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Determination Of Hemoglobin F Level in Normal Pregnant Wom en Reffered To Dar Elber Specialized Medical Center in Khartou m City

A thesis submitted for the partial fulfillment of M Sc degree in medic al laboratory sciences in hematology

By:

Hanan Abaker Turab Mohammed

Bsc in medical laboratory sciences from Shendi University

(hematology 2006)

Supervised By:

Dr. Hamza Ahmed Hassan

Assistant Professor in hematology medical laboratory of Shendi Uni versity

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الآية

Quran VERSE

بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ: ﴿ يَٰٓأَيُّهَا ٱلَّذِينَ ءَامَنُوٓاْ إِذَا قِيلَ لَكُمۡ تَفَسَّحُواْ فِي ٱلۡمَجَٰلِسِ فَٱفۡسَحُواْ يَفۡسَحِ ٱللَّهُ لَكُمۡۖ وَإِذَا قِيلَ ٱنشُزُواْ فَٱنشُزُواْ يَرۡفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمۡ وَٱلَّذِينَ أُوتُواْ ٱلۡعِلۡمَ دَرَجَٰتٖۚ وَٱللَّهُ بِمَا تَعۡمَلُونَ خَبِيرٞ ﴾

صدق الله العظيم

الآية11من سورة المجادلة

Dedication

I dedicate this research

- to my dead father god rest his soul.

-To my beloved mother prolonged god her age.

-To my dear sisters and brothers.

-To my sweet heart my daughter.

-To my evacuation teachers.

-To my driveway buddies.

Acknowledgment

Firstly all thanks the Almighty Allah who helped me to complete this study. I would like to express my great appreciations to patient whose participation made this study possible. Special thank to my supervisor Dr. Hamza Ahmed Hassan for great efforts and guides. Also special thank to D. Ibrahim Elhkider to help and support me . My thanks extended to my teachers and colleagues for their help and support, and Us. Ismail for great help in statistical analysis .aloes great thanks to lab staff of Dar elber specialize medical center and Elzyutona specialize hospital for their help in collection and analysis samples

Abstract

Background: Fetal hemoglobin (Hb F) is normal hemoglobin (Hb) that present in the fetus and usually almost absent in adults .The aim of this study was to determination of Hb F level in normal pregnancy at different stages of pregnancy and pattern of Hb F level change during pregnancy.

Methods: This is Descriptive cross-sectional study conducted at Dar Elber Specialize medical center in Jabel Awlya locality at southern of Khartoum city to determine the level of Hb F in the normal pregnant women in period between (April to September 2018) the study included (60)samples who normal pregnant females,(20) at first trimester,(20) at second trimester and(20) at third trimester of gestation beside that (20) samples of healthy non pregnant females as control group at same ages .

Blood samples were collected from all groups and Hb F levels were measured by using cation exchange high performance liquid chromatography(HPLC)full automated method .Data were collected by using questionnaire and the (SPSS) program was used for data analysis.

Results :The study reveal that the Hb F mean ± SD during pregnancy was (0.96±0.37) while in non pregnant control group was(0.49±0.16), and (p value 0.00), that indicate strong significant increase in the level of Hb F in pregnancy, the increase in Hb F shown in first trimester then lower gradually in second trimester then the third trimester refer to this finding in discussion.

Conclusions :The normal pregnancy associated with significant increase in Hb F ,the high level of Hb F during first trimester that declined gradually in second and third trimester ,there was significant difference in the level of Hb F between three trimester groups.

ملخص الدراسة

مقدمة: الهيموقلوبين الجنيني عبارة عن هيموقلوبين طبيعي يوجد في الجنين وغالبا يختفي عند البالغين .

الهدف من هذه الدراسة تحديد مستوي الهيموقلوبين الجنيني عند الحمل الطبيعي ونمط التغير في مستوي الهيموقلوبين الجنيني اثناء الحمل.

منهجيه الدراسة :اجريت هذ الدراسة (دراسة وصفية مقطعية) في مركز دار البر الطبي التخصصي ب محلية جبل اولياء جنوب مدينة الخرطوم لتحديد مستوي الهيموقلوبين الجنيني في الحمل الطبيعي في الفترة بين (ابريل الي سبتمبر 2018) ومجموعة الدراسة عبارة عن 60 عينة من النساء الحوامل 20 في الثلث الاول من الحمل و 20 في الثلث الثاني من الحمل و20 في الثلث الثالث من الحمل بجانب ذلك 20 عينة من النساء الغير حوامل كمجموعة ضابطة من نفس الاعمار .

تم جمع العينات من جميع هذه المجموعات وتم قياس مستوي الهيموقلوبين الجنيني لها بأستخدام جهاز(HPLC),تم جمع المعلومات بواسطة الاستبيان و من ثم استخدام الحزم الاحصائية للعلوم الاجتماعية الذي يعرف ببرامج(SPSS) لتحليل بيانات الدراسة .

النتائج: كشفت هذه الدراسة ان الحمل الطبيعي يترافق مع زيادة عالية في مستوي الهيموقلوبين الجنيني حيث وجد المتوسط للهيموقلوبين الجنيني عند النساء الحوامل حوالي 0.37±0.96 والمتوسط في الغير حوامل حوالي 0.16±0.49 هذه الزيادة واضحة في الجزء الاول من الحمل بعد ذلك تبدأ تقل تدريجيا الثلث الثاني ثم الثلث الثالث من الحمل .

الخلاصة: الحمل الطبيعي يترافق مع زيادة عالية في مستوي الهيموقلوبين الجنيني وهذه الزيادة واضحة في الثلث الاول من الحمل بعد ذلك تقل تدريجيا في الثلث الثاني ثم الثالث للحمل ,هنالك فرق واضح في مستوي الهيموقلوبين الجنيني في مختلف مراحل الحمل الثلاث.

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List of abbreviations

Hb	Hemoglobin
Hb F	Fetal hemoglobin
Hb A	Adult hemoglobin
Hb A2	Adult hemoglobin type 2
α	Alpha
γ	Gamma
β	Beta
δ	Delta
ε	Epsilon
ζ	Zeta
LMB	Last normal menstrual period
HPLC	High preformace liquid chromatography
BPG	Bisphosphoglyceric acid
DPG	Diphosphoglyceric acid
N	Nitrogen
C	Carbon
PNH	Paroxysmal nocturnalhemoglobinuria
HPFH	Hereditary persistence of fetal hemoglobin
ESI-MS	Electrospray- Ionization mass spectrometery
MCV	Mean corpuscular volume
D.W	Distilled water
PGE2	Gylacoprotein enzyme 2
PT	prothrombin time test
APTT	Activated partial thrombplastine time test
TT	thrombin time test
VII	Factor seven
VIII	Factor eight
X	Factor ten
XII	Factor eleven
APC	acquired activated protein c resistance
TAT	thrombin antithrombin
FDP	fibrin degradation products
VWF	Von Willebrand factor
SPSS	Statistical package for the social sciences
ANOVA	Analysis of variance
CV	Coefficient of variation

Unitsabbrevition

Min	Minutes
Rev	Revolution counter

cu mm	Cubic milimeter
mmHg	Millimter mercury
P50	Partial pressure of oxygen when is 50% saturated
G/ L	Gram/ liter
g/ dl	Gram/ dice liter

Chapter One
Introduction
Objectives
Rationale

1.1. Introduction

1.1.1.Fetal hemoglobin :

Fetal hemoglobin protein structure formed by 2 alpha subunits and two gamma subunits .

Fetal hemoglobin , or foetal hemoglobin(also hemoglobin F , HbF a_2 y_2) the main oxygen transport protein in the human fetus during the last seven months of development in the uterus and persists in the new born until roughly 6 months old functionally , fetal hemoglobin differs most from adult hemoglobin in that it is able to bind oxygen with greater affinity than adult form , giving the developing fetus better access to oxygen from the mothers blood stream .

In new born, fetal hemoglobin in nearly completely replaced by adult hemoglobin by approximately 6 months post nattily, except in a few thalassemia cases in which there may be delay in cessation of Hb production until 3-5 years of age , in adults , fetal hemoglobin production can be reactivated pharmacologically. [(1)]which is useful in the treatment of diseases such as sickle cell disease .

After the first to 12 weeks of development , the fetus primary form of hemoglobin switches from embryonic hemoglobin to fetal hemoglobin at birth , fetal hemoglobin comprise 50-90% of an infants hemoglobin . this levels decline after six months as adult hemoglobin synthesis is activated , while fetal hemoglobin synthesisdeactivated , Adult hemoglobin (Hb A) in particular takes over as the permanent form of hemoglobin in normal children , However hemoglobin F has been traced even in adult's blood (< 1% of all hemoglobin) . [(2)]

pregnancy is associated with an increased rate of erythropoiesis and this increased demand on the erythron may cause a transit increased in F cell production with increased Hb F in blood as seen in certain clinical condition with similar increased demand on the erythron .[(3)]

1.1.2Defination of pregnancy:

Pregnancy, also known as gestation, is the time during which one or more offspring develops inside a woman. [(4)]Pregnancy can occur by sexual intercourse or assisted

reproductive technology. [6] Childbirth typically occurs around 40 weeks from the last menstrual period (LMP). This is just over nine months, where each month averages 29½ days. [4,5] When measured from fertilization it is about 38 weeks. An embryo is the developing offspring during the first eight weeks following fertilization, after which, the term fetus is used until birth. [13] Symptoms of early pregnancy may include missed periods, tender breasts, nausea and vomiting, hunger, and frequent urination. [7] Pregnancy may be confirmed with a pregnancy test. [8]

Pregnancy is typically divided into three trimesters.The first trimester is from week one through 12 and includes conceoption, which is when the sperm fertilizes the egg. The fertilized egg then travels down the fallopian tube and attaches to the inside of the uterus, where it begins to form the embryo and placenta. [4] During the first trimester, the possibility of miscarriage (natural death of embryo or fetus) is at its highest. [8]The second trimester is from week 13 through 28. Around the middle of the second trimester, movement of the fetus may be felt. At 28 weeks, more than 90% of babies can survive outside of the uterus if provided with high-quality medical care. The third trimester is from 29 weeks through 40 weeks. [4] Prenatal care improves pregnancy outcomes. Prenatal care may include taking extra folic acid, avoiding drugs and alcohol, regular exercise, blood tests, and regular physical examinations. [9] Complications of pregnancy may include disorders of high blood pressure, gestational diabetes, iron-deficiency anemia, and severe nausea and vomiting among others. [10] In the ideal childbirth labor begins on its own when a woman is "at term". [11] Pregnancy is considered at full term when gestation has lasted 39 to 41 weeks.After 41 weeks, it is known as late term and after 42 weeks post term. Babies born before 39 weeks are considered early term while those before 37 weeks are preterm. [4] Preterm babies are at higher risk of health problems such as cerebral palsy. Delivery before 39 weeks by labor induction or caesarean section is not recommended unless required for other medical reasons. [12]

1.2. Rationale

Pregnancy is associated with an increase rate of erythropoiesis and this increased demand on the erythron may cause a transient increase in F cell production with increased Hb F in blood .As seen in certain clinical condition with similar increased demand on the erythron .Very few studies have considered the level of Hb F in the normal pregnancy .The aim of this study attempt to determine the level of Hb F in the normal pregnant women in khartoum city and pattern of Hb F level change during pregnancy .

1.3.Objectives

1.3.1General Objectives

To determine the level of Hb F in normal pregnant women

2.3.1Specific Objectives

1-To compare the level of Hb F in normal pregnant women to non pregnant women

2-To determine the level of Hb F in the different trimesters

Chapter Two
Literature Review

2.Literature Review

2.1. Definition of hemoglobin

Hemoglobin: The oxygen-carrying pigment and predominant protein in the red blood cells. Hemoglobin forms an unstable, reversible bond with oxygen. In its oxygenated state it is called oxyhemoglobin and is bright red. In the reduced state it is called deoxyhemoglobin and is purple-blue. Each hemoglobin molecule is made up of four heme groups surrounding a globin group. Heme contains iron and gives a red color to the molecule. Globin consists of two linked pairs of polypeptide chains. The development of each chain is controlled at a separate genetic locus. Changes in the amino acid sequence of these chains results in abnormal hemoglobins . For example, hemoglobin S is found in sickle-cell disease, a severe type of anemia in which the red cells become sickle-shaped when oxygen is in short supply. When red blood cells die, the hemoglobin within them is released and broken up: the iron in hemoglobin is salvaged, transported to the bone marrow by a protein called transferrin and used again in the production of new red blood cells; the remainder of the hemoglobin becomes a chemical called bilirubin that is excreted into the bile which is secreted into the intestine, where it gives the feces their characteristic yellow-brown colour . [13]

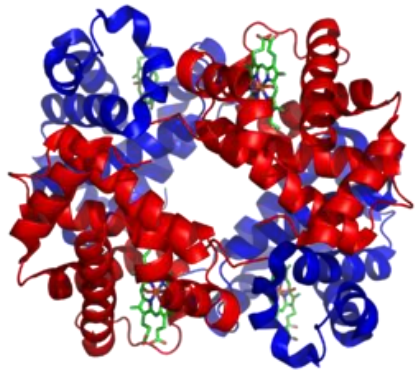

Fig.2.1 Structure of human hemoglobin. α and β subunits are in red and blue, and the iron-containing heme groups in green. [18]

2.2. Type of normal hemoglobin :

The hemoglobins present during normal development—ζ2γ2 (Hb Portland-1), ζ2ε 2 (Hb Gower-1), and α2ε 2 (Hb Gower-2) during the embryonic period; α2γ2 (fetal Hb or HbF) during the fetal stage; and α2β2 (adult Hb or Hb A) as well as small amounts of α2δ2 (HbA2) in adults ,have some different properties that confer specific advantages during the various stages of development. For example, even though these hemoglobins have very similar overall structural architectures, they have differences in O_2 affinity and in their interactions with allosteric effectors. [14,15] That arise from amino acid substitutions at strategic positions to control the manner in which the subunits fit together . [16] Knowledge of how these subunit interactions differ for various hglobins is important in understanding their physiological properties and is reported here.

Oxygenated blood is delivered to the fetus via the umbilical vein from the placenta , which is anchored to the wall of the mother's uterus the chorion acts as barrier between the maternal and fetal circulation so that where is no admixture of maternal and fetal blood , fetal hemoglobin's affinity for oxygen is substantially greater than that of adult hemoglobin , Notably the P50 value for fetal hemoglobin is lower than adult hemoglobin (i.e the partial pressure of oxygen at which protein is 50% saturated : lower values indicate greater affinity) the P50 of fetal hemoglobin is roughly 19 mm Hg – where adult hemoglobin is approximately 26.8 mm Hg . As result of " oxygen saturation curve " left-shifted for oxygen is explain by the lack of fetal hemoglobin is interaction with 2,3-BPF or 2,3 DPG) in adult red blood cells this substance decrease the affinity of hemoglobin for oxygen-2,3BPG is also present in fetal red blood cells , but interacts less efficiently with fetal hemoglobin , this due to a change in a single amino acid (residue 143) found in the 2,3-BPG binding pocket , from histidine to serine which gives rise to greater oxygen affinity than adult hemoglobin . [17]

2.3. Structure and genetic of fetal hemoglobin：

Most types of normal hemoglobin, including hemoglobin A, hemoglobin A2, as well as hemoglobin F, are tetramers composed of four protein subunits and four heme prosthetic groups. Whereas adult hemoglobin is composed of two α (alpha) and two β (beta) subunits, fetal hemoglobin is composed of two α subunits and two γ (gamma) subunits, and is commonly denoted as α2γ2. Because of its presence in fetal hemoglobin, the γ subunit is commonly called the "fetal" hemoglobin subunit. In humans, the gamma subunit is encoded on chromosome 11, as is the beta subunit. There are two similar copies of the gamma subunit gene: γG which has a glycine at position 136, and γA which has an alanine. The gene that codes for the alpha subunit is located on chromosome 16 and is also present in duplicate.iseases such as sickle-cell disease .[18]

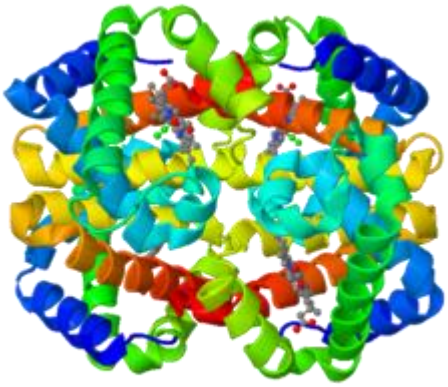

Fig.2.2 Fetal hemoglobin protein structure, formed by 2 alpha subunits (top) and two gamma subunits (bottom), as well as their four heme groups. Each polypeptide chain (ribbon) is rainbow-colored from blue to red (N- to C-termini) . [18]

2.4.Clinical significance of Hb F:

Treatment of sickle-cell disease

Increasing the body's production of fetal hemoglobin is used as a strategy to treat sickle-cell disease.

When fetal hemoglobin production is switched off after birth, normal children begin producing adult hemoglobin (HbA). Children with sickle-cell disease instead begin producing a defective form of hemoglobin called hemoglobin S which aggregates together and forms filaments that cause red blood cells to change their shape from round to sickle-shaped. These defective red blood cells have a greater tendency to stack on top of one another and block blood vessels. These invariably lead to so-called painful vaso-occlusive episodes, which are a hallmark of the disease. If fetal hemoglobin remains the predominant form of hemoglobin after birth, the number of painful episodes decreases in patients with sickle-cell disease. Hydroxyurea promotes the production of fetal hemoglobin and can thus be used to treat sickle-cell disease .[1,19]

The fetal hemoglobin's reduction in the severity of the disease comes from its ability to inhibit the formation of hemoglobin aggregates within red blood cells which also contain hemoglobin S. Combination therapy with hydroxyurea and recombinant erythropoietin—rather than treatment with hydroxyurea alone—has been shown to further elevate hemoglobin F levels and to promote the development of HbF-containing F-cells . [20]

2.5Causes of Increase Hemoglobin F in Adults:

Table (1) : Main pre-analytical subject-related factors that may increaseHb F levels (adapted and modified ref . [21]

Hereditary disorders	**Acquired conditions**
Homozygous β-thalassemia	Pernicious anemia
Heterozygous β-thalassemia	Paroxysmal nocturnal hemoglobinuria (PNH)
HPFH, homozygous	Refractory normoblastic anemia
HPFH, heterozygous	Sideroblastic anemia
δβ thalassemia, homozygous	Pure red cell aplasia
δβ thalassemia, heterozygous	Aplastic anemia
Sickle cell anemia	Pregnancy
Some other hemoglobinopathies (HbC, HbE, HbLepore, some unstable Hbs) (variable)	Recovery after bone marrow transplant, marrow hypoplasia, leukemia chemotherapy and transient erythroblastopenia; treatment with hydroxyurea, 5-aza-2′-deoxycytidine, butyrates, ertythropoietin
Trisomy D	Hyperthyroidism
Hereditary spherocytosis	Juvenile chronic myeloid leukemia
Some Hb variants (Hb Rainier, Hb Bethesda) alkali resistant a	Acute leukemias Erythroleukemia
Hb variants with isolectric point identical to that of HbFb	Benign monoclonal gammopathies Cancer with marrow metastases, Hepatoma
Hb variants with retention time similar to that of HbF	Chronic renal disease

2.6. Methods of determination of Hb F:

The classical method for the determination of fetal hemoglobin is that based on the alkali denaturation.[33] This method relies on the resistance to denaturation by alkali of Hb F compared to Hb A, the denaturation being activated by the ionisation of buried, weakly acidic side chains (one tyrosine and two cysteines) present in Hb A and not in Hb F .[34] This is only a relative difference, and the conditions have been there optimized over time in order that during the time of exposure to alkali most of the Hb A is denaturated while the Hb F is largely unaffected. Before the exposure to alkali, all the hemoglobin forms are transformed in the more stable cyanmethemoglobin form by means of treatment with Drabkin's reagent. An optimized version of the preliminary method has been proposed by Pembrey .[35] It is important to remember that the alkali denaturation is a kinetic test to be performed only under well standardized conditions in order to obtain reproducible results. Today the most common approach to the quantification of HbF is based on the separation of this hemoglobin from other hemoglobin fractions by cation exchange HPLC on dedicated commercial apparatus .[36,37] Often with direct loading from the primary tube. Capillary electrophoresis .[38] Is becoming a valid alternative, while proposed immunochemical methods are still not sufficiently validated. As a matter of fact, it should be recalled that while the by the alkali denaturation resistance all Hb F is measured, in HPLC the acetylated fraction is eluted before the main fraction of HbF, thus leading to a value significantly lower. This fact, although not clinically relevant, may be important when performing method comparison analyses. The analysis of fetal Hb has been recently investigated by electrospray-ionization mass spectrometry (ESI-MS) .[28]Technique already widely diffused to the analysis of hemoglobin variants also in screening programs .[29] The method has a good reproducibility (CV □5%) and can be used to accurately measure the fetal γ-chain masses in neonatal cord blood.

The determination of red cell containing Hb F is used to detected cells containing fetal hemoglobin in mixtures of cells containing adult hemoglobin. This is useful to

define the distribution of fetal hemoglobin in red cells in presence of HPFH, in which the distribution of Hb F is almost pancellular, or for the detection of fetal erythrocytes in maternal blood following transplacental hemorrhages. The acid elution test originally proposed by Keihauer is still used. [30] although more sensitiveand specific method based on monoclonal antibodies and flow cytometry have been recently proposed .[31]

Finally, estimation of Hb F in neonatal blood has been also proposed by hemoxymetry, through the determination of P50 on the HbO2 dissociation curve, based on linear assumption. This method has never been well validated, and showed significant overestimation respect to HPLC.[32]

2.7.Haematological change during the pregnancy:

2.7.1. Red Blood Cells:

During pregnancy, the total blood volume increases by about 1.5 liters, mainly to supply the demands of the new vascular bed and to compensate for blood loss occurring at delivery .[33] Around one liter of blood is contained within the uterus and maternal blood spaces of the placenta. Increase in blood volume is, therefore, more marked in multiple pregnancies and in iron deficient states. Expansion of plasma volume occurs by 10–15 % at 6–12 weeks of gestation. [34,35]

During pregnancy, plasma renin activity tends to increase and atrial natriuretic peptide levels tend to reduce, though slightly. This suggests that, in pregnant state, the elevation in plasma volume is in response to an under filled vascular system resulting from systemic vasodilatation and increase in vascular capacitance, rather than actual blood volume expansion, which would produce the opposite hormonal profile instead (i.e., low plasma renin and elevated atrial natriuretic peptide levels). [36,37]

Red cell mass (driven by an increase in maternal erythropoietin production) also increases, but relatively less, compared with the increase in plasma volume, the net result being a dip in hemoglobin concentration. Thus, there is dilutional anemia. The drop in hemoglobin is typically by 1–2 g/dL by the late second trimester and

stabilizes thereafter in the third trimester, when there is a reduction in maternal plasma volume (owing to an increase in levels of atrial natriuretic peptide). Women who take iron supplements have less pronounced changes in hemoglobin, as they increase their red cell mass in a more proportionate manner than those not on hematinic supplements.

The red blood cell indices change little in pregnancy. However, there is a small increase in mean corpuscular volume (MCV), of an average of 4 fl in an iron-replete woman, which reaches a maximum at 30–35 weeks gestation and does not suggest any deficiency of vitamins B12 and folate. Increased production of RBCs to meet the demands of pregnancy, reasonably explains why there is an increased MCV (due to a higher proportion of young RBCs which are larger in size). However, MCV does not change significantly during pregnancy and a hemoglobin concentration <9.5 g/dL in association with a mean corpuscular volume <84 fl probably indicates co-existent iron deficiency or some other pathology. [38]

Post pregnancy, plasma volume decreases as a result of diuresis, and the blood volume returns to non-pregnant values. Hemoglobin and hematocrit increase consequently. Plasma volume increases again two to five days later, possibly because of a rise in aldosterone secretion. Later, it again decreases. Significant elevation has been documented between measurements of hemoglobin taken at 6–8 weeks postpartum and those taken at 4–6 months postpartum, indicating that it takes at least 4–6 months post pregnancy, to restore the physiological dip in hemoglobin to the non-pregnant values. [39]

2.7.3. White Blood Cells:

White blood cell count is increased in pregnancy with the lower limit of the reference range being typically 6,000/cumm. Leucocytosis, occurring during pregnancy is due to the physiologic stress induced by the pregnant state. [40]

Neutrophils are the major type of leucocytes on differential counts .[41]This is likely due to impaired neutrophilic apoptosis in pregnancy. [42]

The neutrophil cytoplasm shows toxic granulation. Neutrophil chemotaxis and

phagocytic activity are depressed, especially due to inhibitory factors present in the serum of a pregnant female. [31] There is also evidence of increased oxidative metabolism in neutrophils during pregnancy. Immature forms as myelocytes and metamyelocytes may be found in the peripheral blood film of healthy women during pregnancy and do not have any pathological significance .[44] They simply indicate adequate bone marrow response to an increased drive for erythropoesis occurring during pregnancy. [45]

Lymphocyte count decreases during pregnancy through the first and second trimesters and increases during the third trimester. There is an absolute monocytosis during pregnancy, especially in the first trimester, but decreases as gestation advances. Monocytes help in preventing fetal allograft rejection by infiltrating the decidual tissue (7th–20th week of gestation) possibly, through PGE2 mediated immunosuppression. [46] The monocyte to lymphocyte ratio is markedly increased in pregnancy. Eosinophil and basophil counts, however, do not change significantly during pregnancy. [47]

The stress of delivery may itself lead to brisk leucocytosis. Few hours after delivery, healthy women have been documented as having a WBC count varying from 9,000 to 25,000/cumm. By 4 weeks post-delivery, typical WBC ranges are similar to those in healthy non-pregnant women .[48]

2.7.3. Platelets:

Large cross-sectional studies done in pregnancy of healthy women (specifically excluding any with hypertension) have shown that the platelet count does decrease during pregnancy, particularly in the third trimester. This is termed as “gestational thrombocytopenia.” It is partly due to hemodilution and partly due to increased platelet activation and accelerated clearance .[47]

Gestational thrombocytopenia does not have complications related to thrombocytopenia and babies do not have severe thrombocytopenia (platelet count ≤20,000/cumm). It has hence been recommended that the lower limit of platelet count in late pregnancy should be considered as 1.15 lac/cumm. [33]

The platelet volume distribution width increases significantly and continuously as gestation advances, for reasons cited before. Thus, with advancing gestation, the mean platelet volume becomes an insensitive measure of the platelet size.

Post delivery platelet count increases in reaction to and as a compensation for increased platelet consumption during the process of delivery.

2.7.4. Hemostatic Profile:

Pregnancy is associated with significant changes in the hemostatic profile. Fibrinogen and clotting factors VII, VIII, X, XII, vWF and ristocein co-factor activity increase remarkably as gestation progresses. Increased levels of coagulation factors are due to increased protein synthesis mediated by the rising estrogen levels. In in vitro experiments, pregnant plasma has been demonstrated to be capable of increased thrombin generation. [(42)] Thus, pregnancy is a prothrombotic state. In pregnancy, aPTT is usually shortened, by up to 4 s in the third trimester, largely due to the hormonally influenced increase in factor VIII. However, no marked changes in PT or TT occur. [(47)]

There are changes in the levels and activity of the natural anticoagulants also. Levels and activity of Protein C do not change and remain within the same range as for non-pregnant women of similar age. Levels of total and free (i.e., biologically available) Protein S, decrease progressively with the advancement of gestation. Antithrombin levels and activity are usually stable throughout the pregnancy, fall during labor and rise again soon after delivery. Acquired activated Protein C (APC) resistance has been found to occur in pregnancy, even when Factor V Leiden and antiphospholipid antibodies are not present .[(48)]

This has been attributed to the high factor VIII and factor V activity and low free Protein S levels. Hence, APC sensitivity ratio does not serve as a screening test for Factor V Leiden during pregnancy. Coagulation factors remain elevated for up to 8–12 weeks post partum and assays for them may be falsely negative during this period. Markers of hemostatic activity which are clinically relevant are thrombin–antithrombin complexes (TAT) and prothrombin fragments (F 1 + 2), which reflect

in vivo thrombin formation, as also, tests which demonstrate plasmin degradation of fibrin polymer to yield fragments, namely D-dimer and fibrin degradation products (FDP) assay. TAT levels increase with gestation; in early pregnancy the upper limit of normal is similar to the adult range of 2.63 g/L, whereas by term, the upper limit of normal is 18.03 g/L. D-dimer levels are markedly increased in pregnancy, with typical reference range being tenfold higher in late pregnancy than in early pregnancy or in the nonpregnantstate .[33]

The increase in D-dimers reflects the overall increase in total amount of fibrin during pregnancy consequent to increased thrombin generation, increased fibrinolysis or a combination of both. [47]

This also explains why the D-dimer assay is not reliable for predicting the possibility of venous thrombo-embolism in pregnant patients .[45]

2.2. Previous studies

-In December 2009 Ibrahim M, Mohamed H Qari et al from hematology department collage of medicine King Abd ulaziz university hospital conduct study found d significant increase (p value 0.001)was observed in level of maternal Hb F ,there was no significant difference in Hb F level in three trimester groups, correlation studies between the gestation age of level of Hb F showed no significant increase of Hb F with advancing pregnancy (p o.081).[49]

-In october2012 yamada , T. Morikawa ,M . et al from jaban conducted study found on Hb F value 0f 0.082%(0.47%) during first trimester decreased significantly to 0.66%(0.35%) during second trimester .[50]

-Done by James RF,Szumski R et al Hb F fraction remain within the normal range during the first trimester after 15 weeks of gestation increase in70% in pregnancy women .[51]

-In 5015 Seaed R and Babiker R in sudan from Alzaiem Alazhari university conducted study found on existence of slight increase at the first three months compare with the second and third three months (p value 0.001),this increase lower gradually as the age of pregnancy rise because average of Hb F at first three months is 1.156 while at second three months 0.9500 and at third three months is 0.8866.[52]

Chapter Three
Material & Methods

3. Material and Methodology :

3.1. Study Design :

This is descriptive cross-sectional analytical study to determination level of Hb F in normal pregnant women reffered to Khartoum city during period of (April 2018 to September 2018) .

3.2. Study Area :

The study area carried out at Dar Elber Specialize Medical complex which located in southern of Khartoum city.

3.3 .Study Population :

A total of(60) samples collected of study group of normal pregnant women and (20) samples of non pregnant women .

3.4. Inclusion Criteria:

All normal pregnant women who not suffering from diabetes or other hemoglobinpathis.

3.5. Exclusion criteria:

Diabetic , Sicklier pregnant women or other hemoglobinpathy .

-Control Group non pregnant women.

3.6. Sample Size :

Required blood sample were collected from 80 women ,20 women at first trimester of pregnancy ,20 women in second trimester and 20 women in third trimester of pregnancy in addition to 20 from non pregnant women.

3.7. Data collection tools:

Data were collected using self –administrated pre-coded questionnaire to obtain information which helped in study.

3.8. Collected sample and preparation :

Two and half milliliters of venous blood were collected using sterile disposable plastic syringe after cleaning the venipuncture area with ethanol (70%),the blood

added to vacationer with EDTA anticoagulant and centrifuge at 1200g (3000rev\min) for 30 min and supernatant was discharge , then washed the packed cell with normal saline three time , centrifuge at 1200g (3000rev\min) for1 min every time, 2volumes of washed packed cell lysed in 1 volume of D.W then centrifuge at 1200g(3000rev\min)for 30 min . The proportions of Hb F in the hemolysate was measured by full automated cation exchange high pereformace liquid chromatography (HPLC-TOSOH G7〞 Jaban〞) . [53]

3.9. Methods:

Measurement of Hb F done by using HPLC G7Analyzer

3.9.1. Principle:

HPLC-723G7, HLC-723G8 and HLC-723GX and HLC-723G11 are fully automated high performance The liquid chromatography instrument-reagent systems that rapidly and precisely separate hemoglobins found naturally in blood. Charged hemoglobins and other hemoglobin components are eluted at varying times depending upon the net charge of the molecule in relation to a gradient of increasing ionic strength passed through a non-porous cation exchange column (negatively charged beads). Cation exchange columns employ the differences in ionic interactions between hemoglobin components to separate them into a total of fractions .

A step gradient featuring three elution buffers with differing salt concentration is used to separate when assaying Hb F, s-A_{1c} ,Total A_1 and Hb A_2 ,according to the manufacture catalog

3.9.2. Procedure and Reagent:

Procedure and Reagent available at appendix no3

3.10. Ethical consideration :

The laboratory testing and data was collected after obtained oral informed consent from the study participations.

3.11. Data analysis:

The collected data code in master sheet and proceed using SPSS version 11.5. (mean , stander deviation and stander error mean , P. value by using independent T test . ANOVA test was used . The data were finally presented in tables

Chapter Four
Results

4. Results:-

-This is study included 60 pregnant females and 20 un pregnant females as control group at ages between 19-44 years from Khartoum city.

The measurement of Hb F was carried out at the first three months , second three months and third three months of gestation by using full automated HPLC G7 in Elzaytouna specialized hospital.

-In this study the level of Hb F during pregnancy showed a strong significant increase of Hb F level when compared to age matched un pregnant females (P value 0.00), the mean of Hb F during pregnancy 0.96±0.37% while in the un pregnant control group it was 0.49±0.16% . Table (4.1)

-There was significant difference when the HbF level compared between the three trimester groups using ANOVA test(p □ 0.05). Also bivariate analysis between the first and second and third, and second and third groups showed significant difference . Table (4.2)

-According to an age the case group divided into two groups one from 19 to 31years and another from 32-44 years the level of Hb F when compared between this tow groups showed no significant difference (p value 0.916). Table(4.3)

Table (4.1) mean of HbF comparison across the study group

Parameters	**Group**	**Mean±SD**	***P-value***
Hb F	Cases	0.96±0.37	0.000
	Control	0.49±0.16	

Table (4.2) mean of HbF comparison across pregnancy trimester

Parameters	Group	Mean ±SD	P-Value
First trimester with Second trimester	First trimester	1.21±0.39	0.028
	Second trimester	0.97±0.34	
First trimester with Third trimester	First trimester	1.21±0.39	0.001
	Third trimester	0.86±0.27	
Second trimester with third trimester	Second trimester	0.97±0.34	0.284
	Third trimester	0.86±0.27	

Table (4.3) mean of HbF comparison across age group

Parameters	Age	Mean±SD	*P-Value*
HbF	19-31 Years	1.02±0.33	0.916
	32-44 Years	1.01±0.39	

Chapter Five
Discussion
Conclusion
Recommendations

5.1. Discussion :-

-In this study the average of Hb F during pregnancy was 0.96±0.37,while in non pregnant control group was 0.49±0.16 this reflect the fact pregnancy associated with significantly higher of Hb F level .pregnancy accompanied by a marked increase in the circulating blood volume by 40%, an increase in the number of adult F cell production result of an increased demand for red cells. [54] Same study conducted in 72 chinese women done by George T.C.Chan found significant increase in the level of Hb Fin the normal pregnancy women this increase showed in first trimester evidenced by greater stander deviation but second trimester comedown to non pregnant value and stays at this level throughout the third trimester.

-The increase of Hb F shown in first trimester the mean 1.21±0.39 while decrease gradually in second trimester to 0.97±0.34 then in third trimester 0.86±o.27that reflect the increase level of Hb F during pregnancy related to age of gestation, HbF level was significant increase during first trimester compared with those during second and third trimester consistent with result of previous studies in which the proportion of red cell containing Hb F peak during pregnancy between 2and7 month of gestation ,the increase in the red cell containing Hb F during the earlier stages of pregnancy suggested to be due to an increase number of adult f cells. [55,56]

However fetal hemoglobin entering the maternal circulation may contribute to some extent the elevated of HbF level in first trimester .

-There was no significant difference Hb F level when compare case group according to age that reflect the increase of Hb F level related to pregnancy not to age .

5.2. Conclusion:-

The finding of this study suggest that :

-The fact normal pregnancy associated with significant increase in HbF , the high level of Hb F shown during the first trimester that declined gradually in second and third trimester .there was significant difference in the level of Hb F between three trimester groups

5.3. Recommendation :-

Beside the finding of this study we recommended that :

1-Another study with bigger sample size should be done.

2-Molecular technique should be used to detected the genetic variation.

3- further investigation by Hb F electrophoresis should be done

4-Other advance techniques should be done

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Appendix

Appendix I

University of Shendi

Faculty of Graduate studies

Haematology Department

Questionnaire

Title of Research:

Determination of hemoglobin F level in normal pregnant women in Khartoum town During Period From April to September 2018.

.This questionnaire used only for purpose of the research

Patient No:…….

Age:……...

Month of pregnancy:……

Number of trimester:…….

The result……..

Appendix II

إقرار بالموافقة

الاسم:----------

العمر----------

العنوان----------

أوافق بمحض ارادتى بلمشاركة فى البحث العلمى المتعلق ب دراسة تحديد مستوى الهيموقلوبين الجنينى في الحمل الطبيعى لدى الحوامل فى مركز دار البر الطبى التخصصى .

الباحثة :حنان ابكر تيراب محمد

بعد ان شرحت لي بانه لا يترتب عليه إي أذي جسدى او نفسى واعلم أن المشاركة فى هذا البحث لن تؤثر على بأى حال من الاحوال في الرعاية الصحية التي أتلقاها كما أنه يحق لى بدون ابداء اسباب الإنسحاب من هذا البحث .

البحث بإشراف:

د.حمزة أحمد حسن محمد التوم

التوقيع:----------

التاريخ:----------

Appendix III

P0500902

Automated Glycohemoglobin Analyzer HLC-723®G7

Instructions For Use

G7 Variant Elution Buffer HSi

TOSOH

TOSOH CORPORATION
BIOSCIENCE DIVISION
Shiba-Koen First Bldg.
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EC REP **TOSOH EUROPE N.V.**
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B-3980 Tessenderlo, Belgium
Phone: +32 13 66 88 30 Fax: +32 13 66 47 49

Supplied by **TOSOH BIOSCIENCE, INC.**
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South San Francisco, CA 94080, USA
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CE

TOSOH

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Safety Precautions

To help protect you and/or your property from potential damage and ensure personal safety, please read this IFU thoroughly before using the product.

[Notational Convention]

Notation	Explanation
⚠ CAUTION	Indicates a hazard with a low level of risk which, if not avoided, could result in minor or moderate injury.

⚠ CAUTION

- **Use only in well ventilated areas**

  In case of insufficient ventilation, flammable and toxic solvents can cause fire, explosion, or poisoning.

- **First Aid**

  Skin exposure

  Wash exposed area with plenty of soap and water.

  Eye exposure

  Open eyes as wide as possible and wash with clean water for at least 15 minutes. Immediately call for medical attention.

  Ingestion

  Please wash mouth with excess water and immediately call for medical attention.

- **Do not spill solvents**

  Spillage and leakage can cause fire, electric shock, poisoning, injury, and corrosion. Wear appropriate protective gear when cleaning up a spill.

- **Wear eye protection and protective gloves**

  Organic solvents and acids are harmful and should not come in direct contact with the skin.

- **Handle package with care**

  Inappropriate handling may cause rupturing and/or spattering of the product.

- **Only use this product as intended**

  This product is intended for *in vitro* diagnostic use for the measurement of HbA_{1c} in blood specimens.

  US federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.

- **Proper disposal**

  Dispose in accordance with local laws and regulations.

NOTE

Keep this IFU with the product for future reference.

Европейски потребители / Evropští zákazníci / Europæiske kunder / Europäische Kunden / Ευρωπαίοι πελάτες / European customers / Clientes europeos / Euroopa kliendid / Consommateurs européens / Európai ügyfelek / Clienti europei / Europos klientai / Eiropas klienti / Europeiske kunder / Klanci europejscy / Clientes europeus / Clienți europeni / Európski zákazníci / Evropski kupci / Europeiska kunder / Avrupa'dan müşteriler

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RO Instrucțiuni de utilizare în diverse limbi sunt disponibile pe site-ul nostru: www.tosohbioscience.eu, la secțiunea "Service & Support" (Service și asistență tehnică). O versiune pe hârtie poate fi obținută comandând-o prin fax, la +32 13 66 47 49, sau prin e-mail, la info.raqa.eu@tosoh.com. Versiune Actuală: G7V-0100901

SK Návod na použitie vo viacerých jazykoch je dostupný na našej web stránke: www.tosohbioscience.eu, v sekcii "Service & Support". Vytlačenú verziu možno získať faxom +32 13 66 47 49 alebo cez e-mail: info.raqa.eu@tosoh.com Aktualna Verzia: G7V-0100901

SL Večjezična navodila za uporabo so na voljo na naši spletni strani: www.tosohbioscience.eu v razdelku "Service & Support" (Servis in podpora). V papirni obliki jih lahko prejmete po faksu: +32 13 66 47 49 ali elektronski pošti: info.raqa.eu@tosoh.com. Aktualna Različica: G7V-0100901

SV Bruksanvisning på flera språk finns att tillgå på vår webbplats: www.tosohbioscience.eu under rubriken "Service & Support". En pappersversion kan erhållas per fax +32 13 66 47 49 eller e-post: info.raqa.eu@tosoh.com. Aktuell Version: G7V-0100901

TR Bir çok dilde Kullanma Talimatları'na www.tosohbioscience.eu adresindeki web sitemizin, "Service & Support" bölümünden ulaşabilirsiniz. Basılı sürümünü +32 13 66 47 49 numaralı faks ya da info.raqa.eu@tosoh.com e-posta adresinden isteyebilirsiniz. Mevcut Sürüm: G7V-0100901

Symbols on the product labels

European Conformity

Manufacturer

Authorized representative in the European Community

Supplied by

In vitro diagnostic medical device

Consult instructions for use

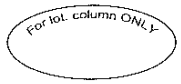
Catalogue number / Part number

Batch code / Lot number

Temperature limitation

Use by date

Net volume (after reconstitution for lyophilized material)



For specified column lot only

CONTENTS

Revised in June, 2016

1. Introduction

Based on the principle of High-Performance Liquid Chromatography assay, the G7 Variant Elution Buffer HSi is designed exclusively for use with the Automated Glycohemoglobin Analyzer HLC-723G7 (referred to as HLC-723G7 in the IFU). It is not designed for and should never be used with any other type of system.

The G7 Variant Elution Buffer HSi is intended for *in vitro* diagnostic use for the measurement of Hemoglobin A_{1c} (HbA_{1c}) in blood specimens. Hemoglobin A_{1c} measurements are used in the clinical management of diabetes to assess the long-term efficacy of diabetic control.
Glycohemoglobin (GHb) is a general term for whole blood glucose complexes which are nonenzymatically bound to the α and β chains of human hemoglobin.
The most quantitatively prevalent complex is called HbA_{1c}, in which glucose binds to the N-terminus of the β chain.

HbA_{1c} is nonenzymatically synthesized in two steps:

Step 1. The glucose aldehyde group and the free amino group on the valine in the N-terminus of the β chain react to form the Schiff bond (aldimine, labile A_{1c} (L-A_{1c})).
Step 2. A stable ketoamine form (stable HbA_{1c} (s-A_{1c})) is produced by a reaction known as amadori rearrangement.

Since, in the measurement of HbA_{1c}, L-A_{1c} (the intermediate product of this process) changes rapidly in response to whole blood glucose concentration, the s-A_{1c} complex is used to measure HbA_{1c}. Because, unlike L-A_{1c}, s-A_{1c} does not fluctuate in response to physiological factors, s-A_{1c} provides a better indication of the average glucose level over the last 1 to 3 months, in order to monitor diabetes control.

In the past, accurate measurement of s-A_{1c} was possible only after removing L-A_{1c} by pretreatment. Now, by using the TSKgel® G7 Variant HSi, s-A_{1c} and L-A_{1c} can be rapidly separated, allowing the accurate measurement of s-A_{1c}, without pretreatment.

The hemoglobins found in a normal adult are hemoglobin A, which comprises about 97 % of the total hemoglobin. On the other hand, various hemoglobin abnormalities have also been reported in different regions, especially in area with a large immigrant population.

Abnormal hemoglobins or hemoglobin variants may co-elute with other hemoglobin components and lead to misinterpretation of s-A_{1c} values. The HLC-723G7, when used with the TSKgel G7 Variant HSi and the G7 Variant Elution Buffer HSi will produce s-A_{1c} values that will not be affected by most commonly-known hemoglobin variants.

2. Prior to Use

Inspect the packaging and the exterior of the aluminum pack for any signs of damage prior to use. If any damages are visible, contact your local Tosoh sales representative.

Confirm that the following document is included in the package.
• Instructions For Use 1 copy (in each box)

3. Warnings and Precautions

1) This product is for *in vitro* diagnostic use only.
2) This product is intended for use on the Automated Glycohemoglobin Analyzer HLC-723G7 Hemoglobin A_{1c} (s-A_{1c}) 2.2 min. analysis method only.
3) Do not use this product beyond the expiration date.

4. Content

The following types of elution buffers are designed for exclusive use with the HLC-723G7.

Catalogue No.	Description	Package content
0019552	G7 Variant Elution Buffer HSi No. 1 (S)	10 × 800 mL
0019553	G7 Variant Elution Buffer HSi No. 2 (S)	10 × 800 mL
0019554	G7 Variant Elution Buffer HSi No. 3 (S)	10 × 800 mL

Each contains less than 0.05 % sodium azide as a preservative.

Assay mode : Hemoglobin A_{1c} (s-A_{1c}) 2.2 min. analysis method
Applicable analyzers : Automated Glycohemoglobin Analyzer HLC-723G7

5. Related Components

	Catalogue No.
Hemoglobin A1c Calibrator Set	0018767
Hemoglobin A1c Control Set	0021974
TSKgel G7 Variant HSi	0019680
HSi Hemolysis & Wash Solution (L)	0018431
HSi Hemolysis & Wash Solution (LL)	0019550

6. Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at 4 to 30 ℃. The expiration date indicated on the package box and aluminum pack labels.
Stable for 3 months after opening when stored at 4 to 25 ℃.

7. Specimens

Whole blood samples.

8. Assay Principle

Separation is achieved with the TSKgel G7 Variant HSi by utilizing differences in the ionic interactions between the cation exchange group on the resin surface and the hemoglobin components. HbF, s-A_{1c} and Total A_1 and Hb Variants can be separated by performing a step-wise elution of three-types G7 Variant Elution Buffer HSi (G7 Variant Elution Buffer HSi No. 1, 2 and 3) with different salt concentrations.

9. Installation

Be sure to read the IFU included with the TSKgel G7 Variant HSi, Hemoglobin A1c Calibrator Set, Hemoglobin A1c Control Set, G7 Variant Elution Buffer HSi, HSi Hemolysis & Wash Solution as well as the HLC-723G7 Operator's Manual.

1) Press the STOP key on the HLC-723G7 system operation panel to enable the STAND-BY mode.
2) Remove the cap from the elution buffer pack.
3) Make sure to insert the tube matching the color of the elution buffer being installed.
4) Gently squeeze the elution buffer pack by hand to remove all excess air, then tighten cap firmly into place to create a vacuum that will prevent air from entering during operation.
5) Press the REAGENT CHANGE key on the Mainte screen.
6) Press the keys of the reagent to replace on the REAGENT CHANGE screen.
7) Press the CHANGE key. A confirmation message will be displayed.
   If everything is all right, press the OK key.

10. Assay Procedures

Refer to the HLC-723G7 Operator's Manual for detailed instructions.

11. Precautions for Use

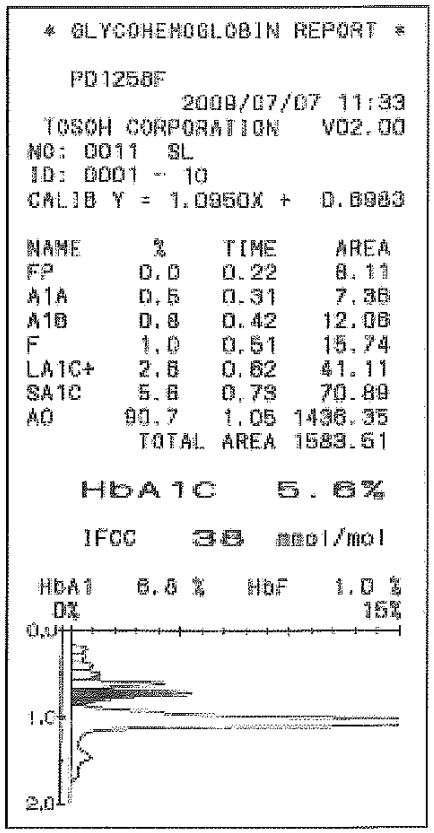
1) Carefully read the instructions contained in this IFU and related Instructions For Use provided with HLC-723G7, TSKgel G7 Variant HSi, Hemoglobin A1c Calibrator Set, Hemoglobin A1c Control Set and HSi Hemolysis & Wash Solution.
2) The G7 Variant Elution Buffer HSi is designed exclusively for use in combination with the HLC-723G7 analyzer system, TSKgel G7 Variant HSi and HSi Hemolysis & Wash Solution indicated below, never in any other combination.
   • Automated Glycohemoglobin Analyzer HLC-723G7
   • TSKgel G7 Variant HSi
   • HSi Hemolysis & Wash Solution (L), (LL)
3) Always use the G7 Variant Elution Buffer HSi in combination with the TSKgel G7 Variant HSi of the identical lot number. The column lot number is indicated by a single uppercase alphabetical character (A, B, etc.) on the label of column box. The elution buffer label displays an alphabetic character corresponding to the column lot number, as shown below.

4) Return the elution buffer to room temperature before use.
5) When first opening the aluminum pack to insert the suction tube, gently squeeze the elution buffer pack by hand to remove all excess air, and tighten bottle cap firmly to create a vacuum that will prevent air from entering during operation.
6) Never use reagents that have exceeded the expiration date printed on their labels. Assay results for expired reagents will not be reliable. Also note that reagents must be used within three months after opening (provided that the container is correctly maintained in vacuum state).
7) When there is leftover reagent in the aluminum pack that must be removed from the analyzer and stored, again gently squeeze the elution buffer pack by hand to remove all excess air, and tighten the bottle cap firmly to create a vacuum that will prevent air from entering during storage and store between 4 and 25 ℃.
8) Always replace with an aluminum pack when elution buffer is

almost empty. Avoid refilling leftover elution buffer into aluminum pack as this can produce unreliable assay results.

9) For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state and federal regulations.

12. Reference Data (from HLC-723G7 Assay Examples)



13. Measurement Values

Measurement values (%) indicate the percentage of each peak in relation to the Total Area (excluding the front peak (FP)). Note the minimum unit of measurement displayed is 0.1 %.

14. Evaluation of Results

Quality Control

In order to monitor and evaluate the precision and analytical performance, it is recommended that control samples should be run daily. If one or more control sample value(s) is out of the acceptable range, it is necessary to investigate the validity of calibration curve before reporting patient results. Standard laboratory procedures should be followed in accordance to the strictest regulatory agency under which the laboratory operates.

15. Expected Values

Reference Ranges (nondiabetic) : HbA_{1c} 4.0-6.0 % (MEAN 5.0 %, SD 0.5 %)

Ref American Diabetes Association. Standards of medical care for patients with diabetes mellitus. Diabetes Care 2000; 23 (Suppl. 1), S32-42.

The values referred to within this IFU have been determined with a NGSP certified method. It is known that the relationship between HbA_{1c} results from the NGSP network (%) and the IFCC network (mmol/mol) is expressed by using the following equation (See http://www.ifcchba1c.net/IFCC 08.asp):

$$NGSP = 0.09148 \times IFCC + 2.152$$

16. Performance Characteristics

Dilution / Linearity

The study demonstrates that the assay is linear in samples with Total Area values from 500 to 4500.

Total Area	HbA_{1c} (%)	Total Area	HbA_{1c} (%)
255	5.6	551	10.2
568	5.7	1155	10.3
1162	5.7	1711	10.2
1775	5.7	2312	10.2
2420	5.7	2975	10.3
3056	5.7	3350	10.3
3909	5.7	4191	10.3
4553	5.7	4686	10.3

Recovery / Linearity
Two samples were mixed in specified ratios to produce 5 samples with a range of HbA_{1c}% levels. The study demonstrates linearity in the measurement of HbA_{1c}%.

Sample A (mL)	Sample B (mL)	Measurement value	Theoretical value	Recovery %
0.0	1.0	4.2	4.2	100
0.3	0.7	9.2	9.3	99
0.5	0.5	12.5	12.7	98
0.7	0.3	15.8	16.1	98
1.0	0.0	20.8	20.8	100

Comparative Analysis
Patient specimens were analyzed with the HLC-723G7 analyzer assay and another commercially available glycohemoglobin assay. About a half of the samples were from healthy individuals with HbA_{1c} results less than 6 %.
The remaining samples were from diabetics in the abnormal range. The comparison yielded the following correlation statistics between these two methods. These two methods are certified by NGSP.

Slope : 1.0988
Y-Intercept : 1.1646
Correlation Coefficient : 0.9924
Range of Samples (%) : 5-13
n : 100

17. Precision

Intra-Assay
The intra-assay (within run) precision coefficient of variation was evaluated with diluted blood, two controls and two whole blood specimens with two HbA_{1c} levels (low HbA_{1c} < 6 %, and high HbA_{1c} > 8 %).

(n=20)

	MEAN (HbA_{1c})	SD	CV %
Control (L)	4.9	0.04	0.8
Control (H)	8.7	0.04	0.5
Diluted	4.0	0.03	0.9
Whole	4.1	0.02	0.5
Whole	13.4	0.12	0.9

Inter-Assay
The inter-assay (between run) precision coefficient of variation was evaluated by testing two control specimens (HbA_{1c} < 6 % and HbA_{1c} > 8 %) once a day for 20 days.

(n=20)

	MEAN (HbA_{1c})	SD	CV %
Control (L)	4.9	0.06	1.2
Control (H)	9.1	0.07	0.8

18. Interferences

A substance is considered to interfere when the recovery of a known specimen falls outside a 10 % acceptability range.

1) Icterus, as indicated by free and conjugated bilirubin concentrations up to 200 mg/dL, does not interfere with the assay.
2) Lipemia, as indicated by triglyceride concentrations up to 2000 mg/dL, does not interfere with the assay.
3) Concentrations of up to 20 mg/dL of ascorbic acid, salicylic acid (aspirin), sodium cyanate, and acetaldehyde, do not interfere with the assay.
4) The presence of HbD, HbS, HbC, Labile HbA_{1c}, Carbamyled Hb does not interfere with the assay. High HbF may interfere with the assay.
5) A reduced concentration of HbA_{1c} can be caused through the limited life span of the red blood cells. Clinical interpretation have to be done with care.
6) A shoulder on the HbA_0 peak can be obtained through the presence of HbE[2][3].
7) Total Areas < 500 of the chromatgrams can be obtained through low concentrations of hemoglobin.
8) Hemoglobin concentrations up to 4500 mg/dL do not interfere with the assay.
9) Hemolysed samples does not interfere with the assay[2][3].
10) Since a high number of Hb Variants is observed worldwide, care should be taken when interpreting the results. Designation of a particular name cannot exclude the presence of other variants.

19. Specimen Collection and Handling

A whole blood sample is required for the assay.
No special preparation is necessary. A venous blood sample is collected aseptically. The blood samples in primary tubes, which contain EDTA, NaF, Heparin and Citric acid may be stored at 25 ℃ for 24 hours prior to analysis, or at 4 ℃ for 14 days.

20. Reference

(1) Gremmels H-D, Richter A, Watzke I. Evaluation of the Hemoglobin A1c-Analyzer TOSOH HLC-723 G7. Clin. Lab. 2003; 49: 243-250.
(2) Study: Hopital Pitie-Salpetriere, Paris, 2001.
(3) Study: Newcastle upon Tyne Hospital: Royal Victoria Infirmary, 2001.