special machines that analyze the different components of blood in less than a minute. –A major portion of the complete blood count is the measure of the concentration of white blood cells, red blood cells, and platelets in the blood. –CBC, lists a number of many important values. Typically, it includes the following: 1–Red blood cell count (RBC or erythrocyte count) 2–Hemoglobin (Hbg) 3–Hematocrit (Hct) 4–Blood indices: a–Mean corpuscular volume (MCV). b–Mean corpuscular hemoglobin (MCH) c–Mean corpuscular hemoglobin concentration (MCHC). 5–White blood cell count (WBC or leukocyte count) 6–WBC differential count 7–Platelet count Analysis of complete blood count (CBC) Chapter I: Blood Practical 14 1– RBCs count: –The normal RBCs count differs according to age and sex: In adult males 5 – 5.5 million / mm3 In adult females 4.5 – 5 million / mm3 In new born 6 – 8 million / mm3 In children 3.5 – 4.5 million / mm3 Average 5 millions –Lower values than normal occur in anaemia and higher values than normal occur in polycythemia. 2– Hemoglobin content: –The normal HB content differs according to age and sex: In adult male 13.5 – 18 average 16 gm %. In adult female 11.5 – 16 average 14 gm %. In new born may reach 18 gm %. Average 15 gram % –Lower values than normal occur in anaemia and

higher values than normal occur in polycythemia. 3- Hematocrit (Hct) value: - it‟sthe percent ratio of RBC's volume to the total blood volume. In adult male 46 % In adult female 42 % In newly born 60 % Average 45 % -Lower values than normal occur in anaemia and higher values than normal occur in polycythemia. Chapter I: Blood Practical 15 4-Blood indices: -they are a group of values designed to assess the type of anaemai: a-Mean corpuscular volume (MCV): Def: is the average volume of a single RBC in micron (μ 3) Equation MCV = haematocrite value x 10 RBCs count in millions/mm3 Normal values 45 x 10 / 5 = 90 μ 3 + 7 i.e 83 to 97 μ 3 Abnormalities -lower values than 83 μ 3 occur in microcytic anaemia as iron deficiency anaemia. -higher values than 97 μ 3 occur in macrocytic anaemia as megaloblastic anaemia. b- Mean corpuscular HB (MCH): Def: is the average amount of HB a single RBC in pg. Equation MCV = Hb content x 10 RBCs count in millions/mm3 Normal values 15 x 10 / 5 = 30 pg + 3 i.e 27 to 33 pg. Abnormalities -lower values than 27 pg occur in microcytic anaemia as iron deficiency anaemia. -higher values than 33 pg occur in macrocytic anaemia as megaloblastic anaemia. N.B: anemia may be: 1-Microctytic anemia e.g iron deficiency anaemia. 2-Macrocytic anemia e.g megakoblastic anemia. 3-Normocytic anemia e,g aplastic anemia. 3- Mean corpuscualr haemoglobin concentration (MCHC): Def: is the average HB concentration in a single RBC. Equation MCHC= Hb content x 100 Haematocrite value Normal values 15 / 45 x 100 = 33 % + 2 i.e 31 to 35 pg. Abnormalities higher values than 35 % occur in hereditary spherocytosis (a type of hereditary haemolytic anaemia). Chapter I: Blood Practical 16 -normally from 150,000 to 400,000 /mm3 . -higher values400,000 are called thrombocytosis. -lower values150,000 are called thrombocytopenia. 5-White blood cell count (WBC or leukocyte count): -Number: 4000 - 11,000 /mm3 6-WBC differential count: Granular leukocytes Neutrophil 60 - 70% of leukocytes. -Higher values are called neutrophilia and occurs in bacterial infection. -Lower values are called neutropenia Eosinophils 1 - 5% of leukocytes -Higher values5% are called esinophilia and occur in allergy and parasitic infection. -Lower values are called esinopenia Basophils 0 - 1 % of leukocytes -Higher values are called basophilia -Lower values are called basopenia A Granular leukocytes Monocytes 3-8% of leukocytes -Higher values are called monocytosis -Lower values are called monocytopenia. Lymphocytes 20-30 % of leukocytes -Higher values are called lymphocytosis -Lower values are called lymphopenia. 7-Platelet count: Chapter I: Blood Practical 17 Question 1: A male patient 26 years old has the following CBC: -HB =10 gm% - Haematocrite value =35% -RBCs count 3,5 million RBCS/mm3 Comment on : 1) HB content:

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..... 2) RBCs count:

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..... 3) Calculate MCV:

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..... 4) Calculate MCH:

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..... 5) Haematocrite value:

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..... 6) What is the type of anemia in this

condition if present: .....

..... 7) What is the commonest cause of this type of anemia: .....

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Question 2: A female patient 26 years old has the following CBC: -HB =8 gm% -Haematocrite value =30% -RBCs count 3,7 million RBCS/mm3 Comment on : 1) HB content: .....

..... 2) RBCs count: .....

..... 3) Calculate MCV: .....

..... 4) Calculate MCH: .....

..... 5) Haematocrite value: .....

..... 6) What is the type of anemia in this condition if present: .....

..... 7) What is the commonest cause of this type of anemia: .....

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Question 3: A male patient 26 years old has the following CBC: -HB =11 gm% -Haematocrite value =30% -RBCs count 3,4 million RBCS/mm3 Comment on : 1) HB content: .....

..... 2) RBCs count: .....

..... 3) Calculate MCV: .....

..... 4) Calculate MCH: .....

..... 5) Haematocrite value: .....

..... 6) What is the type of anemia in this condition if present: .....

..... 7) What is the commonest cause of this type of anemia: .....

..... Chapter I: Blood Practical 20

Question 4: A male patient 26 years old has the following CBC: -HB =11 gm% -Haematocrite value =32% -RBCs count 3,7 million RBCS/mm3 Comment on : 1) HB content: .....

..... 2) RBCs count:

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..... 3) Calculate MCV:

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..... 4) Calculate MCH:

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..... Question 5: A male patient 26 years old has the following CBC: -HB =20 gm% -Haematocrite value =60 % -RBCs count 7 million RBCS/mm3 Comment on : 1) HB content:

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..... Chapter I: Blood Practical 21 2) RBCs count: .....

..... 3) Calculate MCV:

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..... 4) Calculate MCH:

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..... Chapter I: Blood Practical 25 I-Bleeding

time –Haemostasis refers to the process of stoppage of bleeding after blood vessels are punctured, cut, or otherwise damaged. Haemostasis involves the following 4 steps: 1. Vasoconstriction (contraction of injured blood vessels). 2. Platelets plug formation. 3. Formation of a blood clot. 4. Fibrinolysis (dissolution of the clot). Tests for Hemostasis: The commonly used tests include: 1. Bleeding time (BT). 2. Clotting time. 3. Prothrombin time (PT). 4. Prothrombin concentration. 5. Activated partial thromboplastin time (APTT). 6. International normalized ratio (INR). Definition: –It is the time needed for bleeding to stop from small puncture. Significance: –give idea about the degree of vasoconstriction (vascular function) and functions of platelets. 4– Tests for Haemostasis Chapter I: Blood Practical 26 II– Coagulation (clotting) time Technique: Duke 's method: Materials: – Disposable sterile lancets – Filter paper – Stop watch – %07 alcohol – cotton Procedure 1. The skin of fingertip or earlobe is cleaned with 70% alcohol, and then a puncture is made by using disposable lancet. 2. Start the stopwatch and note the time needed to stop bleeding. 3. Gently remove completely the blood coming from the wound at 30 seconds interval until bleeding stops. 4. Every time use fresh area of the filter paper. Significance – Normally: about 2–6 minutes. – It is prolonged in: a) purpura (platelet deficiency, or vessel wall defects). b) Von willbrand disease. c) Use of anti–platelets as aspirin. Bleeding time is usually normal in hemophilia. Definition: –It is the time needed for clot formation. Slide method: ● Clean filter papers. ● Clean glass slide Chapter I: Blood Practical 27 ● Lancet ● Stopwatch. Procedure: 1. Clean a fingertip with 70% alcohol and dry it. 2. Puncture it with a sterile disposable lancet fairly deep, so that, blood flows freely. 3. Put a drop of blood on the glass slide. 4. After 1 min, try to dip the tip of lancet on blood drop every 30 seconds until a thread of fibrin appears between the tip of lancet and drop of blood. 5. The time interval between putting the blood into slide and the appearance of fibrin threads is the coagulation time. Significance: – Gives idea about the efficiency and timing of blood clot formation. – Normally: 5–8 minutes. – It is prolonged in: a) hemophilia. b) deficiency of clotting factors e.g vit K deficiency & liver diseases c) use of oral anticoagulants, – Clotting time is usually normal in purpura. Chapter I: Blood Practical 28 –Prothrombin time is used as a measure of the extrinsic pathway of coagulation (factors I, II, V, VII, and X). Procedure: 1. Blood is collected and immediately Na citrate, so that none of prothrombin is converted into thrombin. 2. Take 1.0 ml of citrated plasma in a sterile test tube and keep it in a water bath at 37□c. 3. Add 1.0 ml of thrombokinase reagent (thromboplastin) and then add 1.0 ml of 1% CaCl2. 4. By using stopwatch, record the time passes between the addition of CaCl2 and the appearance of a fine mesh of fibrin. 5. The time required for coagulation is the “prothrombin time”. Steps of prothrombin time Significance: – Normally; prothrombin time is 12–16 seconds. – It is prolonged if there is a deficiency of one or more factors of the extrinsic pathway of coagulation e.g. in liver diseases , oral anticoagulants and vitamin K deficiency. III– Prothrombin time Chapter II: Body Temperature Practical 29 CHAPTER II: BODY TEMPERATURE