بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Association Between Serum Lipid Profile and Blood Group Types in Healthy Sudanese Individuals

العلاقة بين مستوى الدهون العام في الدم ونوع فصيلة الدم لدى الأشخاص الأصحاء في السودان

A dissertation submitted in partial fulfillment for the requirements of

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لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا ۚ لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ ۗ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا ۚ رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا ۚ رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ ۖ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا ۚ أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ

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Dedication

To my eyes, I see from it

My mother

To the spinal cord of my family

My father

To whom bright my life

To everyone teach me one letter

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Abstract

Background: lipids are playing avital role in all aspects of life, and the ABO system is the most important system in a transfusion process.

The aim of this study was to associate between serum levels of total cholesterol, triglycerides, HDL-C, LDL-C and ABO blood group in healthy Sudanese individuals.

Materials and methods: This is a cross- sectional study conducted during the period from March 2019 to August 2020, the study includes 200 healthy individuals (50 of them were A blood group, 50 were O blood group, 50 were B blood group and 50 were AB blood group) in Khartoum state, to determine the ABO blood group type and serum cholesterol, triglyceride, HDL-C , LDL-C . The estimation of serum lipid profile was done by using Cobas 311 chemistry analyzer; Statistical analysis was done by using SPSS.

The Results: Showed that a significant increase in the means of serum cholesterol (with p value = 0.004) and triglycerides (P value = 0.000) between different blood groups with the highest mean of serum cholesterol (mean ± SD: 176.0 ± 42.5 mg/dl) and triglycerides (mean ± SD: 191.2 ± 130.6 mg/dl) in AB blood group. The study also find positive correlation between the serum cholesterol and age in blood group O, B, AB (R=0.399, P value=0.004), (R=0.431, P value=0.002), (R=0.403, P value=0.004) respectively. And also find positive correlation between the serum triglycerides and age in blood group A and O (R=0.463, P value=0.001), (R=0.408, P value=0.003) respectively. And positive correlation between LDL-C and age in blood group B and AB (R=0.385, P value=0.006), (R=0.463, P value =0.001) respectively.

Conclusion: Individuals with AB blood group found to have high levels of serum cholesterol and triglycerides when compared to other blood groups, and positive correlation between age and cholesterol , triglycerides and LDL-C in many blood groups.

المستخلص

خلفية الدراسه : تلعب الدهون دور مهم وحيوي في جميع وجوه الحياة , وأيضا معرفة نوع فصيلة الدم بنظام (أ,ب,و) مهم جدا في عمليات نقل الدم .

الهدف من هذه الدراسة هو ايجاد العلاقة بين مستوى الكوليسترول الكلي والدهون الثلاثيه والكوليسترول عالي والكوليسترول منخفض الكثافه ونوع فصيله الدم لدي الاشخاص الأصحاء في السودان.

مواد و طرق البحث :أجريت هذه الدراسة المقطعيه في الفترة بين مارس 2019 وحتى أغسطس2020م في الاشخاص الاصحاء في ولاية الخرطوم لمعرفة نوع فصيلة الدم وقياس مستوى الدهون العام في الدم باستخدام جهاز التحليل الكيما ئي (كوباس 311) و حللت النتائج احصائياً باستخدام الكمبيوتر.

شملت الدراسة 200 شخص سليم، منهم 50 شخص فصيلة دمهم (أ) ، و 50 شخص فصيلتهم (ب) ، و50 شخص فصيلتهم (و) ، و 50 شخص فصيلتهم (أب).

النتيجة :أظهر التحليل الأحصائي للعينات ان هنالك فرق ذو دلاله أحصائيه في متوسط معدل الكوليسترول بقيمة أحتماليه 0.004 و الدهون الثلاثية بقيمة أحتماليه 0.000 بين فصائل الدم المختلفة مع وجود أكبر متوسط للكولسترول (176.0±42.5) و الدهون الثلاثية (191.2±130.6) في الأشخاص ذوي الفصيلة (أب) .

أيضاً توجد علاقة طردية موجبه بين العمر ومستوى الكوليسترول في فصائل الدم أ - أب – و (القيمة الإحتماليه = 0.002 ، 0.004 ، 0.004 بالتتابع). و مستوى الدهون الثلاثية في فصائل الدم أ – و (القيمة الإحتمالية = 0.001 ، 0.003 بالتتابع). و الكوليسترول منخفض الكثافة في فصائل الدم أ – أب (القيمة الإحتمالية = 0.006 ، 0.001 بالتتابع).

خلاصة الدراسة : وجود أعلي معدل للكوليسترول و الدهون الثلاثية في الأشخاص ذوي الفصيلة (أب)، و زيادة طردية لكل من معدل الكوليسترول ، الدهون الثلاثية و الكوليسترول منخفض الكثافة مع الزيادة في العمر .

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List of Abbreviations

CVD	Cardio Vascular Disease
IHD	Ischemic Heart Disease
MI	Myocardial Infarction
VWF	Von -Willebrand Factor
GWAS	Genome-Wide Associations
IAP	Intestinal Alklaine Phosphatase
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
VLDL	Very Low Density Lipoprotein
CM	Chylomicron
NEFAS	Non Esterified Fatty Acids
HMG-COA	Hydroxy Methyle Glutaryl COA
ABC1	Adenosine Triphosphate Binding Cassette
LCAT	Lecithin-Cholesterol Acyl Transferase
SRB-1	Scavanger Receptor Type B1
CHD	Coronary Heart Disease
PVD	Priephral Vascular Disease
RBCs	Red Blood Cells
BGS	Blood Groups
Ig	Immunoglobulin
Rh	Rhesus Factor

Chapter One

Introduction

CHAPTER ONE

1 Introduction, rationale and objectives

1. 1 Introduction

ABO system one of the most important parts in blood transfusion process ,and it is found that there is a close relationship between ABO system and other many diseases such as diabetes mellitus (Kamil et al., 2010), breast cancer (Meo et al., 2017) and gastric cancer (Edgren et al., 2010).

Between the different populations ,the frequency of the ABO blood groups differs, for example, in Sudan the (O blood group) is most frequent one ,then followed by A ,B ,and AB blood groups (Shahata et al., 2012).

The relationship between ABO blood group and serum lipid profile has been investigated in various populations .Serum lipid is the biochemical marker that strongly involved in the development of CVD in particular, serum cholesterol.

As (Suliman., 2011) reported in his research, the Cardiovascular diseases (CVD) one of the most important causes of morbidity and mortality in Sudan . Different studies presented that, the ABO- blood groups act as risk factor that participate in formation of cardiovascular diseases (Wu et al., 2008, Abdollahi et al., 2009) such as ischemic heart disease IHD (Whincup et al., 1990) thrombosis (Rumley et al., 1999) (Franchini et al., 2007) and MI (Myocardial Infarction) (Galeazzi and Gualandri, 1975, Lin et al., 2017) .

(Lin et al., 2017) suggested that blood group (A) subjects are the most susceptible for MI , other studies reported that blood type B (Stakisaitis et al., 2002) or AB (Bartimaeus and Waribo., 2017) also have susceptibility. A British study conducted by (Mitchell., 1977) reported that cities with high incidence of group O are the highest in mortality rates that caused by CVD .

Blood groups other than O group found to be with high level of Von-Willebrand Factor (VWF) which has been associated with increased

thrombotic risk (Ohira et al., 2007) and incidence of major IHD events (Meade et al., 1994) and ,it is known that plasma VWF level reported to be approximately 25% higher in non-O blood individuals(Franchini et al., 2007).

In the last recent years the Genom-Wide association (GWAS) has confirmed ABO as a locus for venous thrombo embolism (VTE), MI and multiple cardiovascular biomarkers (Zeller et al., 2011).

Many researchers revealed that healthy people of blood type A have higher mean of serum cholesterol than other groups and consider A blood type as a risk factor for CVD (Gali et al., 2010) ,This is imputable to many researches presented evidence that individuals of A-blood type have lowest activity of intestinal alkaline phosphatase (IAP)than other blood group (Domar et al., 1991) , this is in the accordance to the evidence that IAP is involved in the regulation of the lipid absorption process (Nakano et al., 2007).

Also , other studies have been done found the triglyceride level is higher in individuals with B antigen (B + AB) than other blood groups (Contiero et al., 1994) by contrary, also negative association of B and AB blood groups with triglyceride level was found (Algroom et al., 2015).

(Ureme et al.,2018) showed that a subjects of O blood group have higher level of LDL-C and lower HDL-C to non-O blood groups ,Whereas, other study have not been provided any association between total cholesterol , HDL-C and LDL-C and ABO blood group (Contiero et al., 1994).

1.2. Rationale

Different studies on different populations have yielded that serum lipid profile is significantly affected according to the blood group type (Contiero et al., 1994) (Bartimaeus and Waribo, 2017). The serum lipid play major rule in assessment of heart diseases as a risk factor, and the presence of abnormal Serum lipid levels in certain blood group more than others acts as an alarm for them to change their life style and pay attention for its regular assessment in sake of prevention, to the best of our knowledge no published data were found concerning this association between serum lipid and blood group in Sudanese population, That's why we attempt to do this study.

1.3 Objectives

1.3.1 General objective:

To determine the association between serum levels of total cholesterol, triglycerides, HDL-C, LDL-C and ABO blood group in Sudanese individuals.

1.3.2 Specific objectives:

1. To measure level of serum cholesterol, triglycerides, HDL-C and LDL-C in different blood group types individuals.
2. To compare levels of Serum cholesterol, triglycerides, HDL-C and LDL-C among different ABO blood types.
3. To correlate between levels of serum cholesterol, triglycerides, HDL-C and LDL-C and age according to blood group type.

Chapter Two

Literature Review

CHAPTER TWO

2 Literature review

2.1 ABO blood group system:

The ABO blood group system (BGS) is the most important human BGS in transfusion practice and was the first to be discovered by Karl Landsteiner in 1900. He was able to identify three different patterns of reactivity, which were later reclassified as groups A, B, and O (Quinely ., 2011). Von Decastello and Sturli discovered group AB (the rarest of the common ABO types) defined by antigens present on the surface of red blood cells (RBCs), A, B, O, and AB represent the four major groups in the ABO system, all normal, healthy people older than 3 months of age have naturally occurring antibodies to the ABO antigens that they lack. These antibodies were first called naturally occurring because they were thought to arise without antigenic stimulation (Quinley ., 2011).

2.1.1 Inheritance of the ABO Blood Groups:

The inheritance of ABO genes follows simple Mendelian genetics. ABO, like most other blood group systems, is codominant in expression. One position, or locus, on each chromosome 9 is occupied by an A, B, or O gene, the O gene is considered an amorph, as no detectable antigen is produced in response to the inheritance of this gene, therefore, the group O phenotype is an autosomal recessive. An individual who has the phenotype A (or B) can have the genotype AA or AO (or BB or BO the phenotype and genotype are the same in an AB individual because of the inheritance of both the A and B gene (Harmening ., 2012) .

In blood group A formation, the A gene (AA or AO) codes for the production of α-3-N-acetylgalactosaminyltransferase which transfers an N-acetyl-D-galactosamine sugar to the H substance, this sugar is responsible for a specificity. Individuals who are blood group B inherit a B gene (BB or BO)

that codes for the production of 3-D galactosyl transferase, which attaches D-galactose (Gal) sugar to the H substance, this sugar is responsible for B specificity (Catovsky et al ., 2005).

When both A and B genes are inherited, the B enzyme (3-D galactosyl transferase) seems to compete more efficiently for the H substance than the A enzyme 3-Nacetylgalactosaminyltransferase). Individuals who are blood group O inherit at least one H gene (genotype HH or Hh) and two O genes (Quinely 2011). The H gene elicits the production of an enzyme called α-2-L-fucosyltransferase, which transfers the sugar L-fucose to an oligosaccharide chain on the terminal galactose of type 2 chains, therefore, L-fucose is the sugar responsible for H specificity, the O blood group has the highest concentration of H antigen, the H gene is present in more than 99.99% of the random population (Harmening ., 2012).

2.1.2 Development of the A, B and H antigens:

The A and B antigens can be detected on the red cells of very young fetuses, but their reactions are weaker than those of adult. Similarly, the H antigen is less well developed at birth than in adult life. After birth, the expression of the A, B and H antigens increases until about 3 years of age, and thereafter, in health, remains stable throughout life (Catovsky et al., 2005).

2.1.3 ABO antibodies:

The antibodies of the ABO system arise shortly after birth on exposure to environmental agents for which antigenic makeup is similar to the A and B antigens. They are primarily IgM in nature, although some quantity of IgG and IgA may also be present,these antibodies follow the general traits of IgM antibodies (i.e., they react best at room temperature or below, are capable of activating complement, and are saline agglutinins), the IgM and IgA versions of the ABO antibodies do not cross the placental barrier. However, IgG versions cross the placenta and may cause hemolytic disease of the newborn (Quinley ., 2011).

Immune antibodies develop in response to the introduction by transfusion or by transplacental passage during pregnancy of red cells possessing antigens that the subject lacks .These antibodies are commonly IgG, although some IgM antibodies may also develop usually in the early phase of an immune response, Immune antibodies react optimally at 37°C (warm antibodies),only IgG santibodies are capable of transplacental passage from mother to fetus and The most important immune anti-body is the Rh antibody, anti-D (Hoffbrand et al ., 2005).

2.2 Lipids:

Lipids are defined as organic compounds that are poorly soluble in water but miscible in organic solvents (Crook., 2012).

Lipids commonly referred to as fats, are ubiquitous constituents of all living cells and have a dual role. First, because they are composed of mostly carbon hydrogen (C–H) bonds, they are a rich source of energy and a efficient way for the body to store excess calories .Because of their unique physical properties, lipids are also an integral part of cell membranes and, therefore, also play an important structural role in cells. Lipids are also precursors for the steroid hormones, prostaglandins, leukotrienes, and lipoxins. The lipids transported by lipoproteins, namely, triglycerides, phospholipids, cholesterol, and cholesteryl esters, are also the principal lipids found in cell membranes (Bishop et al., 2018).

The major lipids present in the plasma are fatty acids**,** triglycerides, cholesterol and phospholipids. Other lipid-soluble substances present in much smaller amounts but of considerable physiological importance include steroid hormones and fat soluble vitamins. Because they are not water soluble, lipids are transported in the plasma in association with proteins (Bishop et al., 2018).

Albumin is the principal carrier of free fatty acids (FFAs), the other lipids circulate in complexes known as lipoproteins. These consist of a non-polar

core of triglyceride and cholesteryl esters surrounded by a surface layer of phospholipids, cholesterol and proteins known as apolipoproteins (Marshal et al., 2012).

2.2.1 Basic Lipid Biochemistry:

Lipid applies to a class of hydrophobic compounds that are soluble in organic solvents and nearly insoluble in water. Chemically, lipids are usually enriched in carbon and hydrogen and after hydrolysis typically yield fatty acids or complex alcohols, which are usually esterified with fatty acids. Some lipids are more complex, containing other chemical groups, such as sialic, phosphoryl, amino, or sulfate groups. The presence of these charged or polar groups makes these lipids amphipathic, which gives them the property of having an affinity for both water and organic solvents; this is an important feature in their ability to form cell membranes (Burtis et al., 2012).

2.2.2 Biomedical importance:

Lipids are playing a vital role in virtually all aspects of life: Serve as hormones or hormone precursors, aiding in digestion, providing a source of metabolic fuel and energy storage, acting as functional and structural components in cell membranes, and forming insulation to allow nerve conduction or to prevent heat loss (Burtis et al., 2012).

2.2.3 Classification of clinically important lipids:

2.2.3.1 Fatty Acids:

Fatty acids are simply linear chains of C–H bonds that terminate with a carboxyl group (COOH). They are variable in length and can be classified as short chain (4 to 6 carbon atoms), medium chain (8 to 12 carbon atoms), long chain (12 to 18 carbon atoms), or very long chain (>20 carbon atoms) fatty acids (Burtis et al ., 2012).

They may be saturated, containing no double bonds, monounsaturated, with one double bond, or polyunsaturated, with more than one double bond Fatty acids can esterify with glycerol to form triglycerides or be non-esterified

(NEFAs) or free. Plasma NEFAs liberated from adipose tissue by lipase activity are transported to the liver and muscle mainly bound to albumin. The NEFAs provide a significant proportion of the energy requirements of the body (Crook., 2012).

2.2.3.2 Triglycerides:

Triglycerides are more correctly called ‘tri acylglycerols’, they consist of glycerol esterified with three long chain fatty acids, such as stearic (18 carbon atoms) or palmitic (16 carbon atoms) acids .Triglyceride is present in dietary fat, and can be synthesized in the liver and adipose tissue to provide a source of stored energy; this can be mobilized when required, for example during starvation. Although the majority of fatty acids in the body are saturated, certain unsaturated fatty acids are important as precursors of prostaglandins and in the esterification of cholesterol .Triglycerides containing both saturated and unsaturated fatty acids are important components of cell membranes (Marshal et al., 2012).

2.2.3.3 Phospholipids:

Phospholipids are similar in structure to triglycerides except that they only have two esterified fatty acids. The third position on the glycerol backbone instead contains a phospholipid head group. There are several types of phospholipid head groups, such as choline, inositol, inositol phosphates, glycerol, serine, and ethanolamine, which are all hydrophilic in nature. The various types of phospholipids are named based on the type of phospholipid head group present , Phosphatidylcholine (often referred as lecithin) has a choline head group and is the most common phospholipid found on lipoproteins and in cell membranes, Because phospholipids contain both hydrophobic fatty acid C–H chains and a hydrophilic head group, they are by definition amphipathic lipid molecules and, as such, are found on the surface of lipid layers or on the surface of lipoprotein particles (Bishop et al., 2018).

2.2.3.4 Cholesterol:

Cholesterol is an unsaturated steroid alcohol containing four rings (A, B, C, and D), and it has a single C–H side chain tail similar to a fatty acid in its physical properties. The only hydrophilic part of cholesterol is the hydroxyl group in the A-ring. Cholesterol is, therefore, also an amphipathic lipid and is found on the surface of lipid layers along with phospholipids. Cholesterol can also exist in an esterified form called cholesteryl ester, with the hydroxyl group conjugated by an ester bond to a fatty acid, in the same way as in triglycerides (Bishop et al ., 2018).

Cholesterol is synthesized in most tissues of the body from acetyl-CoA in the microsomal and cytosolic compartments of the cell. More than 25 enzymes are involved in the formation of cholesterol from acetyl-CoA. The principal steps include the conversion of acetyl-CoA derived from either the β-oxidation of fatty acids or the oxidative decarboxylation of pyruvate to β-hydroxy β-methyl glutaryl CoA (HMG-CoA). It is also unique in that, unlike other lipids, it is not readily catabolized by most cells and, therefore, does not serve as a source of fuel (Marchal et al., 2012).

Cholesterol can, however, be converted in the liver to primary bile acids, such as cholic acid and chenodeoxycholic acid, which promote fat absorption in the intestine by acting as detergents. A small amount of cholesterol can also be converted by some tissue, such as the adrenal gland, testis, and ovary to steroid hormones, such as glucocorticoids, mineralocorticoids, and estrogens. Finally, a small amount of cholesterol, after first being converted to 7-dehydrocholesterol, can also be transformed to vitamin D3 in the skin by irradiation from sunlight (Bishop et al., 2018).

2.2.3.5 Prostaglandins:

Prostaglandins and related compounds are derivatives of fatty acids, primarily arachidonate. This group consists of prostaglandins, thromboxanes, some hydroperoxy and hydroxyl fatty acid derivatives, and leukotrienes , although

their full physiologic role is not completely known, these lipids are known to affect a wide variety of biological functions. In general, they are extremely potent, producing physiologic actions at concentrations as low as 1 μg/L (Burtis et al., 2012).

2.2.3.6 Sphingolipids:

Sphingolipids, a fourth class of lipids found in humans, are derived from the amino alcohol sphingosine. This dihydric 18-carbon alcohol contains an amino group at C-17. A fatty acid containing 18 or more carbon atomscan be bound to the amino group through an amide linkage to form ceramide. This is the intermediary step in the formation of three important sphingolipids, sphingomyelin, galactosylceramide, and glucosylceramide (Burtis et al., 2012). The glycosylceramides can have additional monosaccharide moieties, such as galactose, N-acetylgalactosamine, and N-acetylneuraminic acid to formcomplex globosides and gangliosides. These complex sphingolipids form the major lipids of cell membranes, particularly in the central nervous system (Burtis et al., 2012).

2.2.3.7 Terpenes:

Terpenes are polymers of the five-carbon isoprene unit; they form the backbone of vitamins A, E, and K and the dolichols, which play an important role in protein glycosylation (Marchal et al., 2012).

2.2.4. Lipoproteins:

Lipoproteins are typically spherical in shape and range in size from as small as 10 nm to more than 1 μm .As the name implies, lipoproteins are composed of both lipids and proteins, called apo lipoproteins, the amphipathic cholesterol and phospholipid molecules are primarily found on the surface of lipoproteins as a single monolayer, whereas the hydrophobic and neutral triglycerides and cholesteryl ester molecules are found in the central or core region and thus are micelles. Because the main role of lipoproteins is the delivery of fuel to peripheral cells, the core of the lipoprotein particle

essentially represents the cargo that is being transported by lipoproteins (Bishop et al., 2018). The size of the lipoprotein particle correlates with its core neutral lipid content. The larger lipoprotein particles have correspondingly larger core regions and, therefore ,contain relatively more triglyceride and cholesteryl ester, the larger lipoprotein particles also contain more lipid relative to protein and thus are lighter in density (Rae et al ., 2018).

2.2.4.1 Classification of lipoproteins:

Lipoproteins are classified on the basis of their densities as demonstrated by their ultra-centrifugal separation. Density increases from chylomicrons (CM, of lowest density) through lipoproteins of very low density (VLDL), intermediate density (IDL) and low density (LDL), to high density lipoproteins (HDL). HDL can be separated, on the basis of density, into two metabolically distinct subtypes: HDL2 (density 1.064–1.125) and HDL3 (density 1.126–1.21), Distinct subtypes of LDL (LDL-I, II and III, in increasing order of density) are also recognized (Marshal et al., 2012).

2.2.4.2. Lipoprotein metabolism:

Chylomicron

Chylomicrons are formed in the intestinal mucosa after a fat containing meal, and reach the systemic circulation via the thoracic duct. They then Transfer apo A to HDL and acquire apo C and apo E from HDL, the apo C II activates lipoprotein lipase in the tissues, and triglycerides are progressively removed from the hydrophobic core of the chylomicrons (Rae et al., 2018). As the size of the particles decreases, the more hydrophilic surface components (apo C, UN esterified cholesterol and phospholipid) transfer to HDL. The triglyceride poor chylomicron remnants are taken up by the liver, where they are catabolised (Rae et al., 2018).

Very low density lipoprotein VLDL

VLDL are formed from triglycerides synthesized in the liver either de novo or by re esterification of free fatty acids. VLDL also contain some cholesterol, apo B, apo C and apo E, They are synthesized in the liver and transport endogenous triglyceride from the liver to other tissues where they are removed by the action of lipoprotein lipase. At the same time, cholesterol, phospholipids and apo C and apo E are released and transferred to HDL.VLDL are the principal transport form of endogenous triglycerides and initially share a similar fate to chylomicrons, triglycerides being removed by the action of LPL (Marchal et al 2012). As the VLDL particles become smaller, phospholipids, free cholesterol and apolipoproteins are released from their surfaces and taken up by HDL, thus converting the VLDL to denser particles, IDL, under normal circumstances, there are very few IDL in the circulation because of their rapid removal or conversion to LDL (Marchal et al., 2012).

Low Density Lipoprotein (LDL)

The LDL particles are rich in cholesterol esters, probably derived from HDL; apoB100 is the only apolipoprotein. LDL is removed from the circulation by two processes; one regulated, the other unregulated (Rae et al., 2018).

The regulated mechanism involves the binding of LDL to specific apoB100 receptors present on the ‘surface pits’ of hepatocytes and other peripheral tissue cells. The entire LDL particle is incorporated into the cell by invagination of the cell membrane. Inside the cell, the particle fuses with lysosomes, apoB is then broken down and the cholesterol esters are hydrolysed , thereby making unesterified cholesterol available to the cell, the size of the intracellular cholesterol pool regulates: the rate of cholesterol synthesis in the cell, through the effect of cholesterol on HMG CoA reductase and the number of LDL apoB receptors on the cell surface, the unregulated mechanism involves receptor independent mechanisms of

cholesterol uptake by cells; these are present particularly in macrophages, these mechanisms are brought into operation especially when plasma cholesterol is increased (Rae et al., 2018).

High Density Lipoprotein HDL

The HDL is synthesized in both hepatic and intestinal cells and secreted from them as small, nascent HDL particles rich in free cholesterol, phospholipids, apoA and apoE. This cholesterol acquisition is stimulated by adenosine triphosphate binding cassette protein 1 (ABC1) (Bishop et al ., 2018).

The enzyme lecithin cholesterol acyltransferase (LCAT) is present on HDL and catalyses the esterification of free cholesterol and is activated byapoA1, the predominant apolipoprotein of HDL, some HDL particles also contain apoA2, Most of this esterified cholesterol is transferred to LDL, VLDL and chylomicron remnants and thus ultimately reaches the liver, the transport of cholesterol from non-hepatic cells to the liver involves HDL particles, in a process called reverse cholesterol transport (Crook ., 2012).

High-density lipoprotein cholesterol is cardio protective not only because of the reverse cholesterol transport system, which helps to remove cholesterol from the peripheral tissues, but also because of the mechanisms that include increased atherosclerotic plaque stability, protection of LDL from oxidation, and maintaining the integrity of the vascular endothelium (Rodwell et al ., 2012).

A plasma HDL cholesterol concentration of less than 1.0 mmol/L confers increased cardiovascular risk and can be raised by various lifestyle changes, such as smoking cessation, regular exercise and weight loss (Crook,. 2012).

Reverse Cholesterol Transport Pathway

As previously described, one of the major roles of HDL is to maintain the equilibrium of cholesterol in peripheral cells by the reverse cholesterol transport pathway, HDL is believed to remove excess cholesterol from cells by multiple pathways. In the aqueous diffusion pathway, HDL acts as a sink

for the small amount of cholesterol that can diffuse away from the cells, although cholesterol is relatively water insoluble, because it is an amphipathic lipid, it is soluble in plasma in micromolar amounts and can spontaneously dissociate from the surface of cell membranes and enter the extracellular fluid, some free cholesterol will then bind to nascent HDL in the extracellular space, and once bound, it becomes trapped in lipoproteins after it is converted to cholesteryl ester by the enzyme LCAT, which resides on nascent HDL and is activated by its cofactor, apo A-I. The nascent HDL is first converted to HDL3 (Bishop et al., 2018). The formation of cholesteryl ester via LCAT increases the capacity of the surface of HDL3 particle to absorb more free cholesterol from cell membranes along with apo C-I, C-II, C-III, and E and phospholipids from VLDL and chylomicrons and is converted to HDL2. HDL2 can then directly deliver cholesterol to the liver by the scavenger receptor type B1 (SRB1) (Marchal et al., 2012).

Another pathway in which HDL mediates the removal of cholesterol from cells involves the ABCA1 transporter, the ABCA1 transporter is a member of the ATP-binding cassette transporter family, which pumps various ligandsacross the plasma membrane, the exact substrate for the ABCA1 transporter is not known, but it is believed that the transporter modifies the plasma membrane by transferring a lipid, which then enables apo A-I that has dissociated from HDL to bind to the cell membrane In a detergent-like extraction mechanism, apo A-I then removes excess cholesterol and phospholipid from the plasma membrane of cells to form a discoidal-shaped HDL particle, the newly formed HDL is then competent to accept additional cholesterol by the aqueous diffusion pathway and is eventually converted into spherical HDL by the action of LCAT (Bishop et al., 2018).

2.2.5. Lipid Absorption

Because fats are water insoluble, special mechanisms are required to facilitate the intestinal absorption of the 60 to 130 g of fat consumed per day in a

typical Western diet, during the process of digestion, pancreatic lipase, by cleaving off fatty acids, first converts dietary lipids into more polar compounds with amphipathic properties ,thus, triglycerides are transformed into monoglycerides and diglycerides; cholesterol esters are transformed into free cholesterol; and phospholipids are transformed into lysophospholipids ,these amphipathic lipids in the intestinal lumen form large aggregates with bile acids called micelles (Bishop et al ., 2018).Lipid absorption occurs when the micelles come in contact with the microvillus membranes of the intestinal mucosal cell. Short-chain free fatty acids, with 10 or fewer carbon atoms, can readily pass directly into the portal circulation and are carried by albumin to the liver, the absorbed long-chain fatty acids, monoglycerides, and diglycerides are re esterified in intestinal cells to form triglycerides and cholesteryl esters, the newly formed triglycerides and cholesteryl esters are then packaged into chylomicrons by the microsomal transfer protein, along with apo B48 (Rodwell et al., 2018).

Triglyceride absorption is efficient, greater than 90% of dietary triglycerides are taken up by the intestine. In contrast, only about half of the 500 mg of cholesterol in the typical diet is absorbed each day, specific transport system, involving the ABCG5 and ABCG8 transporters, has been described that prevents excess absorption of dietary cholesterol and plant sterols. Individuals with defective ABCG5 or ABCG8 transporters have a disease called sitosterolemia and have a predisposition for atherosclerosis and xanthomatosis (Crook., 2012) . ABCG5 and ABCG8 are also present in the liver and defects in these proteins also impair the elimination of plant sterols into bile for removal from the body, the newly synthesized chylomicrons in the intestine pass into the lymphatic ducts and eventually enter the circulation, chylomicrons interact with proteoglycans, such as heparin sulfate, on the luminal surface of capillaries in various tissues, such as skeletal muscle, heart, and adipose tissue. The proteoglycans promote the binding of LPL, which

hydrolyzes triglycerides on chylomicrons, the free fatty acids and glycerol generated by the hydrolysis of triglycerides by LPL can then be taken up by cells and used as a source of energy , excess fatty acids, particularly in fat cells (adipocytes), are re esterified, The hormones epinephrine and cortisol play a key role in the mobilization and hydrolysis of triglycerides from adipocytes, whereas insulin right after a meal prevents lipolysis by adipocytes and promotes fat storage and glucose utilization (Bishop et al ., 2018).

During lipolysis of chylomicrons, there is a transfer of lipid (mainly triglyceride) and apolipoproteins (apo A-I and C-II) onto HDL, and chylomicrons are converted within a few hours after a meal into chylomicron remnant particles , chylomicron remnants are rapidly taken up by the liver and lysosomal enzymes break down to release free fatty acids, free cholesterol, and amino acids. Some cholesterol is converted to bile acids, both bile acids and free cholesterol are directly excreted into the bile, but not all of the excreted cholesterol and bile salt exit the body and the remainder appearing in the stool, as fecal neutral steroids (Bishop et al., 2018).

2.2.6 Lipid abnormalities:

2.2.6.1 DysLipidemias

Diseases associated with abnormal lipid concentrations are referred to as dyslipidemias, they can be caused directly by genetic abnormalities or through environmental/lifestyle imbalances, or they can develop secondarily, as a consequence of other diseases, dyslipidemias are generally defined by the clinical characteristics of patients and the results of laboratory tests and are not necessarily defined by the specific genetic defect associated with the abnormality, many but not all dyslipidemias regardless of etiology, are associated with CHD or arteriosclerosis (Bishop et al., 2018).

2.2.6.2. Arteriosclerosis

The relationship between heart disease and dyslipidemias stems from the deposition of lipids, mainly in the form of esterified cholesterol, in artery

walls, This lipid deposition first results in fatty streaks, which are thin streaks of excess fat in macrophages in the sub endothelial space, autopsy studies have shown that fatty streaks occur in almost everyone older than age 15. Fatty streaks can develop over time into plaques that contain increased number of smooth muscle cells, extracellular lipid, calcification, and fibrous tissue, which can partially block or occlude blood flow (Bishop et al., 2018).

When plaque develops in arteries of the arms or legs, it is called PVD, when it develops in the heart, it is referred to as CAD and when it develops in the vessels of the brain, and it is called cerebro vascular disease. CAD is associated with angina and myocardial infarction, and CVD is associated with stroke, elevated plasma cholesterol levels (> 5.2 mmol/L) are one of the most important factors in promoting atherosclerosis, but it is now recognized that elevated blood triacylglycerol is also an independent risk factor (Rodwell et al., 2018).

Chapter Three

Materials and Methods

CHAPTER THREE

3 Materials and Methods

3.1 Materials

3.1.1 Study Design

This is a descriptive cross sectional study.

3.1.2 Study area and Period

The study was conducted in Khartoum state, Sudan. The study carried out between March 2019 to August 2020.

3.1.3 Ethical considerations

The study was approved by the scientfic committee of Clinical Chemistry Department , collage of Medical Laboratory Sciences in Sudan University Of Science and Technology and informed consent (appendix I) was obtained from all participants and demographic data was collected by a questionnaire (appendix II).

3.1.4 Study Population

The study was conducted on Sudanese apparently healthy individuals, 200 males and females with ages between (20-60 year) and BMI less than 25.

Individuals who have HTN, heart diseases, obesity and gastric diseases were excluded from this study.

3.2 Methods

3.2.1 Sampling

Five ml of venous blood sample was collected into two seperate containers, plain container to obtain serum after centrifugation for lipid profile and EDTA container to obtain whole blood for ABO testing.

3.2.2 Measurement of serum Total cholesterol:

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest- 4-en-3-one and hydrogen peroxide. In the

presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminophenazone to form a red quinoneimine dye (appendix III).

3.2.3 Measurement of triglyceride:

This method is based on using a lipoprotein lipase for hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dye (appendix IV).

3.2.4 Measurement of HDL-C:

In the presence of magnesium ions, dextran sulfate selectively forms water soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG modified enzymes. The cholesterol concentration of HDL cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups .Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. PEG cholesterol In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to 4- cholestenone and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide generated reacts with 4- amino- antipyrine and HSDA to form a purple-blue dye (appendix V).

3.2.5 Calculation of LDL-C:

LDL-C was calculated using Friede wald calculation.

LDL-C = Total cholesterol –HDL-C − Triglycerides/5 (Bishop et al., 2018).

3.2.6 ABO Testing

Slide test was used, human red blood cells possessing A and/or B antigen will agglutinate in the presence of antibody directed toward the antigen. Agglutination of red blood cells with anti-A, anti-B, anti-AB reagents is a positive test result and indicates the presence of the corresponding antigen. Absence of agglutination of red blood cells with anti-A, anti-B, anti-AB

reagents is a negative test result and indicates the absence of the corresponding antigen (appendix VI).

3.3 Quality Control:

Normal and pathological control sera were measured to assure the accuracy of the results.

3.4 Statistical Analysis:

Data was analyzed by using the SPSS computer program, one way ANOVA test was used for comparison between different variables and Pearson's correlation test was used to find correlations, (P-value< 0.05) was considered significant.

Chapter Four

Results

CHAPTER FOUR

4 Results

Chart 4.1 shows the demographic distribution of males and females according to blood group type within individuals under study.

Table 4.1 shows Mean ±SD of serum cholesterol, triglycerides, HDL-C, LDL-C within different blood groups .The result show significant difference ($p<0.05$) in the means of cholesterol and triglycerides within different blood groups and insignificant difference in the means of HDL-C and LDL-C (P value >0.05).

Table 4.2 shows comparison between different blood groups in the mean of Serum cholesterol level .The result shows significant difference in the mean of cholesterol in blood group O when compared with group B (P=0.041) and group B when compared to group AB(P=0.000).

Table 4.3 shows comparison between different blood groups in the mean of Serum Triglycerides level. The result shows significant difference in the mean of triglycerides in blood group A when compared with group AB (P=0.000) and group B when compared to group AB (P=0.000).

Table 4.4 shows comparison between different blood groups in the mean of HDL-C level. The result shows insignificant difference in the mean of HDL-C within different blood groups.

Table 4.5 shows comparison between different blood groups in the mean of LDL-C level. The result shows insignificant difference in the mean of LDL-C within different blood groups.

Table 4.6 shows the correlation between age and S. Lipid profile according to the blood group type. The result shows that presence of significant positive correlation between the age and cholesterol level in the blood group O, B and AB. significant positive correlation between the age and triglycerides level in

blood group A and O and significant positive correlation between the age and LDL-C level in blood group B and AB.

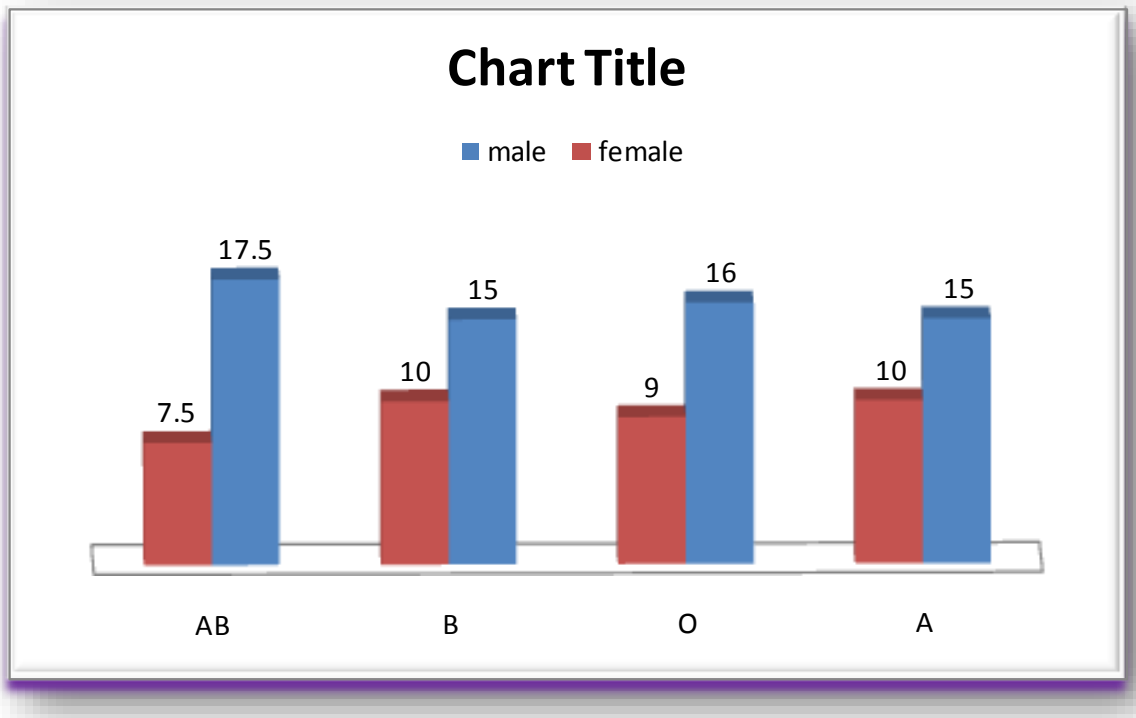

Chart 4.1: Demographic distribution (percentage %) of subjects between different blood groups.

Table 4.1: Mean ± SD of cholesterol, Triglycerides, LDL-C, HDL-C for different blood groups.

	A	O	B	AB	Sig.
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Cholesterol	161.5 ± 39.2	163.8 ± 32.6	148.4 ± 35.0	176.0 ± 42.5	0.004
Triglyceride	117.0± 63.5	134.8 ± 101.5	111.2 ± 65.1	191.2 ± 130.6	0.000
HDL-C	42.5 ± 11.6	41.1 ± 9.1	40.4 ± 11.0	40.3 ± 13.3	0.733
LDL-C	97.6 ± 34.8	95.7 ± 26.8	85.8 ± 27.1	96.8 ± 35.5	0.205

ANOVA test was used.

P value < 0.05 considered significant

Table 4.2: ANOVA table of comparison of means of total cholesterol among the blood groups

	Comparing groups		Mean Difference	Sig.
Cholesterol mg/dl	A	O	-2.360	0.754
		B	13.100	0.083
		AB	-14.560	0.054
	O	B	15.460	0.041
		AB	-12.200	0.106
	B	AB	-27.660	0.000

ANOVA test was used

P. value less than 0.05 is considered to be significant

Table 4.3: ANOVA table of comparison of means of Triglycerides (TG) amongst the blood groups

	Comparing groups		Mean Difference	Sig.
TG mg/dl	A	O	-17.84	0.346
		B	5.78	0.760
		AB	-74.20	0.000
	O	B	23.62	0.213
		AB	-56.36	0.003
	B	AB	-79.98	0.000

ANOVA test was used

p. value less than 0.05 is considered to be significant

Table 4.4 : ANOVA table of comparison of means of HDL-C amongst the blood groups

	Comparing groups		Mean Difference	Sig.
HDL-C mg/dl	A	O	1.40	0.539
		B	2.18	0.339
		AB	2.28	0.318
	O	B	0.78	0.732
		AB	0.88	0.700
	B	AB	0.10	0.965

ANOVA test was used

p. value less than 0.05 is considered to be significant

Table 4.5: ANOVA table of comparison of means of LDL-C amongst the blood groups

	Comparing groups		Mean Difference	Sig.
LDL-C mg/dl	A	O	1.88	0.765
		B	11.80	0.061
		AB	0.80	0.899
	O	B	9.92	0.115
		AB	-1.08	0.864
	B	AB	11.00	0.081

ANOVA test was used

p. value less than 0.05 is considered to be significant

Table 4.6: Correlation between study variables and age within different blood groups

		blood group A	blood group O	blood group B	blood group AB
		Age	**Age**	**Age**	**Age**
Cholesterol mg/dl	R. value	0.03	0.399	0.431	0.403
	p. value	0.837	0.004	0.002	0.004
TG mg/dl	R .value	0.463	0.408	0.181	0.101
	p. value	0.001	0.003	0.208	0.486
HDL-C mg/dl	R. value	-0.192	0.001	0.210	-0.124
	p. value	0.182	0.994	0.144	0.392
LDL-C mg/dl	R .value	-.0160	0.175	0.385	0.463
	p. value	0.910	0.224	0.006	0.001

Pearson's correlation test was used.

P. Value less than 0.05 considered as significant.

Chapter Five

Discussion, Conclusion and Recommendations

CHAPTER FIVE

5 Discussion, Conclusion and Recommendations

5.1 Discussion

The association between ABO and serum lipid profile has been studied in many different populations (Polychronopoulou et al., 1977; Contiero et al., 1994).

Many studies have been done found blood type A individuals have high cholesterol level comparing to other blood groups (Gali et al ., 2010) , high triglycerides level in B and AB individuals (Meade et al .,1994) and higher level of HDL-C and lower LDL-C in O blood group individuals (Ureme et al., 2018).

The present study showed significant increase in the mean of cholesterol and triglyceride between different blood groups, with the highest cholesterol and triglyceride mean show in AB blood group. This result agree with the study done by (Bartimaeus and Waribo., 2017) were found the prevalence of CVD risk is highest in blood group AB . Also (Contiero et al., 1994) study in Italian population showed triglyceride levels were higher in Band AB subjects (Meade et al., 1994) reported significantly higher incidence of ischemic heart disease in AB blood group.

Many researchers have been presented the explanation for the association between cholesterol level and ABO blood group. As reported by (Namazi et al., 2014) found that the association depend upon the role of cholesterol in the erythrocyte membrane from its ability to modulate the biophysical properties of the membrane bilayer ,cholesterol in membranes is also important in regulation of the membrane protein transporters and cell functions .

On the other hands many studies reported that serum cholesterol concentration is the highest in blood group A individuals (Beckman et al.,

1970), and serum triglyceride level were not associated with the ABO system (Polychronopoulou et al., 1977).these studies were disagree with our present study.

The result obtained from this study also reported a significant increase in the mean of serum cholesterol between blood group O and B and blood group B and AB also significant increase in the mean of triglyceride level between AB and other blood groups.

Also there is no significant association between LDL-C and HDL-c and ABO blood group. This presentation agree with the study done by (Contiero et al., 1994).

This study show there is a significant positive correlation between age and cholesterol in O,B,AB blood group, age with triglyceride in A and O blood group, and age with LDL-c in B and AB blood group.

Many factors as lifestyle, diet, and environmental factors may be attributed to get this result.

5.2 Conclusion:

Individuals with AB blood group had increased levels of serum cholesterol and triglycerides when compared to other blood groups, no association between LDL-C and HDL-C levels and blood group type. Serum cholesterol, triglycerides and LDL-C levels increased with increasing in age in all blood groups. .

5.3 Recommendations

1. Further cohort studies to get accurate results should be done.

2. Further studies include genetic testing should be done to study variants responsible for lipid changes according to blood group type and to assess the relevance of the findings to other ethnic groups.

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Appendices

الموافقه المستنيره

انا الباحث يسريه حسين علي من جامعه السودان للعلوم والتكنلوجيا كلية المختبرات الطبيه اقوم بعمل بحث علمي لدراسة علاقة مستوي الدهون في الدم مع نوع فصيلة الدم لتفادي خطر الاصابه بامراض القلب في المستقبل.

اختياري لك للمشاركه في هذا البحث لانك تنطبق عليك كل الشروط المطلوبه للمشاركه في البحث وهي شخص سليم غير مصاب بالسكري , الضغط ، امراض القلب والبدانه .

اولا سنقوم باخذ التاريخ الوراثي لك والتاكد من خلوك من بعض الأمراض التي قد تعيق مادة البحث ومن ثم اخذ عينة دم (5مل) واجراء الفحوصات المعملية اللازمه, مشاركتك في البحث لن تعرضك لاي نوع من الاخطار غير الخطر البسيط الناتج من الوخذ بالأبره.

كل المعلومات التي قمت بمشاركتها لنا ستكون في سريه تامه ولن يطلع عليها الا فريق البحث وذلك بعمل شفرة رقميه لن يظهر فيها اسمك.

بعد مشاركتك في البحث سنقوم بتحليل النتائج والخروج بحلول وتوصيات تساعد بعض فصائل الدم المعرضه لخطر لاصابه بامراض القلب من تفادي الاصابه قبل حدوثها والوقايه منها .

كل نتائج الفحوصات الخاصه بك سنقوم باعطائها لك ولن يطلع عليها أي شخص غير فريق البحث

مشاركتك في هذا البحث طوعيه وغير اجباريه ورفضك المشاركه لن يوثر عليك .لاتوجد أي فوائد ماديه او معنويه ستجنيها من المشاركه في البحث ولك كل الحق من الانسحاب في أي وقت من المشاركه , لكل مشارك الحق في التواصل مع فريق البحث والاستفسار عن المعلومات والنتايج من البحث وذلك بالاتصال علي الرقم 0918222514 .

فورم اقرار المشاركه بالبحث:

لقد قمت بالاطلاع علي البحث الذي تم شرحه لي والاجابه علي كل الاسئله التي طرحها علي الباحثين وانا اقر بالموافقه علي المشاركه طواعيه في هذه الدراسه واعلم بحقي في الانسحاب في أي وقت من المشاركه دون ان يوثر دلك علي حقوقي

اسم المشارك:.....

رمزالمشارك:.....

توقيع المشارك:.....

توقيع الباحث:.....

Sudan University of science and technology

Collage of Graduate Studies

Clinical Chemistry Department

Association between Serum Lipid Profile and Blood Group Types in Healthy Sudanese Individuals

العلاقة بين مستوى الدهون العام في الدم ونوع فصيلة الدم لدى الأشخاص الأصحاء في السودان

Questionnaire

Participant code: …….

Age: …….. Gender: ……

Height:……..Cm Weight…….Kg

BMI…….

Exclusion Criteria:

Hypertension …… Obesity …… Gastric diseases…

Heart Diseases……..

Clinical Investigations:

ABO grouping ……..

S. cholesterol:……. mg/dl

…….mmol/l

S. triglycerides: : …….. mg/dl

……..…..…….mmol/l

HDL-C: ……. mg/dl

…….mmol/l

LDL-C: …….

mg/dl…….mmol/l

Date…….

Signature……

Appendix III

003039773190c501V12.0

CHOL2

Cholesterol Gen.2
Order information
Analyzer(s) on which cobas c pack(s) can be used
03039773 190 Cholesterol Gen.2 (400 tests)

10759350 190 Calibrator f.a.s. (12 x 3 mL)
System- ID 07 6726 3 Roche/Hitachi cobas c 311, cobas c 501/502
Code 401

Code 401

Code 300

Code 300

Code 301

Code 301

Code 300

Code 300

Code 301

Code 301

Code 304

Code 305

Code 391

Code 391

Code 391

Code 392

Code 392

Code 392

System- ID 07 6869 3
10759350 360 Calibrator f.a.s. (12 x 3 mL, for USA)

12149435 122 Precinorm U plus (10 x 3 mL)

12149435 160 Precinorm U plus (10 x 3 mL, for USA)

12149443 122 Precipath U plus (10 x 3 mL)

12149443 160 Precipath U plus (10 x 3 mL, for USA)

10171743 122 Precinorm U (20 x 5 mL)
10171735 122 Precinorm U (4 x 5 mL)
10171778 122 Precipath U (20 x 5 mL)
10171760 122 Precipath U (4 x 5 mL)
10781827 122 Precinorm L (4 x 3 mL)
11285874 122 Precipath L (4 x 3 mL)
05117003 190 PreciControl ClinChem Multi 1 (20 x 5 mL)

05947626 190 PreciControl ClinChem Multi 1 (4 x 5 mL)

05947626 160 PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)

05117216 190 PreciControl ClinChem Multi 2 (20 x 5 mL)

05947774 190 PreciControl ClinChem Multi 2 (4 x 5 mL)

05947774 160 PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)

04489357 190 Diluent NaCl 9 % (50 mL)

English

Test principle

Enzymatic, colorimetric method.

System information

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest- 4- en- 3- one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4- aminophenazone to form a red quinone- imine dye.

For cobas c 311/501 analyzers:

CHO2I: ACN 798: ID/MS Standardization

CHO2A: ACN 433: Abell/Kendall Standardization

For cobas c 502 analyzer:

CHO2I: ACN 8798: ID/MS Standardization

CHO2A: ACN 8433: Abell/Kendall Standardization

CE

Cholesterol esters + H

2

O

cholesterol + RCOOH

Intended use

CHOD

POD

In vitro test for the quantitative determination of cholesterol in human serum and plasma on Roche/Hitachi cobas c systems.

Cholesterol + O_2

cholest- 4- en- 3- one + H O_2

2

Summary

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for atherosclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.

2 H

O

2 2

+ 4- AAP + phenol

quinone- imine dye + 4 H O

2

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in

absorbance.

Reagents – working solutions

R1

PIPES buffer: 225 mmol/L, pH 6.8; Mg^{2+}: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4- aminophenazone: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3 %;

cholesterol esterase (Pseudomonas spec.): ≥ 25 µkat/L
(≥ 1.5 U/mL); cholesterol oxidase (E. coli): ≥ 7.5 µkat/L
(≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 µkat/L

REF	CONTENT	

Safety data sheet available for professional user on request.
The assay is optionally standardized against Abell/Kendall and isotope dilution/mass spectrometry. The performance claims and data presented here are independent of the standardization.
For USA: For prescription use only.
2016-12, V 12.0 English

Precipath U (20 x 5 mL)
Precipath U (4 x 5 mL)
Precipath U plus (10 x 3 mL)
Precipath U plus (10 x 3 mL, for USA)
Precipath L (4 x 3 mL)
PreciControl ClinChem Multi 1 (20 x 5 mL)
PreciControl ClinChem Multi 1 (4 x 5 mL)
PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)
PreciControl ClinChem Multi 2 (20 x 5 mL)
PreciControl ClinChem Multi 2 (4 x 5 mL)
PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)
Diluent NaCl 9 % (50 mL)
English
GK
System information
For cobas c 311/501 analyzers:
TRIGL: ACN 781
glycerol + ATP
glycerol‑3‑phosphate + ADP
dihydroxyacetone
Mg2+
GPO
For cobas c 502 analyzer:
TRIGL: ACN 8781
glycerol‑3‑phosphate + O
2
phosphate + H O
2 2
Intended use
In vitro test for the quantitative determination of triglycerides in human serum and plasma on Roche/Hitachi cobas c systems.
peroxidase
H
2
O
2
+ 4‑aminophenazone
4‑(p‑benzoquinone‑monoimino)
‑phenazone + 2 H O + HCl
Summary[1,2,3,4,5,6]
+ 4‑chlorophenol
2
Triglycerides are esters of the trihydric alcohol glycerol with 3 long‑chain fatty acids. They are partly synthesized in the liver and partly ingested in food.
Reagents - working solutions
The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.
R1
PIPES buffer: 50 mmol/L, pH 6.8; Mg : 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP: ≥ 1.4 mmol/L;
4‑aminophenazone: ≥ 0.13 mmol/L; 4‑chlorophenol: 4.7 mmol/L;
lipoprotein lipase (Pseudomonas spec.): ≥ 83 µkat/L;
glycerol kinase (Bacillus stearothermophilus): ≥ 3 µkat/L; glycerol phosphate oxidase (E. coli): ≥ 41 µkat/L;
2+
The enzymatic triglycerides assay as described by Eggstein and Kreutz still required saponification with potassium hydroxide. Numerous attempts were subsequently made to replace alkaline saponification by enzymatic hydrolysis with lipase. Bucolo and David tested a lipase/protease mixture; Wahlefeld used an esterase from the liver in combination with a particularly effective lipase from Rhizopus arrhizus for hydrolysis.
peroxidase (horseradish): ≥ 1.6 µkat/L; preservative, stabilizers
R1 is in position B.
This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4‑aminophenazone and 4‑chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.
Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory

reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

REF	CONTENT	

Precipath U
Venipuncture should be performed prior to the administration of Metamizole. Venipuncture immediately after or during the administration of Metamizole may lead to falsely low results. A significant interference may occur at plasma Metamizole concentrations above 0.05 mg/mL.
Human serum 1
Human serum 2
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]
Intermediate precision
Mean
SD
CV
%
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
mmol/L (mg/dL) mmol/L (mg/dL)
Precinorm U
1.39 (123)
2.33 (206)
1.18 (104)
2.95 (261)
0.03 (3)
0.04 (4)
0.02 (2)
0.05 (4)
2.0
1.6
1.9
1.8
ACTION REQUIRED
Precipath U
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. The latest version of the carry‑over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. cobas c 502 analyzer: All special wash programming necessary for avoiding carry‑over is available via the cobas link, manual input is required in certain cases.
Human serum 3
Human serum 4
Method comparison
Triglycerides values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared with those

Appendix IV

0020767107322c501V12.0

TRIGL

Triglycerides
determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).
A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.
Sample size (n) = 71
Symbols
Passing/Bablok[19]
y = 1.015x - 0.005 mmol/L
τ = 0.976
Linear regression
y = 1.001x + 0.018 mmol/L
r = 0.999
Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223‑1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):
Contents of kit
The sample concentrations were between 0.560 and 9.13 mmol/L (49.6 and 808 mg/dL).
Volume after reconstitution or mixing
References
GTIN
Global Trade Item Number

1
Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995.

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2
Eggstein M, Kreutz FH. A new determination of the neutral fats in blood serum and tissue. I. Principles, procedure, and discussion of the method. Klin Wschr 1966;44(5):262-267.

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

3
4
5
6
Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973;19(5):476-482.
Wahlefeld AW, Bergmeyer HU, eds. Methods of Enzymatic Analysis. 2nd English ed. New York, NY:Academic Press Inc 1974;1831.
Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:24-27.
Siedel J, Schmuck R, Staepels J, et al. Long term stable, liquid ready-to-use monoreagent for the enzymatic assay of serum or plasma triglycerides (GPO-PAP method). AACC Meeting Abstract 34. Clin Chem 1993;39:1127.

Additions, deletions or changes are indicated by a change bar in the margin.

AppendixV

0004399803190c501V9.0

HDLC3

HDL-Cholesterol plus 3rd generation
Order information
Analyzer(s) on which cobas c pack(s) can be used
04399803 190 HDL‑Cholesterol plus 3rd generation 200 tests

12172623 122 Calibrator f.a.s. Lipids (3 x 1 mL)
System‑ID 07 6833 2 Roche/Hitachi cobas c 311, cobas c 501/502
Code 424
12172623 160 Calibrator f.a.s. Lipids (3 x 1 mL, for USA)

10781827 122 Precinorm L (4 x 3 mL)
Code 424
Code 304
11778552 122 Precipath HDL/LDL‑C (4 x 3 mL)
Code 319
05117003 190 PreciControl ClinChem Multi 1 (20 x 5 mL)

05947626 190 PreciControl ClinChem Multi 1 (4 x 5 mL)

05947626 160 PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)

05117216 190 PreciControl ClinChem Multi 2 (20 x 5 mL)

05947774 190 PreciControl ClinChem Multi 2 (4 x 5 mL)

05947774 160 PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)

04489357 190 Diluent NaCl 9 % (50 mL)
Code 391
Code 391
Code 391
Code 392
Code 392
Code 392
System ID 07 6869 3
English
The cholesterol concentration of HDL‑cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approx. 40 %).
System information

For cobas c 311/501 analyzers:

HDLC3: ACN 435
Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.
For cobas c 502 analyzer:

HDLC3: ACN 8435
PEG‑cholesterol esterase
HDL‑cholesterol esters +

H_2O

HDL‑cholesterol + RCOOH

Intended use
In vitro diagnostic test for the quantitative determination of the HDL‑cholesterol concentration in human serum and plasma on Roche/Hitachi cobas c systems.
In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4‑cholestenone and hydrogen peroxide.

Summary
PEG‑cholesterol oxidase
High density lipoproteins (HDL) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is

transformed to bile acids which are excreted into the intestine via the biliary tract. Monitoring of HDL‑cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL‑cholesterol concentrations and the risk of atherosclerotic disease. Elevated HDL‑cholesterol concentrations are protective against coronary heart disease, while reduced HDL‑cholesterol concentrations, particularly in HDL‑cholesterol +

REF	CONTENT

0004399803190c501V9.0

HDLC3

HDL-Cholesterol plus 3rd generation
The intrinsic pink color of the cholesterol reagent does not interfere with the test.
Sample volumes
Sample
Sample dilution

Sample Diluent (NaCl)
Storage and stability
Normal
2.5 µL

12.5 µL

2.5 µL
–
–
HDLC3
Decreased

Increased
15 µL

–
135 µL

–
Shelf life at 2‑8 °C:
See expiration date
on cobas c pack
label.
cobas c 501 test definition
On‑board in use and refrigerated on the analyzer: 12 weeks
Assay type
2‑Point End
Diluent NaCl 9 %
Reaction time / Assay points 10 / 10‑47
Shelf life at 2‑8 °C:
See expiration date
on cobas c pack
label.
Wavelength (sub/main)
700/600 nm

Increase
Reaction direction
Units
mmol/L (mg/dL, g/L)

Diluent (H O)

2
On‑board in use and refrigerated on the analyzer: 12 weeks
Reagent pipetting
Specimen collection and preparation
For specimen collection and preparation only use suitable tubes or collection containers.
R1

R2
150 µL

50 µL
–
–
Only the specimens listed below were tested and found acceptable.
Serum.
2

MONOCLONAL BLOOD GROUPING REAGENTS

CE 0843

Rapid Labs

DIRECTIONS FOR USE

Anti-A, Anti-B and Anti-A,B Monoclonal:

For Tube, DiaMed-ID, Ortho BioVue, Microplate and Slide Techniques.

SUMMARY

In 1900, Landsteiner discovered the serum of some people would agglutinate the red cells of others. Four common phenotypes are now recognised: O, A, B and AB. Subgroups of A and B have since been identified.

Forward Group				Reverse Group			ABO	
A	B	A,B	A_1	A_2	B	O		Caucasian
+	0	+	0	0	+	0	A	42
0	+	+	+	+	0	0	B	10
0	0	0	+	+	+	0	O	44
+	+	+	0	0	0	0	AB	4

PRINCIPLE

The reagents will cause direct agglutination (clumping) of test red cells that carry the corresponding ABO antigen. No agglutination generally indicates absence of the corresponding ABO antigen (see Limitations).

REAGENT

Rapid Labs Monoclonal IgM ABO blood grouping reagents contain mouse monoclonal antibodies diluted in a phosphate buffer containing sodium chloride, EDTA and bovine albumin. Each reagent is supplied at optimal dilution for use with all the recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see Vial Label.

Product	Cell Line/Clone	Colour	Dye Used
Anti-A	9113D10	Blue	Patent Blue
Anti-B	9621A8	Yellow	Tartrazine
Anti-A,B	152D12 + 9113D10	Colourless	None

STORAGE

Reagent vials should be stored at 2 - 8°C on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. This reagent has undergone transportation stability studies at 37°C and −25°C as described in document EN13640:2002.

SAMPLE COLLECTION AND PREPARATION

Blood samples drawn with or without anticoagulant may be used for antigen typing. If testing is delayed, then store specimens at 2-8 °C. EDTA and citrate samples should be typed within 7 days after collection. Samples collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS or Isotonic saline before being tested. Blood samples showing evidence of lysis may give unreliable results.

PRECAUTIONS

1. The reagents are intended for in vitro diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagents past the expiration date (see Vial Label).
4. Do not use the reagents if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. The reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. The reagents contain < 0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
8. No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

DISPOSAL OF REAGENT AND DEALING WITH SPILLAGES

For information on disposal of the reagent and decontamination of a spillage site see Material Safety Data Sheets, available on request.

CONTROLS AND ADVICE

1. It is recommended a positive control and a negative control be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. Since these reagents do not contain macromolecular potentiators, it is very unlikely that false positive reactions are caused with IgG coated cells.
3. Blood specimens of weak A or B subgroups (e.g Ax) may give rise to false negative or weak reactions when tested using slides, microtitre plates or gel cards. It is advisable to re-test weak subgroups using tube technique.
4. Individuals older than six months should have their ABO blood-grouping results confirmed by testing their serum or plasma against known group A and B cells before their ABO blood group can be confirmed.
5. In the Recommended Techniques one volume is approximately 50µl when using the vial dropper provided.
6. The use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
7. The user must determin the suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Applicator sticks.
- Automatic plate reader.
- DiaMed ID-Cards (Neutral).
- DiaMed ID-Centrifuge.
- DiaMed ID-CellStab or ID-Diluent 2.
- Glass microscope slides.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Microplate centrifuge.
- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.
- Ortho 0.8% Red Cell Diluent.
- Plate shaker.
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Positive and negative control red cells:
- Anti-A: group A2B (positive control) and group O (negative control). Anti-B: group A1B (positive control) and group O (negative control). Anti-A,B: group A1B (positive control) and group O (negative control).
- Test tube centrifuge.
- Validated "U" well microplates.
- Volumetric pipettes.

RECOMMENDED TECHNIQUES

A. Tube Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume of Rapid Labs Anti-ABO reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at room temperature for 1 minute.
4. Centrifuge all tubes for 10 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination
6. Any tubes, which show a negative or questionable result, should be incubated for 15 minutes at room temperature.
7. Following incubation, repeat steps 4 and 5.

B. DiaMed-ID Micro Typing Technique

1. Prepare a 0.8% suspension of washed red cells in ID-CellStab or ID Diluent 2.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50µl of test red cell suspension and 25µl of Rapid Labs Anti-ABO reagent.
4. Centrifuge ID-Card(s) in the DiaMed gel card centrifuge.
5. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique

1. Prepare a 0.8% suspension of washed test red cells in 0.8% Ortho Red Cell Diluent.
2. Remove aluminium foil from as many reaction chambers as needed.
3. Place in appropriate reaction chamber: 50µl of test red cell suspension and 40µl of Rapid Labs Anti-ABO reagent.
4. Centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
5. Read macroscopically for agglutination.

D. Microplate Technique, using "U" wells

1. Prepare a 2-3% suspension of washed test red cells in PBS or Isotonic saline.
2. Place in the appropriate well: 1 volume Rapid Labs Anti-ABO reagent and 1 volume test red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination.
4. Incubate at room temperature for 15 minutes (time dependant on user).
5. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
6. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker
7. Read macroscopically or with a validated automatic reader.
8. Any weak reactions should be repeated by the tube technique.

E. Slide Technique

1. Prepare a 35-45% suspension of test red cells in serum, plasma or PBS or Isotonic saline.
2. Place on a labelled glass slide: 1 volume of Rapid Labs Anti-ABO reagent and 1 volume of test red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2-minute period, maintaining slide at room temperature.
5. Read macroscopically after 2 minutes over a diffuse light and do not mistake fibrin as agglutination.
6. Any weak reactions should be repeated by tube technique

INTERPRETATION OF TEST RESULTS

1. Positive: Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the appropriate ABO antigen on the test red cells.
2. Negative: No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of the appropriate ABO antigen on the test red cells.
3. Discrepancies: If the results obtained with reverse group don't correlate with forward group, further investigation is required.

STABILITY OF THE REACTIONS

1. Read all tube and microplate tests straight after centrifugation.
2. Slide tests should be interpreted within two minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
3. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. ABO antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
2. When using Monoclonal Anti-A,B, blood specimens of weak A or B subgroups (e.g Ax) may give rise to false negative or weak reactions when tested using slides, microtitre plates or gel cards. It is advisable to re-test weak subgroups using the tube technique.
3. Rapid Labs monoclonal Anti-A and monoclonal Anti-B are not validated to detect Ax and A3 or Bx and B3 antigens respectfully and we therefore do not claim reactivity of the monoclonal Anti-A or Anti-B reagent against these weak A and B sub-groups.
4. Stored blood may give weaker reactions than fresh blood.
5. False positive or false negative results may also occur due to:
   - Contamination of test materials
   - Improper storage, cell concentration, incubation time or temperature
   - Improper or excessive centrifugation
   - Deviation from the recommended techniques
   - Cord samples contaminated with Wharton's jelly

SPECIFIC PERFORMANCE CHARACTERISTICS

1. The reagents have been characterised by all the procedures mentioned in the Recommended Techniques.
2. Prior to release, each lot of Rapid Labs Monoclonal Anti-A, Anti-B and Anti-A,B is tested by the Recommended Techniques against a panel of antigen-positive red cells to ensure suitable reactivity.
3. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
4. The potency of the reagents has been tested against the following minimum potency reference standards obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-A reference standard 03/188 And / Or Anti-B reference standard 03/164
5. Rapid Labs Anti-B does not react with "Acquired-B" red cells.
6. Rapid Labs Monoclonal ABO reagents do not detect cryptantigens such as T, Tn or Cad.
7. The Quality Control of the reagents was performed using red cells that had been washed at least twice with PBS or Isotonic saline prior to use.
8. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

DISCLAIMER

1. The user is responsible for the performance of the reagents by any method other than those mentioned in the Recommended Techniques.
2. Any deviations from the Recommended Techniques should be validated prior to use[8].

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2. Messeter L et al. Mouse monoclonal antibodies with Anti-A, Anti-B and Anti-A,B specificities, some superior to human polyclonal ABO reagents. Vox Sang 1984; 46, 185-194
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AVAILABLE REAGENT SIZES

	Vial/ Pack Size	Catalogue Number
Anti-A Monoclonal	10 ml 10 X 10 ml	BC-A10 BC-A10X10
Anti-B Monoclonal	10 ml 10 X 10 ml	BC-B10 BC-10X10
Anti-A,B Monoclonal	10 ml 10 X 10 ml	BC-AB10 BC-AB10X10

TABLE OF SYMBOLS


Consult instructions for use

For *in vitro* diagnostic use only

Catalogue Number

Batch/Lot Number


Store at


Expiry Date


Manufacturer


Date of manufacture


Manufactured by: Rapid Labs Ltd

Unit 2 & 2A Hall Farm Business Centre, Church Road,
Little Bentley, Colchester, Essex, CO7 8SD, United Kingdom

Email: medical@rapidlabs.co.uk
Website: www.rapidlabs.co.uk