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Evaluation of Selected Haematological and Biochemical Predictors for Ischaemic Heart Disease in Shendi Locality River Nile State, Sudan

A Thesis Submitted for the Requirements of the PhD Degree in Haematology

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قال تعالى: (يَا أَيُّهَا النَّاسُ قَدْ جَاءتْكُم مَّوْعِظَةٌ مِّن رَّبِّكُمْ وَشفَاء لِّمَا فِي الصُّدُورِ وَهُدًى وَرَحْمَةٌ لِّلْمُؤْمِنِينَ...) صدق الله العظيم

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Dedication

-To dear my mother,

Who taught me the meaning of life.

-To my dear father,

Who gave me love and respect.

-To my brothers and sister,

Who bring happiness to my life.

-To my teachers,

who led me to the way of success.

-To my friends and colleagues,

I dedicate this study.

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Abstract

Background: Ischaemic heart disease (IHD), is a group of diseases that includes: stable angina, unstable angina, myocardial infarction, and sudden cardiac death. It is within the group of cardiovascular diseases of which it is the most common type. The aim of the study is to evaluate the haematological and biochemical predicators in ischaemic heart disease patients.

Methods: This is a cross-sectional case control prospective analytical study conducted at El-Mek Nimir University Hospital in Shendi town to evaluate the haematological and biochemical predicators in ischaemic heart disease patients in the period between (January 2015- August 2017). The study included (100) patients who were diagnosed as ischaemic heart disease and the study groups were compared with (100) healthy volunteers as a control group.

Blood samples were collected from the two groups. Complete blood count (CBC), high sensitivity C-reactive protein (hsCRP), D. dimer (DD), renal function tests (urea and creatinine) and serum electrolytes (Na^+, K^+ and Ca^{2+}) were measured. Data was collected using a structured face to face questionnaire and the (SPSS) version (11.5) program was used for data analysis.

Results: The study revealed that the ischaemic heart disease patients were; (32%) male and (68%) female, the mean of age was (61.8 ± 10.4) distributed as (95%) have (40-80) years old, the mean of weight was (68.08 ± 11.912), with a mean range of (50-100) kg.

Complete blood count (CBC) indicated the mean values of Hb, PCV, RBCs, MCV, MCH, MCHC, RDW were (12.3 g/dl), (37.7%), ($4.2x10^{12}$/l), (87.9 fl), (28.9 pg), (32.9 g/dl) and (16.5) respectively.

Also prevailed the mean of TWBCs, Neutrophil, lymphocyte, monocyte, eosinophil, basophil, platelet count and MPV were (8.4×10^9 /l), (66.6%), (25%), (5.9%), (2.5%), (0%), (301.9×10^9 /l) and (8.4) respectively.

The study revealed the mean of hsCRP, D.dimer, urea and Creatinine were (5.896 mg/l), (1247.4 ng/ml), (57.68 mg/dl) and (1.55 mg/dl) respectively.

Serum electrolytes showed the mean of (Na^{+}), (K^{+}) and (Ca^{2+}) were (136.1 mmol/l), (3.87 mmol/l) and (9.73 mg/dl) respectively.

Conclusions: Ischaemic heart disease is responsible for significant changes in haemoglobin, packed cell volume, red blood cells count, total white blood cells count, differential WBCs count , urea , Creatinine , high sensitivity c-reactive protein, D.dimer , (Na^{+}), (K^{+}) and (Ca^{2+}).

Key word: Ischaemic heart disease, CBC, hsCRP, D.Dimer, urea, creatinine, electrolytes and Shendi.

المستخلص

مدخل:مرض القلب الاحتشائي هو مجموعة من الامراض تضم الذبحة الصدرية المستقرة وغير المستقرة واحتشاء عضلة القلب وتوقف القلب الفجائي.وهو من مجموعة امراض القلب الوعائية الاكثر انتشارا. وتهدف الدراسة الي تقييم التنبوءات الدموية والبيوكيميائية في مرضي القلب الاحتشائي.

منهجية الدراسة:أجريت هذه الدراسة المقطعية الحالة-الضابطة التحليلية المتقدمة في مستشفي المك نمر الجامعي بمدينة شندي لتحديد مدى تاثير مرض القلب الاحتشائى علي الخلايا الدموية والاختبارات البيوكيميائية للدم في الفترة ما بين (يناير2015-اغسطس2017م). وكانت عينة الدراسة عبارة عن (100) مريض تم اختيارهم بصورة عشوائية. وقورنت نتائج الدراسة مع (100) متطوع سليم كمجموعة ضابطة.

تم جمع عينات الدم من جميع المرضي وتم تحليلها معمليا لاجراء فحص الدم الكامل وبروتين سى وجزئيات الفبرين المتكسرة ووظائف الكلي (اليوريا والكرياتين) وكهارل الجسم (الصوديوم والبوتاسيوم والكالسيوم) . تم جمع المعلومات بواسطه الاستبيان ومن ثم استخدام برنامج الحزمة الإحصائية للعلوم الاجتماعية الذي يعرف ببرنامج (SPSS) لتحليل بيانات الدراسة.

النتائج:أظهرت الدراسة أن المرضي (32٪) منهم ذكور و (68٪) منهم اناث وكان متوسط أعمارهم (61.8 ± 10.4)، (95٪) منهم أعمارهم من (40-80) سنة. ومتوسط اوزانهم (68.08±11.912) معظمهم بين (50-100) كيلوجرام.

تحليل الدم الكامل اظهر ان متوسط الهيموغلوبين ، وتعداد كريات الدم الحمراء ،الحجم الحشوي للدم ،متوسط حجم الخلية الحمراء ، متوسط الهيموغلوبين في الخلية الحمراء ،متوسط تركيز الهيموغلوبين في الخلية الحمراء ومعامل توزيع حجم الخلية الحمراء هم 12.3 g/dl, 37.7%, $4.2x10^{12}$/l, 87.9 fl, 28.9 pg, 32.9 g/dl,16.5 علي التوالي.

كما اظهرت الدراسة ان متوسط تعداد كريات الدم البيضاء والخلايا العدلة والليمفاوية ووحيدات الخلية والحمضية والقاعدية والصفائح الدموية ومتوسط حجم الصفائح الدموية هم , 66.6% , l/ 10^9 x 8.4 8.4.25% , l/ 10^9 x 301.9, 0%, 2.5% , 5.9% علي التوالي.

وجدت الدراسة ان متوسط بروتين سي المتفاعل عالي التحسس وجزئيات الفبرين المتكسرة واليوريا والكرياتين هم1.55 mg/dl, 57.68 mg/dl, 1247.4 ng/ml, 5.896 mg/l علي التوالي.

الخلاصة:مرض القلب الاحتشائي مسئول عن تغيرات ذو دلالة مهمة التى تحدث في الهيموغلوبين وحجم الكرية الحمراء الحشوي وكريات الدم الحمراء وتعداد كريات الدم البيضاء والتعداد التفريقي للكريات

البيضاء واليوريا والكرياتين وبروتين سي المتفاعل عالي التحسس و جزئيات الفبرين المتكسرة والصوديوم والبوتاسيوم والكالسيوم.

الكلمات المفتاحية:مرض القلب الاحتشائي ، فحص الدم الكامل ، اليوريا ،الكرياتين ،بروتين سي المتفاعل عالي التحسس ، جزئيات الفبرين المتكسرة،الكهارل ، شندي

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List of abbreviations

AMI	Acute myocardial infarction
AV	Atrioventricular node
Bas	Basophil
BFU-E	Burst forming unit-erythroid
BMI	Body mass Index
BMP	Basic metabolic panel
BNP	Brain natriuretic peptide
BP	Blood Pressure
BPM	Beat per minute
BSA	Bovine serum albumin
BUN	Blood urea nitrogen
C\EBPs	Cytosine/enhancer binding proteins
CAB	Coronary artery by pan surgery
CAD	Coronary artery disease
CBC	Complete blood count
CD	Cluster of differentiation
CFU	Colony forming units
CHD	Coronary heart disease
CHF	Congestive heart failure
CIHD	Chronic ischaemic heart disease
Con	Control
CT-Scan	Computed tomography-scan
CVD	Cardiovascular diseases
DF	Dynamic focusing
DM	Diabetes mellitus

DNA	Deoxyribonucleic acid
DVT	Deep vein thrombosis
ECG	Electrocardiogram
Eos	Eosinophil
ESR	Erythrocyte sedimentation rate
FLT3	Fms-like tyrosine kinase receptor3
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte /macrophage-colony stimulating factor
Hb	Haemoglobin
HDL	High density lipoprotein
HER	Embryonic heart rate
HHD	Hypertensive heart disease
HLA	Human leucocytes antigen
hsCRP	High- sensitivity C.reactive protein
HTN	Hypertension
IHD	Ischaemic heart disease
IL	Interleukin
Jak2	Janius 2 – kinase
LBBB	Left bundle branch block
LDL	Low density lipoprotein
LMP	Last normal menstrual period
LYM	Lymphocyte
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
MeSH	Medical subject heading
MI	Myocardial infraction
m-mode	Motion mode

MONO	Monocyte
MPs	Mucopolysaccharides
NADPH	Nicotinamide adenine dinucleotide Phosphate
NANA	N-acetylneuraminic acid
NEUT	Neutrophil
NIH	National Institutes of Health
NRBCs	Nucleated red cells
OCAD	Obstructive artery disease
PAI	Plaminogen activator inhibitor
PAPP-A	Pregnancy-associated plasma protein-A
PBS	Phosphate buffered saline
PCI	Percutaneous coronary intervention
PCV	Packed cell volume
PE	Pulmonary embolism
PM	Particulate matter
PVD	Peripheral vascular disease
Ras	Rat sarcoma
RBCs	Red blood cells
RF	Renal failure
RHD	Rheumatic heart disease
ROMK	Renal outer medullary potassium channel
RR	Relative risk
SA	Sinoatrial node
SPSS	Statistical package for the social sciences
STAT	Signal transducers and activators of transcription
TPO	Thrombopoietin
UK	United kingdom
UN	United nations

VCAM	Vascular cell adhesion molecule
VWF	Von Willebrand factor
WBCs	White blood cells
WHO	World health organization

Units abbreviation

cm	Centimeter
g	Gram
KDa	Kilo Dalton
Km	Kilometer
L	Liter
m	Meter
mEq/L	MilliEquivalent/litre
mg	Micro gram
min	Minutes
Mm Hg	Millimeter mercury
mmol	Millimol
S	Second
Yrs	Years
μm	Micro meter

Chapter one

Introduction

Rationale

Objectives

1. Introduction, Rationale and objectives

1.1. Introduction

1.1.1. Cardiovascular diseases (CVD)

is a class of diseases that involves the heart, the blood vessels (arteries, capillaries, and veins) or both.[1] CVDs refer to any disease that affects the cardiovascular system, principally cardiac diseases, vascular diseases of the brain and kidney, and peripheral arterial diseases.[2] The causes of CVDs are diverse but atherosclerosis and/or hypertension are the most common[8]**.** Additionally, when aging come a number of physiological and biochemical changes that alter cardiovascular function and lead to subsequently increased risk of CVDs, even in healthy asymptomatic individuals.[3]

CVDs are the leading cause of deaths. In 2008, (30%) of all global deaths is attributed to cardiovascular diseases. Deaths caused by CVDs are also higher in low and middle-income countries as over (80%) of all global deaths caused by CVDs occurred in those countries. [17] It is also estimated that by 2030, over (23) million people will die from CVDs annually. [4] [17] The causes, diagnosis, prevention, control and/or treatment of all forms of CVDs remain active fields of biomedical research, with hundreds of scientific studies being published on a weekly basis.

In 2013 coronary artery disease (CAD) was the most common cause of death globally, resulting in (8.14) million deaths (16.8%) up from (5.74) million deaths (12%) in 1990.[6] The risk of death from (CAD) for a given age has decreased between 1980 and 2010 especially in developed countries.[19]

The number of cases of (CAD) for a given age has also decreased between 1990 and 2010. [20] In the United States in 2010 about (20%) of those over (65) had (CAD), while it was present in (7%) of those (45 to 64 yrs), and (1.3%) of those (18 to 45yrs).[21]

Rates are higher among men than women of a given age.[21] The Sudan Household Survey (SHHS) reported a prevalence of (2.5%) for heart diseases. Hypertensive heart disease (HHD), rheumatic heart disease (RHD), ischaemic heart disease (IHD) and cardiomyopathy constitute more than (80%) of CVD in Sudan. [4]

Heart diseases are an important cause of morbidity and mortality in Sudan. The tetrad of hypertension, (RHD), (IHD) and cardiomyopathy constitute the bulk of (CVD). Hypertension is prevalent, with poor control rates. [5]

Most CVDs can be prevented by addressing risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity, high blood pressure, diabetes and raised lipids. More than half of the deaths due to heart disease in 2009 were in men. Coronary heart disease (CHD) is the most common type of heart disease, killing more than (385,000) people annually. [4]

Ischaemic heart disease (IHD) are accompanied by progressive mechanical obstruction, dynamic obstruction, and plaque inflammation, instability, and rupture, followed by superimposed thrombosis. Clinicians have used additional tools to aid clinical assessment and to enhance their ability to identify the “vulnerable” patient at risk for CVD, as suggested by a recent National Institutes of Health (NIH) panel. [13][14]

Biomarkers are one such tool to better identify high-risk individuals, to diagnose disease conditions promptly and accurately, and to effectively prognosticate and treat patients with disease. A biomarker may be

measured on a biosample (as a blood, urine, or tissue test), it may be a recording obtained from a person (blood pressure, electrocardiogram or Holter), or it may be an imaging test (echocardiogram).

A fairly recent emphasis is on the link between low-grade inflammation that hallmarks atherosclerosis and its possible interventions. C-reactive protein (CRP) is a common inflammatory marker that has been found to be present in increased levels in patients at risk for cardiovascular disease.[6] Also osteoprotegerin which involved with regulation of a key inflammatory transcription factor called nuclear factor (NF-κB) has been found to be a risk factor of cardiovascular disease and mortality.[7]

1.1.2. Complete Blood Count:

The CBC can help detect blood diseases and disorders, such as anaemia, infections, clotting problems, blood cancers, and immune system disorders. This test measures many different components of blood. [16]

Red blood cells carry oxygen (O_2) from lungs to the rest of the body. Abnormal red blood cell levels may be a sign of anaemia, dehydration (too little fluid in the body), bleeding, or another disorder. [20]

White blood cells are part of the immune system, which fights infections and diseases. Abnormal WBC levels may be a sign of infection, blood cancer, or an immune system disorder. A CBC measures the overall number of white blood cells in blood and with differential test looks at the amounts of different types of WBCs. Platelets are blood cell

fragments that help in blood clot. [19] They stick together to seal cuts or breaks on blood vessel walls and stop bleeding.

Abnormal platelet levels may be a sign of a bleeding disorder (not enough clotting) or a thrombotic disorder (too much clotting).[20] Superimposed thrombosis in ischaemic heart disease may be manifested as elevations of circulating D-dimer, plasminogen activator inhibitor-1, and Von Willebrand factor.

Haemoglobin is an iron rich protein in red blood cells (RBCs) that carries oxygen. Abnormal haemoglobin levels may be a sign of anaemia, sickle cell anaemia, thalassaemia , or other blood disorders.

Haematocrit is a measure of how much space (RBCs) take up in blood. A high haematocrit level might mean dehydrated. A low haematocrit level might mean anaemia. Abnormal haematocrit levels also may be a sign of a blood or bone marrow disorder. [20]

Mean corpuscular volume (MCV) is a measure of the average size of RBCs. Abnormal MCV levels may be a sign of anaemia. Several hypotheses concerning the mechanisms for the association of (IHD) with CBC is an increase in blood viscosity. Recent studies have suggested that's high iron intake or high stored body iron might be related to an increased risk of (IHD).

The effect of leucocytosis on IHD can be explained by multiple mechanisms; however, the inflammatory basis of atherosclerosis remain the cornerstone of this relation. leucocytosis can be considered amarker of inflammatory changes in atherosclerotic lesions, because leucocytes play role in initiation and progression of the disease. Leucocyte release cytokines, bringing about further macrophage recruitment and the proliferation of smooth muscle cells within the vascular wall. High

platelet count are clearly associated with an increased risk of thrombosis; however, the effect of increased platelet count that are still within physiologic ranges ramains unclear.

A limited number of studies have shown that high platelet counts and a rapid platelet aggregation response are associated with increase long – term coronary death. It appears that the role of platelets in the pathogenesis of (IHD) is due mainly to their functional properties and their interaction with plasma and tissue factors.

The BMP includes blood glucose, calcium, and electrolyte tests, as well as blood tests that measure kidney function (urea, creatinine).[20]

Blood tests for kidney function measure levels of blood urea nitrogen (BUN) and creatinine. Both of these are waste products that the kidneys filter out of the body. Abnormal (BUN) and creatinine levels may be signs of a kidney disease or disorder. renal dysfunction has been associated with adverse cardiovascular outcomes.

Electrolytes sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) are involved in heart contraction and relaxation. Cardiac nerve impulse conduction begins when (Ca^{2+}) trigger calcium channels to open. Once the channel opens, (K^+) rushes out of the cell and (Na^+) rushes into the cell and this cause the heart to contract. [15]

(Ca^{2+}) is an important mineral in the body. Abnormal (Ca^{2+}) levels in the blood may be a sign of kidney problems, bone disease, thyroid disease, cancer, malnutrition, or another disorder. Electrolytes are minerals that help maintain fluid levels and acid-base balance in the body. They include (Na^+), (K^+), (HCO_3^-), and (Cl^-). [20]

1.2. Rationale

Increasingly reliable estimates of the prevalence and incidence of IHD emphasize the importance of this disease as a contemporary health hazard.

Cardiovascular disease is now the leading cause of death, with CHD accounting for two-thirds of all heart disease deaths. The most recent data on the use of the WBC count and other components of the complete blood count (CBC) to predict CHD risk. An elevated (WBC) is a well-recognized indicator of inflammation. The total number of WBCs and each subtype (for example, neutrophils, monocytes, lymphocytes, and eosinophils) have been implicated as predictors of IHD. Nearly all of the cellular elements in the blood, including WBCs, RBCs, and platelets, are involved in the underlying pathogenesis of IHD .The study proposed to identify haematological changes in IHD factors related to the onset and course of IHD and designed to determine the importance of conventional risk factors, to identify new risk factors and extensive laboratory evaluations performed at baseline to identify the presence and severity of IHD and may help to establish secondary preventive medication in individual patients.

Also Prevalence data were available only for Khartoum state and there are no data available on any other state.

IHD is an increasing global problem carrying heavy socioeconomic costs. It is the major cause of premature death, in women as in men women lagging behind men by some ten years in this age-related disease.

1.3. Objectives

1.3.1. General objective:

- To evaluate of Selected Haematological and Biochemical Predictors for Ischaemic Heart Disease in Shendi Locality River Nile State, Sudan

1.3.2. Specific objectives:

1- To perform complete blood count (Hb, PCV, RBCs, RBCs indices , RDW, WBCs and differential WBCs , platelet count and MPV) and correlate them in IHD.

2- To measure electrolytes (Na^{+}, k^{+}, Ca^{2+}) in ischaemic heart disease.

3- To estimate the blood urea & serum creatinine in IHD.

4- To determine high sensitivity C. reactive protein (hs-CRP) in IHD patients**.**

5- To measure plasma D.dimer in IHD patients.

Chapter Two
Literature Review

2. Literature Review

2.1. Characteristics of blood:

Blood defined as vital intravascular fluid circulates throughout heart and blood vessels, and classified as connective tissue

Blood compose of two portions, solid portion constituted (45 %); consist of white blood cells, red blood cells, and platelets. Fluid portion of the plasma which constituted about (55%).

Plasma defined as yellowish fluid in which blood cells suspended and obtained by centrifugation of some portion of anticoagulated blood, plasma contains blood clotting factors.

On the other hand serum is yellowish fluid obtained from clotted blood and contains some coagulation factors in excepted fibrinogen.

Plasma composed of plasma proteins, fats, cholesterol, triglyceride, lipoproteins, vitamins (A, B, C and E) and immunoglobulin. Complement proteins (C1-C9). Electrolytes (Na^+, K^+, and CL^-), trace elements (Fe, Zn, and Mg) , enzymes and hormones. The physical properties of blood are including volume are (5-6L) in adult. pH (7.34-7.45). Saline (9%), Viscosity is (4.5 -5.5poise)

The functions of blood are carrying of gases, nutritive enzymes, Hormones and immunity agents such as macrophages, microphages, leukocytes, immunoglobulin and complement. Regulation of the pH, acid base balance and body fluid distribution. Excretions of waste products through execratory organs. Contains coagulation factors, and regulatory mechanism to prevent loss of blood and thrombosis. [106]

Evaluation of the blood components perform by quantitative and qualitative laboratory assessment, to ensure normality or detection of any

defect within blood components, is a concept of clinical haematology diagnostic laboratory.

2.1.1. Erythropoiesis:

Red blood cells (RBCs) are specialized cells that deliver O_2 to tissues and remove CO_2 from the human body. Erythropoiesis, the "making of red cells," involves many different genes and gene products that lead to the production of the mature red cell. Erythropoietin begins at the level of the multipotent stem cell, which then undergoes commitment and differentiation. [106]

2.1.2. Haemoglobin

Haemoglobin (Hb) is the molecule responsible for the transport of oxygen. Under physiological conditions, three types of haemoglobins exist:

- Haemoglobin A ($\alpha 2\beta 2$): major adult haemoglobin (96–98 %).
- Haemoglobin F ($\alpha 2\gamma 2$): during fetal development, (60–80 %) at birth, (0.5–0.8%) during adult life.
- Haemoglobin A2 ($\alpha 2\delta 2$): normally(1.5 – 3%).

The haemoglobin molecule has a molecular weight (MW) of (64,500 KDa) and consists of four polypeptide chains, each carrying a heme group. The heme synthesis starts with the amino acid glycine. Later, porphobilinogen, uroporphyrinogen, coproporphyrinogen and protoporphyrin are formed as intermediate steps. Iron (Fe^{2+}) is supplied from serum transferrin and combines with protoporphyrin to form heme. One heme molecule then binds with one globins chain to form the haemoglobin molecule that avidly binds O_2.

2.1.3. The Red Blood Cells:

The normal erythrocyte has a diameter of about (8 μm) and a biconcave disc form that provides the red cell with a maximum surface-for-gas exchange as well as optimal deformability. The bipolar lipid layer of the red cell membrane is stabilized on the inner side by the attachment of the structural proteins actin and spectrin. Defects of these proteins lead to haemolytic anaemia. The outer layer is covered with mucopolysaccharides (MPs) that form part of the structure of blood group antigens. The N-acetylneuraminic acid (NANA) found in these glycoprotein's results in a negative charge of the cell surface.

Because red cells have lost their nuclei, they are no longer capable of synthesizing proteins, including enzymes. Red cells remain viable and functional for an average of (120 days**).** [(106)]

2.1.4. Granulopoiesis:

Under the influence of cytokines a myeloid progenitor cell is formed. This cell then differentiates into the morphologically recognizable myeloid precursors: myeloblasts, promyelocytes, myelocytes, and metamyelocytes.

Normally these cells do not appear in peripheral blood. Myeloblasts are rather large cells (12–20 μm) in diameter and have a large nucleus with fine chromatin and several nucleoli. No cytoplasmic granules are present.

The normal marrow contains up to (5%) of myeloblasts. Cell division of myeloblasts results in the formation of promyelocytes, slightly larger neutrophilic precursors with granules in their cytoplasm. These cells in turn give rise by cell division to myelocytes, which have smaller granules (secondary or specific granules). At this stage, a differentiation of the myelocytes into the neutrophil, eosinophil, and basophil series can be recognized. Further cell division produces metamyelocytes. These cells can no longer divide and have a somewhat indented nucleus and numerous granules in their cytoplasm. Between the mature neutrophil and

the metamyelocyte, so-called “juvenile,” “stab,” or “band” forms are observed in which the nucleus is not yet fully segmented Such cells occur normally in the peripheral blood (8%) of circulating neutrophils) and are increased under hematopoietic stress, such as during infections. The normal number of neutrophilic granulocytes in the peripheral blood is about (2500–7500/μL). Neutrophilic granulocytes have a dense nucleus split into (2 to 5 lobes) and a pale cytoplasm. The cytoplasm contains numerous pink blue or gray-blue granules. Two types of granules can be distinguished morphologically: primary or azurophilic granules, which appear at the promyelocyte stage, and secondary granules, which appear later. The primary granules contain myeloperoxidase, acid phosphatase, and acid hydrolases, whereas lysozyme, lactoferrin, and collagenase are found in the secondary granules. All granules are of lysosomal origin

Eosinophils, which make up (1–4%) of the peripheral blood leukocytes, are similar to neutrophils but with somewhat more intensely stained reddish granules.

In absolute terms, eosinophils number up to (400/μL). Eosinophilic cells can first be recognized at the myelocyte stage. Eosinophils have a role in allergic reactions, in the response to parasites, and in the defense against certain tumor.

Basophils are seen less frequently than eosinophils; under normal conditions, fewer than (100 cells/μL) are found in the peripheral blood. Basophils have receptors for irnmunoglobulin (IgE) and, in the cytoplasm, characteristic dark granules overlie the nucleus. Degranulation of basophils results from the binding of IgE and allergic or anaphylactic reactions are associated with the release of histamine and heparin.

Mast Cells are similar to basophil, are derived from bone marrow CD34+ progenitors, have receptors for (IgE), and store histamine. Mast cells typically migrate into and mature in connective tissues. Mast cells participate in allergic and immunological reactions.

As already mentioned, monocytes are derived from the myeloid progenitor cell (CFU-GM), which replicates and differentiates into monocytes and, later, macrophages under the influence of certain growth factors. After commitment to the monocytic lineage has been made, the cell goes through distinct monoblast and promonocyte stages before developing into a mature monocyte. Circulating monocytes make up to (2–6%) of all leukocytes (in absolute numbers 200–800/μL).

Monocytes are larger than most other cells of the blood (diameter 15–20 pm). The cytoplasm is abundant and stains blue, with many fine vacuoles. Fine granules are often present. The nucleus is large and often indented with clumped chromatin. Monocytes and macrophages can phagocytose pathogens, present antigens, and secrete many cytokines.

Macrophages after several hours of transit in the blood, the monocytes migrate into different tissues, where they differentiate into macrophages. Macrophages are larger than monocytes, and have an oval nucleus, prominent nucleoli, a blue cytoplasm, and phagocytic vesicles. The different types of macrophages (e.g., Kupffer cells in the liver, alveolar macrophages in the lung, osteoclasts in the bone, macrophages in the bone marrow, peritoneal macrophages) are known as components of the reticuloendothelial system. Macrophages are long-lived (life span at least 10 days or much longer) and secrete numerous cytokines, enzymes, and enzyme inhibitors. **(106)**

2.1.5. Lymphatic tissues and immune response:

The common or pluripotent haematopoietic stem cell differentiates at an early stage into lymphoid and myeloid progenitor cells. From these lymphoid stem cells, the two main classes of lymphocytes, B- and T-cells, develop. The lymphocytes populate the major lymphatic organs, but can also be found circulating in the peripheral blood. Two types of lymphoid organs can be distinguished: the central lymphoid organs (bone

marrow, thymus) and the peripheral lymphoid organs (lymph nodes, tonsils, spleen, and mucosa-associated lymphoid tissue). The central lymphoid organs are the original site of lymphopoiesis and lymphoid maturation, whereas the peripheral lymphoid organs specialize in trapping antigen and initiating adaptive immune responses. In the peripheral blood, (80–85%) of the lymphoid cells belong to the T-cell lineage, whereas in the peripheral lymphoid tissues most lymphoid cells belong to the B-cell lineage.

2.1.6. Megakaryopoiesis :

Platelets are small cell fragments (average size 3 – 4 μm) that are important for haemostasis and coagulation. The normal platelet count is between (150,000 and 450,000/μL). Platelets derive from megakaryocytes, which are very large cells with a large, multilobulated nucleus. The mean DNA content of megakaryocytes is at least eight times that of other somatic cells. One megakaryocytic can produce at least several thousand platelets. The formation and release of platelets is related to a preformed structure in the cytoplasm of megakaryocytes, the so-called “demarcation membrane "system." Megakaryocytes are derived from megakaryocytic progenitors, which in turn originate in the haematopoietic stem cell. Megakaryocytes are mainly found in the bone marrow but can transit to many organs, including the lung, where part of the platelet release occurs. The maturation of megakaryocytes and the production of platelets occurs under the influence of thrombopoietin (TPO). (TPO) acts, together with certain other cytokines like (IL-6 and IL-11), on early megakaryocyte progenitors as well as mature megakaryocytes. Under physiological conditions, the serum levels of TPO are low at normal or elevated platelet counts and high in individuals with low platelet counts. **(106)**

2.2. The heart

Is a hollow muscular organ that pumps blood throughout the blood vessels to various parts of the body by repeated, rhythmic contractions.[21] It is found in all animals with a circulatory system, which includes the vertebrates.[22]

The vertebrate heart is principally composed of cardiac muscle and connective tissue. Cardiac muscle is an involuntary striated muscle tissue specific to the heart and is responsible for the heart's ability to pump blood.

The average human heart, beating at (72) beats per minute, will beat approximately (2.5) billion times during an average (66 yrs) lifespan, and pumps approximately (4.7-5.7 L) of blood per minute. It weighs approximately (250 to 300 g) in females and (300 to 350 g) in males.

2.2.1. Structure:

The structure of the heart can vary among the different animal species. [24] The adult human heart has a mass of between (250 & 350 g) and is about the size of a fist.[25] It is located anterior to the vertebral column and posterior to the sternum.[26]

It is enclosed in a double-walled sac called the pericardium. The pericardium's outer wall is called the parietal pericardium and the inner one the visceral pericardium. Between them there is some pericardial fluid which functions to permit the inner and outer walls to slide easily over one another with the heart movements. Outside the parietal pericardium is a fibrous layer called the fibrous pericardium which is attached to the mediastinal fascia. [27] This sac protects the heart and anchors it to the surrounding structures.

The outer wall of the human heart is composed of three layers; the outer layer is called the epicardium, or visceral pericardium since it is also the inner wall of the pericardium. The middle layer is called the myocardium and is composed of contractile cardiac muscle. The inner layer is called the endocardium and is in contact with the blood that the heart pumps.[28] Also, it merges with the inner lining (endothelium) of blood vessels and covers heart valves.[29]

The human heart has four chambers, two superior atria and two inferior ventricles. The atria are the receiving chambers and the ventricles are the discharging chambers. During each cardiac cycle, the atria contract first, forcing blood that has entered them into their respective ventricles, then the ventricles contract, forcing blood out of the heart. The pathway of the blood consists of a pulmonary circuit and a systemic circuit, [30] which function simultaneously. Deoxygenated blood from the body flows via the vena cava into the right atrium, which pumps it through the tricuspid valve into the right ventricle, whose subsequent contraction forces it out through the pulmonary valve into the pulmonary arteries leading to the lungs. Meanwhile, oxygenated blood returns from the lungs through the pulmonary veins into the left atrium, which pumps it through the mitral valve into the left ventricle, whose subsequent strong contraction forces it out through the aortic valve to the aorta leading to the systemic circulation.[21, 22]

In the human body, the heart is usually situated in the middle of the thorax with the largest part of the heart slightly offset to the left, although sometimes it is on the right, underneath the sternum.

The heart is usually felt to be on the left side because the left heart (left ventricle) is stronger (it pumps to all body parts). The left lung is smaller than the right lung because the heart occupies more of the left

hemithorax. The heart is fed by the coronary circulation and is enclosed by a sac known as the pericardium; it is also surrounded by the lungs. The pericardium comprises two parts: the fibrous pericardium, made of dense fibrous connective tissue, and a double membrane structure (parietal and visceral pericardium) containing a serous fluid to reduce friction during heart contractions. The heart is located in the mediastinum, which is the central sub-division of the thoracic cavity. The mediastinum also contains other structures, such as the esophagus and trachea, and is flanked on either side by the right and left pulmonary cavities; these cavities house the lungs.[31]

The apex is the blunt point situated in an inferior (pointing down and left) direction. A stethoscope can be placed directly over the apex so that the beats can be counted. It is located posterior to the 5^{th} intercostal space just medial of the left mid-clavicular line. In normal adults, the mass of the heart is (250–350 g), or about twice the size of a clenched fist (it is about the size of a clenched fist in children), but an extremely diseased heart can be up to (1000 g) in mass due to hypertrophy. It consists of four chambers, the two upper atria and the two lower ventricles.

2.2.2. Functioning

In mammals, the function of the right side of the heart is to collect de-oxygenated blood, in the right atrium, from the body (via superior and inferior vena cavae) and pump it, through the tricuspid valve, via the right ventricle, into the lungs (pulmonary circulation) so that carbon dioxide can be exchanged for oxygen. This happens through the passive process of diffusion. The left side collects oxygenated blood from the lungs into the left atrium. From the left atrium the blood moves to the left ventricle, through the bicuspid valve (mitral valve), which pumps it out to the body (via the aorta). On both sides, the lower ventricles are thicker and

stronger than the upper atria. The muscle wall surrounding the left ventricle is thicker than the wall surrounding the right ventricle due to the higher force needed to pump the blood through the systemic circulation.[32]

Starting in the right atrium, the blood flows through the tricuspid valve to the right ventricle. Here, it is pumped out the pulmonary semilunar valve and travels through the pulmonary artery to the lungs. From there, oxygenated blood flows back through the pulmonary vein to the left atrium. It then travels through the mitral valve to the left ventricle, from where it is pumped through the aortic semilunar valve to the aorta. The aorta forks and the blood are divided between major arteries which supply the upper and lower body. The blood travels in the arteries to the smaller arterioles and then, finally, to the tiny capillaries which feed each cell. The (relatively) deoxygenated blood then travels to the venules, which coalesce into veins, then to the inferior and superior venae cavae and finally back to the right atrium where the process began. [32]

The heart is effectively a syncytium, a meshwork of cardiac muscle cells interconnected by contiguous cytoplasmic bridges. This relates to electrical stimulation of one cell spreading to neighbowing cells. [32]

Some cardiac cells are self-excitable, contracting without any signal from the nervous system, even if removed from the heart and placed in culture. Each of these cells has their own intrinsic contraction rhythm. A region of the human heart called the sinoatrial (SA) node, or pacemaker, sets the rate and timing at which all cardiac muscle cells contract. The SA node generates electrical impulses, much like those produced by nerve cells, because cardiac muscle cells are electrically coupled by inter-calculated disks between adjacent cells, impulses from the SA node spread rapidly through the walls of the artria, causing both artria to contract in unison.

The impulses also pass to another region of specialized cardiac muscle tissue, a relay point called the atrioventricular node (AV node), located in the wall between the right atrium and the right ventricle. Here, the impulses are delayed for about (0.1s) before spreading to the walls of the ventricle. The delay ensures that the artria empty completely before the ventricles contract. Specialized muscle fibers called Purkinje fibers then conduct the signals to the apex of the heart along and throughout the ventricular walls. The Purkinje fibers form conducting pathways called bundle branches. This entire cycle, a single heart beat, lasts about (0.8s).

The impulses generated during the heart cycle produce electrical currents, which are conducted through body fluids to the skin, where they can be detected by electrodes and recorded as an electrocardiogram (ECG).[17] The events related to the flow or blood pressure that occurs from the beginning of one heartbeat to the beginning of the next is called a cardiac cycle.[32]

The SA node is found in all amniotes but not in more primitive vertebrates. In these animals, the muscles of the heart are relatively continuous and the sinus venosus coordinates the beat which passes in a wave through the remaining chambers. Indeed, since the sinus venosus is incorporated into the right atrium in amniotes, it is likely homologous with the SA node. In teleosts, with their vestigial sinus venosus, the main centre of coordination is, instead, in the atrium. The rate of heartbeat varies enormously between different species, ranging from around (20 beats / min) in codfish to around (600) in hummingbirds.[34]

Cardiac arrest is the sudden cessation of normal heart rhythm which can include a number of pathologies such as tachycardia, an extremely rapid heartbeat which prevents the heart from effectively pumping blood,

which is an irregular and ineffective heart rhythm, and a systole, which is the cessation of heart rhythm entirely. [34]

Cardiac tamponade is a condition in which the pericardium fills with excess fluid or blood, suppressing the heart's ability to beat properly. Tamponade is treated by pericardiocentesis, the gentle insertion of the needle of a syringe into the pericardial sac (avoiding the heart itself) on an angle, usually from just below the sternum, and gently withdrawing the tamponading fluids. [34]

The mammalian heart is derived from embryonic mesoderm germ-layer cells that differentiate after gastrulation into mesothelium, endothelium, and myocardium. Mesothelial pericardium forms the outer lining of the heart. The inner lining of the heart, lymphatic and blood vessels, develop from endothelium. Heart muscle is termed myocardium.[33]

From splanchnopleuric mesoderm tissue, the cardiogenic plates develop cranially and laterally to the neural plates. [35] [36] In the cardiogenic plates, two separate angiogenic cell clusters form on either side of the embryo. The cell clusters coalesce to form an endocardial heart tube continuous with a dorsal aorta and a vitteloumbilical vein. [38][39] As embryonic tissue continues to fold, the two endocardial tubes are pushed into the thoracic cavity, begin to fuse together, and complete the fusing process at approximately (22 days).[20] At (22 days) after conception, the human heart begins beating at 70 to 80 beats per minute and accelerates linearly for the first month of beating. Heart rates measured by motion mode (M-mode) sonography**.** [34] [37]

2.3. Heart diseases:

Is a broad term used to describe a range of diseases that affect heart. The various diseases that fall under the umbrella of heart disease include

diseases of blood vessels, such as CAD; heart rhythm problems (arrhythmias); heart infections; and heart defects born with (congenital heart defects). [44]

The term "heart diseases" is often used interchangeably with "cardiovascular diseases." Cardiovascular disease generally refers to conditions that involve narrowed or blocked blood vessels that can lead to a heart attack, chest pain (angina) or stroke. Other heart conditions, such as infections and conditions that affect your heart's muscle, valves or beating rhythm, also are considered forms of heart disease. [45]

Many forms of heart disease can be prevented or treated with healthy lifestyle choices. [46]

2.3.1. Types

- Coronary artery disease (coronary heart disease and ischaemic heart disease).
- Cardiomyopathy - diseases of cardiac muscles.
- Hypertensive heart disease - diseases of the heart secondary to high blood pressure.
- Heart failure.
- Cor pulmonale - a failure at the right side of the heart with respiratory system involvement.
- Cardiac dysrhythmias - abnormalities of heart rhythm
- Inflammatory heart disease:
    - Endocarditis – inflammation of the inner layer of the heart, the endocardium. The structures most commonly involved are the heart valves.
    - Inflammatory cardiomegaly.

    - Myocarditis – inflammation of the myocardium, the muscular part of the heart.
- Valvular heart disease.
- Cerebrovascular disease - disease of blood vessels that supplies to the brain such as stroke.
- Peripheral arterial disease - disease of blood vessels that supplies to the arms and legs.
- Congenital heart disease - heart structure malformations existing at birth.
- Rheumatic heart disease - heart muscles and valves damage due to rheumatic fever caused by streptococcal bacterial infections.

2.3.2. Risk factors

Evidence suggests a number of risk factors for heart disease: age, gender, high blood pressure, high serum cholesterol levels, tobacco smoking, excessive alcohol consumption, sugar consumption,[47][48] family history, obesity, lack of physical activity, psychosocial factors, diabetes mellitus, air pollution.[49] While the individual contribution of each risk factor varies between different communities or ethnic groups. The consistency of the overall contribution of these risk factors to epidaemiological studies is remarkably strong.[50]

2.3.2.1. Age

Age is by far the most important risk factor in developing CVDs, with approximately a tripling of risk with each decade of life.[51] It is estimated that (82 %) of people who die of CHD are (65yrs) and older.[52] At the same time, the risk of stroke doubles every decade after age**.** [55] [53]

Multiple explanations have been proposed to explain why age increases the risk of CVDs. One of them is related to serum cholesterol level.[54] In

most populations, the serum total cholesterol level increases as age increases. In men, this increased level is around age (45to 50 yrs). In women, the increase continues sharply until age (60 to 65 yr).[54]

Aging is also associated with changes in the mechanical and structural properties of the vascular wall, which leads to the loss of arterial elasticity and reduced arterial compliance and may subsequently lead to coronary artery disease.[55]

2.3.2.2. Sex

Men are at greater risk of heart diseases than pre-menopausal women.[56][57] Once post menopause, it has been argued that a woman's risk is similar to a man's[16] although more recent data from the World Health Organization (WHO) and United Nations (UN) disputes this.[56]

Among middle-aged people, coronary heart disease is (2) to (5) times more common in men than in women.[14] In a study done by the WHO, sex contributes to approximately (40%) of the variation in the sex ratios of coronary heart disease mortality.[57] Another study reports similar results that gender difference explains nearly half of the risk associated with CVDs [58] One of the proposed explanations for the gender difference in CVD is hormonal difference.[59] Among women, estrogen is the predominant sex hormone. Estrogen may have protective effects through glucose metabolism and haemostatic system, and it may have a direct effect on improving endothelial cell function.[60]

The production of estrogen decreases after menopause, and may change the female lipid metabolism toward a more atherogenic form by decreasing the high density lipoprotein (HDL) cholesterol level and by increasing low density lipoprotein (LDL) and total cholesterol levels.[14] Women who have experienced early menopause, either naturally or

because they have had a hysterectomy, are twice as likely to develop heart disease as women of the same age group who have not yet gone through menopause.

Among men and women, there are differences in body weight, height, body fat distribution, heart rate, stroke volume, and arterial compliance.[15] In the very elderly, age related large artery pulsatility and stiffness is more pronounced in women.[15] This may be caused by the smaller body size and arterial dimensions independent of menopause.[61]

2.3.2.3. Air pollution

Particulate matter (PM) has been studied for its short- and long-term exposure CVD. Currently, $PM_{2.5}$ is the major focus, in which gradients are used to determine CVD risk. For every (10 μg/m^3) of $PM_{2.5}$ long-term exposure, there was an estimated (8-18%) CVD mortality risk.[18] Women had a higher relative risk (RR) (1.42) for ($PM_{2.5}$) induced CAD than men (0.90) did.[62] Overall, long-term PM exposure increased rate of atherosclerosis and inflammation. In regards to short-term exposure (2 hrs), every (25μg /m^3) of $PM_{2.5}$ resulted in a (48%) increase of CVD mortality risk.[19] Additionally, after only (5 days) of exposure, a rise in systolic (2.8 mmHg) and diastolic (2.7 mmHg) blood pressure occurred for every (10.5 μg /m^3) of ($PM_{2.5}$).[63] Other research has implicated $PM_{2.5}$ in irregular heart rhythm, reduced heart rate variability (decreased vagal tone), and most notably heart failure.[19] [20] $PM_{2.5}$ is also linked to carotid artery thickening and increased risk of acute myocardial infarction (AMI). [64][65]

2.3.3. Pathophysiology of heart diseases

Population based studies show that atherosclerosis, the major precursor of CVD, begins in childhood. The pathobiological determinants of

atherosclerosis in youth study demonstrated that intimal lesions appear in all the aortas and more than half of the right coronary arteries of youths aged (7–9 yrs).[66]

This is extremely important considering that (1 in 3) people will die from complications attributable to atherosclerosis. In order to stem the tide, education and awareness that cardiovascular disease poses the greatest threat, and measures to prevent or reverse this disease must be taken.

Obesity and Diabetes mellitus are often linked to CVD,[22] as are a history of chronic kidney disease (CKD) and hypercholaesterolemia.[23] In fact, cardiovascular disease is the most life threatening of the diabetic complications and diabetics are (2- to 4-fold) more likely to die of cardiovascular-related causes than non diabetics. [67][68][69]

2.3.4. Screening

- Screening ECGs (either at rest or with exercise) are not recommended in those without symptoms who are at low risk.[70] In those at higher risk the evidence for screening with ECGs is inconclusive.[70] [108] [109] [111] Some biomarkers may add to conventional cardiovascular risk factors in predicting the risk of future CVD; however, the clinical value of some biomarkers is still questionable. [71][72] Currently, biomarkers which may reflect a higher risk of CVD include:

- Coronary artery calcification. [73]
- Carotid intima-media thickness.
- Carotid total plaque area. [74]
- Higher fibrinogen and PAI-1 blood concentrations.
- Elevated homocysteine.

- Elevated blood levels of asymmetric dimethylarginine.
- Inflammation as measured by CRP.
- Elevated LDL.[75]
- Elevated blood levels of brain natriuretic peptide (also known as B-type) (BNP**).** [73]

2.4. Definition of ischaemic heart disease:

Coronary artery disease (**CAD**), also known as **ischaemic heart disease** (**IHD**), [76] is a group of diseases that includes: stable angina, unstable angina, myocardial infarction, and sudden cardiac death.[77] It is within the group of CVDs of which it is the most common type.[78] A common symptom is chest pain or discomfort which may travel into the shoulder, arm, back, neck, or jaw.[79] [95] [96] Occasionally it may feel like heartburn. Usually symptoms occur with exercise or emotional stress, last less than a few minutes, and get better with rest**.** [77] Shortness of breath may also occur and sometimes no symptoms are present**.** [79] The first sign is occasionally a heart attack.[80] [93] [94] Other complications include heart failure or an irregular heartbeat. [81] [87] [88]

Risk factors include: high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet, and excessive alcohol, among others.[83][83]Other risks include depression.[84][89][92]The underlying mechanism involves atherosclerosis of the arteries of the heart.[85] A number of tests may help with diagnoses including: ECG, cardiac stress testing, coronary computed tomographic angiography, and coronary angiogram, among others.[86] [104] [105]

2.5. Biomarkers:

Are one such tool to better identify high-risk individuals, to diagnose disease conditions promptly and accurately, and to effectively

prognosticate and treat patients with disease. [101][102] This review provides an overview of the molecular basis of biomarker discovery and selection and the practical considerations that are a prerequisite to their clinical use. [97][117] The term biomarker (biological marker) was introduced in 1989 as a Medical Subject Heading (MeSH) term: "measurable and quantifiable biological parameters (e.g., specific enzyme concentration, specific hormone concentration, specific gene phenotype distribution in a population, presence of biological substances) which serve as indices for health- and physiology-related assessments, such as disease risk, psychiatric disorders, environmental exposure and its effects, disease diagnosis, metabolic processes, substance abuse, pregnancy, cell line development, epidaemiologic studies, etc." In 2001, an (NIH) working group standardized the definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" and defined types of biomarkers. [118]

A biomarker may be measured on a biosample (as a blood, urine, or tissue test), it may be a recording obtained from a person (blood pressure, ECG, or Holter), or it may be an imaging test (ECG) Biomarkers can indicate a variety of health or disease characteristics, including the level or type of exposure to an environmental factor, genetic susceptibility, genetic responses to exposures, markers of subclinical or clinical disease, or indicators of response to therapy. [103] [98] [99] Thus, a simplistic way to think of biomarkers is as indicators of disease trait (risk factor or risk marker), disease state (preclinical or clinical), or disease rate (progression).[16] Accordingly, biomarkers can be classified as antecedent biomarkers (identifying the risk of developing an illness), screening biomarkers (screening for subclinical disease), diagnostic biomarkers (recognizing overt disease), staging biomarkers (categorizing

disease severity), or prognostic biomarkers (predicting future disease course, including recurrence and response to therapy, and monitoring efficacy of therapy). [118][119][120]

2.5.1. Characteristics of an Ideal Biomarker:

The overall expectation of a CVD biomarker is to enhance the ability of the clinician to optimally manage the patient. For instance, in a person with chronic or atypical chest pain, a biomarker (e.g., treadmill stress test or dobutamine stress echocardiogram) may be expected to facilitate the identification of patients with chest pain of an ischaemic etiology (angina**).**[121][127] In a patient presenting to the emergency department with acute severe chest pain (suspected acute coronary syndrome), a biomarker may help to differentiate patients with an acute myocardial infarction (AMI) from those with unstable angina (e.g., troponin I or T), acute pulmonary embolism (e.g., D-dimer or ventilation perfusion scan), or an aortic dissection (e.g., transesophageal echocardiogram) in a timely fashion to facilitate targeted management**.**[122][123] In a patient with an established AMI, a biomarker may be able to assess the likelihood of the following: a therapeutic response (e.g., ECG ST-segment elevation indicating need for thrombolysis); the extent of myocardial damage (e.g., troponin); the severity of underlying coronary disease (e.g., coronary angiography); the degree of left ventricular dysfunction (e.g., echocardiography); the risk of future recurrences (e.g., exercise stress test); and progression to heart failure (e.g., B-type natriuretic peptide (BNP**).**[124][125][126]

2.5.2. Defining Abnormal Biomarker Values:

Defining abnormal values is a critical step before the clinical use of a biomarker. [128][129][130] It is important to characterize the distribution of

the markers in people in the community and in patient samples on whom the biomarker will be tested. Thus, variation in levels with age, sex, ethnicity, and prevalent disease and the relations of biomarkers to known risk factors must be characterized. [131][132][133]

2.5.3. Cardiovascular Biomarkers: Future Directions:

It is generally believed that the biomarker industry will continue to rapidly expand and flourish in the near future. [134][135][136] The burgeoning research in biomarker development mandates a systematic organization of data with the use of standardized taxonomies that facilitate the online sharing of biomarker meta-data among researchers.[137][138][139] Large epidaemiological and clinical studies will be required to assess the cost-effectiveness of biomarkers. Screening biomarkers will likely compete for limited healthcare budgets, and only those with excellent performance characteristics will find utility in primary care settings.[140][141][142] It is conceivable that some biomarkers may find use as over-the-counter tests as the public continues its informed interest in its own health. Biomarkers that are cost-effective in preventing late sequelae of CVD will likely survive such competition. Diagnostic markers will find use in point-of-care testing in emergency departments and by the bedside. Biomarkers that perform well and cost-effectively in the testing of rapid "rule out" or "rule in" strategies and those that help to triage patients into low- and high-risk treatment strategies will be integrated into clinical decision-making protocols. [143][144][145]

Biomarkers (including pharmacogenetic ones) that facilitate choice of the most appropriate drug, that enable titration of drug dose to avoid side effects, and that maximize therapeutic effects are likely to be attractive to clinicians. [146][147] [148]

2.6. Electrolytes and the heart:

2.6.1. Sodium:

Sodium (Na^+) is the major positive ion (cation) in fluid outside of cells. The chemical notation for sodium is (Na^+). When combined with chloride, the resulting substance is table salt. Excess sodium (such as that obtained from dietary sources) is excreted in the urine. (Na^+) regulates the total amount of water in the body and the transmission of sodium into and out of individual cells also plays a role in critical body functions. Many processes in the body, especially in the brain, nervous system, and muscles, require electrical signals for communication. The movement of (Na^+) is critical in generation of these electrical signals. Therefore, too much or too little sodium can cause cells to malfunction, and extremes in the blood sodium levels (too much or too little) can be fatal. **(149)**

- **Increased sodium (hypernatraemia)** in the blood occurs whenever there is excess sodium in relation to water. There are numerous causes of hypernatraemia; these may include kidney disease, too little water intake, and loss of water due to diarrhoea and/or vomiting.
- **A decreased concentration of sodium (hyponatraemia)** occurs whenever there is a relative increase in the amount of body water relative to sodium. This happens with some diseases of the liver and kidney, in patients with congestive heart failure, in burn victims, and in numerous other conditions. **(150)**

A Normal blood sodium level is (135 - 145 mEq/L), or in international units, (135 - 145 mmol/L).

2.6.2. Potassium:

Potassium (K^+) is the major positive ion (cation) found inside of cells. The chemical notation for potassium is (K^+). The proper level of (K^+) is essential for normal cell function. Among the many functions of (K^+) in the body are regulation of the heartbeat and the function of the muscles. A seriously abnormal increase in potassium (hyperkalaemia) or decrease in potassium (hypokalaemia) can profoundly affect the nervous system and increases the chance of irregular heartbeats (arrhythmias), which, with extreme level, can be fatal**.** [(151)]

Increased potassium is known as hyperkalaemia. (K^+) is normally excreted by the kidneys, so disorders that decrease the function of the kidneys can result in hyperkalaemia. Certain medications may also predispose an individual to hyperkalaemia.

Hypokalaemia, or decreased potassium, can arise due to kidney diseases; excessive loss due to heavy sweating, vomiting, or diarrhoea, eating disorders, certain medications, or other causes. [(152)]

The normal blood potassium level is (3.5 - 5.0 mEq/L), or in international units, (3.5 - 5.0 mmol/L).

2.6.3. Calcium:

Overtime, calcium (Ca^{2+}) can accumulate in arteries. It makes them stiffer and less responsive to the demands of the body. Rigid arteries contribute to high blood pressure, angina (chest pain with exertion or stress), and heart failure. (Ca^{2+}) also builds up in plaque formation, the cholesterol-filled pockets that grow inside arteries like tiny pimples. By narrowing arteries, plaque can choke off the supply of blood to heart muscle and

other vital tissues. If a plaque bursts open, it can trigger a heart attack, stroke, or sudden cardiac arrest. [153]

2.7. Heart Health & Kidney Disease:

Kidney failure and heart disease are closely associated. This is because having kidney failure creates certain imbalances in the body, which, if untreated or ignored, can affect the health of blood vessels and the heart.[97] These are additional to the standard risks factors for heart disease, which all members of our community face. Having an unhealthy heart will ultimately affect your long term health and enjoyment of life. Heart disease remains the leading cause of disability and death for people on dialysis and for people who have been transplanted. Diabetes mellitus, a major risk factor for both CVD and renal failure (RF) has become the commonest cause of RF. Microalbuminuria, with or without DM, indicates increased CVD risk even without a decrease in the function of the kidneys. Patients with diabetes and RF are therefore at a significantly increased risk of developing CVD. It is especially important for people with diabetes to reduce all the other risk factors. [154]

The heart can be damaged very early in the course of kidney failure and a number of features of renal disease, particularly high blood pressure, abnormal blood fats (lipids), abnormal levels of calcium and phosphate and the presence of DM are associated with an increased cardiovascular mortality rate. This is why early diagnosis of renal impairment and early management of all factors, which contribute to heart disease, is so important.

High BP enlarges the heart and weakens the heart muscle. Anaemia can also cause a similar effect, as can repeated fluid overload. Early treatment of high BP and anaemia can prevent such damage occurring. Careful observance of fluid intake for dialysis patients will limit damage to the

heart, as the heart will not have to pump extra volumes of fluid at high pressures around the body.

Imbalances in levels of phosphate and calcium cause not only weakness and pain in your bones but can lead to calcification of blood vessel walls, as well as heart valves. High cholesterol can lead to narrowing of blood vessels. Early intervention with medication and diet can prevent or arrest the damage caused by all these factors. Smoking causes damage to blood vessels, the heart and the lungs and should always be avoided. [155]

In the Australian community, being inactive and overweight increases the chances of developing DM type II, having high BP and heart disease. Diabetes is the cause of RF in about (25%) of patients on dialysis in Australia. As well as damaging the kidneys, DM also damages blood vessels and the heart. People with diabetes need early and very careful monitoring because they are particularly prone to CVD. Early detection of microalbuminuria and early treatment can help to prevent such damage. [155]

2.7.1. Blood Urea:

Urea is the waste product of the degradation of amino acids into (CO_2) and ammonia. Urea is synthesized in the liver and transported through blood to the kidney, where it is filtered through the glomerulus. Almost half of the urea is reabsorbed back into the blood by passive transport in the nephron tubule. Azotemia may indicate renal disease or a nonrenal disorder that causes a secondary increase of blood urea as a consequence of disease.

The reference range for the ratio between BUN-to-creatinine is (10:1) to (20:1). Increases in the ratio may be caused by prerenal, renal, and postrenal factors. Prerenal changes include variation of protein intake and dehydration. Renal disorders that affect the BUN-to-creatinine ratio include renal failure and glomerular damage.

The blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood in the form of urea, and a measurement of renal function. Urea is a by- product from metabolism of proteins by the liver and is removed from the blood by the kidneys**.** [(154)]

2.7.2. Serum Creatinine:

Creatine is synthesized in the liver, pancreas, and kidneys from the amino acids arginine, glycine, and methionine. Creatine is transported through the circulatory system to muscle, brain, and other organs, where it is converted to phosphocreatine and acts as an energy reservoir much like ATP. Creatinine is produced as a waste product of creatine and phosphocreatine. Because much of the creatinine is produced in muscle, the amount of creatinine that is measured in blood is proportional to the patient's lean muscle mass. The waste product, creatinine, enters the blood supply, where it is removed through the kidneys.

Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass).

In chemical terms, creatinine is a spontaneously formed by cyclization of creatine. Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR**).** [(154)]

2.8. The C - reactive protein (CRP):

is synthesized by the liver in response to interleukin-6 and well known as one of the classical acute-phase reactants and as a marker of

inflammation [156] [157]. CRP is the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage. The acute-phase response comprises the nonspecific physiological and biochemical responses of endothermic animals to most forms of tissue damage, infection, inflammation, and malignant neoplasia. [158] [159] The serum CRP level may rise from a normal level of (<5 mg/L to 500 mg/L) during the body's general, non-specific response to infectious and other acute inflammatory events. For some time, the measurement of CRP concentration has been used as a clinical tool for monitoring autoimmune diseases and infectious processes, such as rheumatoid arthritis. [160] [161]

2.9. D-Dimer:

a degradation product of cross-linked fibrin formed during activation of the coagulation system, is commonly used to exclude thromboembolic disease in outpatients suspected of having deep venous thrombosis (DVT) and pulmonary embolism (PE). DVT and PE is relatively common and can cause sudden, fatal embolic events in the pulmonary arteries and other regions. [168] [169] Measurement of the D-dimer level in plasma has been used as a screening strategy for subclinical DVT. A systematic review reported that a normal range of a highly sensitive D-Dimer level accurately ruled out DVT in patients classified as having a low or moderate clinical probability of DVT. The DVT is a high-risk factor for the stroke because of advanced age, hemiplegia, and coagulation disorders, and DVT can cause paradoxical embolic stroke via a right-to left shunt. Thus, it is important to monitor the level of D-dimer the incidence and characteristics of DVT in acute stroke patients. [170] [171]

The Plasma D-dimer level has proven to be useful for DVT screening in chronic stroke patients undergoing rehabilitation. [172] [173] National and international scientific organizations have suggested the use of these markers when implementing new diagnostic strategies in patients with

coronary syndrome. Since D-dimer is well known to be an important prognostic indicator of heart diseases, its most definitive role is on monitoring post-treatment clinical status and the post therapeutic evaluation of patients.

2.10. Haematological changes in ischaemic heart disease:

Ischaemic heart disease is the leading cause of death in the United States, and it is estimated that the prevalence of cardiovascular disease will increase by approximately (10%) over the next (20 yrs). During the past (2 decades), extensive research has established that atherosclerosis is an inflammatory disease, a finding that has offered new possibilities for predicting IHD risk. The presence of many types of inflammatory biomarkers, most notably high-sensitivity C-reactive protein (CRP), has been found to be consistent predictors of IHD events. Although an increasing number of novel inflammatory biomarkers are being studied in this context, many offer little improvement in the current risk-prediction models. Whereas measurement of CRP and lipoprotein-associated phospholipase A_2 (Lp-PLA_2) have shown promise as predictors of IHD events, most of the other newly introduced inflammatory risk markers are expensive to test, are not readily available, lack standardization, and have not been confirmed by multiple prospective studies.

We have previously shown that a growing body of evidence supports the usefulness of the white blood cell (WBC) count as a predictor of future coronary events. Herein, we review the most recent data on the use of the WBC count and other components of the complete blood count (CBC) to predict CHD risk. An elevated (WBC) is a well-recognized indicator of inflammation. The total number of WBCs and each subtype (for example, neutrophils, monocytes, lymphocytes, and eosinophils) have been implicated as predictors of IHD. Nearly all of the cellular elements in the

blood, including WBCs, RBCs, and platelets, are involved in the underlying pathogenesis of atherosclerosis. These markers not only play a role in the development of IHD in asymptomatic patients, but they predict recurrent events and death in patients who already have IHD. In addition to the cellular components, an elevated erythrocyte sedimentation rate (ESR) has been shown to be a weak prognostic factor in IHD patients.

2.11. Previous studies:

-Study done by Pirro M, *et al*, showed high plasma CRP was associated with a significant 1.8-fold increase in IHD risk. The median level of (1.77 mg/L). This association remained significant after adjustment for lipid risk factors but not when the simultaneous contribution of non lipid traditional risk factors was taken into account**.** [(107)]

-Study done by Mohammad Madjid, and Omid Fatemi revealed high number of WBCs and sub type and other component of CBC were associated with IHD. [(108)]

-Study done by Onat A , *et al*, in western Turkey showed high CRP in IHD. The mean value of CRP was 1.9 mg/L. [(109)]

-Study done by Buckley DI , *et al*, in U.S showed strong evidence indicates that high CRP (greater than 3.0 mg/dl) is associated with IHD. The summary estimate of relative risk for incident IHD was 1.58 (95% CI, 1.37 to 1.83) for CRP levels greater than 3.0 mg/L compared with levels less than 1.0 mg/L. [(110)]

-Study done by Lowe GD, *et al*, revealed high plasma CRP and D.dimer were associated with IHD**.** [(111)]

-Study done by Lowe GD, Sweetnam PM, Yarnell JW, *et al*, showed mean levels of CRP and D.dimer were significant higher among IHD patients. CRP and D-dimer had additive effects on risk of IHD**.** [(112)]

-Study done by John Danesh, *et al*, showed an increased significant between D-dimer and CHD**.** [113]

-Study done by Yarnell JW, et al, showed an increased Fibrinogen, viscosity, and white blood cell counts are major risk factors for ischaemic heart disease**.** [114]

-Study done by Choudhury, *et al*, revealed that magnesium (Mg^{2+}) and potassium (K^{+}) level were significantly lower in patients with IHD**.** [115]

-Study done by Toshio kobayashi, *et al*, relationship between haematological parameters (high PCV and Hb) incidence of ischaemic heart diseases among Japanese white-collar male workers. [116]

Chapter Three
Materials & Methods

3. Materials and Methods

3.1. Study design:

This is a descriptive, cross-sectional case-control, prospective, hospital based analytical study to evaluate the haematological and biochemical predictors in ischaemic heart disease patients in Shendi locality River Nile State Sudan

during a period of (January 2015—August 2017).

3.2. Study area:

The study was conducted at Almek Nimir University Hospital which located in Shendi town in Sudan. Shendi is a town in Northern Sudan, situated on the east bank of the Nile (150 km) Northeast of Khartoum. Shendi is also about (45 km) southwest of the ancient city of Meroe. Located in the River Nile state, Shendi is the center of the Ja'aliin tribe and an important historic trading center. Its principal suburb on the west bank is Al-Matamma. A major traditional trade route across the Bayuda desert connects Al-Matamma to Marawi and Napata, (250 km) to the Northwest.

3.3. Study population:

A total of (100) samples collected of Study group of ischaemic heart disease patients and (100) samples collected of healthy individuals as control group.

3.4. Inclusion criteria:

Patients of both sexes with ischaemic heart disease (who take drugs or not take), irrespective of treatment patients with no other medical conditions were included in the study.

3.5. Exclusion criteria:

Patients with other severe diseases such as renal failure, liver disease, haematological diseases and other medical conditions or receiving certain treatment that affect the results were excluded from study.

3.6. Data collection tools:

Data was collected using self-administrated pre-coded questionnaire which specifically designed to obtain information that helped in study.

3.7. Blood Sampling:

Venous blood collected using sterile disposable plastic syringe after cleaning the venipuncture area with (70%) ethanol, the blood added to the anticoagulant and gently mix. The sample centrifuge at (1300 rpm) for (15min) to obtain plasma.

3.8. Methods:

3.8.1. CBC was done by using Mindray Haematology Analyzer (Mindray bc-3000):

3.8.1.1. Principle: blood cells can be broadly divided into three categories .red blood cells, White blood cells and platelets. The analyzer measures the number of cells and distinguishing between their types according to size using sheath flow DC detection. Electrical current is passed through a solution; this method measures the changes in electrical resistance that occurs when blood cells pass through detection aperture. This instrument performs haematology analyses according to the RF/DC detection method, Hydro Dynamic Focusing (DC Detection), and sodium lauryl sulphate (SLS) haemoglobin method. The radio frequencies and direct current (RF/DC detection method) detects the volume of blood cells by changes in direct- current resistance.

3.8.1.2. Procedure:

RBCs count, Hct, Hb concentration, haematimetric indices (MCV, MCH, and MCHC), RDW, WBCs and platelets counts were measured by using an automatic blood cell counter (Mindray -3000 analyzers). The assay was performed according to the instructions provided by the

manufacturer. The analyzer was controlled by normal control, abnormal high and abnormal low.

the EDTA blood samples were aspirated into analyzer through a sample probe, and the counting was started automatically, the results were displayed on the screen within (20) second, the print key was pressed to print out the results.

3.8.2. Blood Films:

Blood films were made from samples collected from all participants

3.8.2.1. Blood Film Preparation:

Ideally, films prepared directly from blood collection into EDTA. Adequate mixing of the specimen is necessary prior to film preparation. Using a wooden stick or glass capillary, a small drop of well-mixed blood was placed in the centre line of a slide about 1 cm from one end. Without delay, a spreader was placed in front of the drop at an angle of about 30^{o} to the slide; it was moved backwards to make contact with the drop. The blood was run quickly along the contact line. With a steady movement of the hand spread the drop of blood along the slide. After the film has been made, was dry in the air. The patient's identification was written in pencil on the edge of the film at its origin.

3.8.2.2. Fixation of films:

To preserve the morphology of the cells, films fixed as soon as possible after they have dried. It is important to prevent contact with water before fixation is complete. Methyl alcohol (methanol) is the choice. The films was fixed, placed them in a covered staining jar or tray containing the alcohol for 2-3 minutes.

3.8.2.3. Blood Film Staining:

Blood films were stained using Leishman's . The main components of Leishman's stain are:

1. A cationic or basic dye such as azure B, which binds to anionic sites and gives a blue-grey colour to nucleic acids (DNA or RNA), nucleoproteins, granules of basophils and weakly to granules of neutrophils.
2. An anionic or acidic dye such as eosin Y, which binds to cationic sites .Staining carried out in a staining rack

3.8.2.4. Microscopic Examination of Blood Films:

Every film first inspected at low power (x10) before general examination was undertaken with the x 40 lens. The x100 oil-immersion lens generally reserved for examining.

3.8.3. B.urea, S. creatinine and S.Ca^{2+} using automated chemistry analyzer, Mindray BS 120):

3.8.3.1. Principle of operation:

After the tray is loaded with samples, a pipette aspirates a precisely measured aliquot of sample and discharges it into the reaction vessel; a measured volume of diluents rinses the pipette. Reagents are dispensed into the reaction vessel. After the solution is mixed (and incubated, if necessary), it is aspirated into a flow cell, where its absorbance is measured by a flow-through colorimeter.

The analyzer then calculates the analyte's chemical concentrations.

3.8.3.2. Operating Procedure:

The sample tube has been loaded into the analyzer; reagents were already being stored in the instrument. The desired tests were programmed and the required tests (Total protein, Albumin, Total Bilirubin, Direct Bilirubin, Urea and Creatinine) were run, the results were displayed on-screen, printed out, stored in the analyzer's internal memory. [144]

3.8.3.3. Blood Urea:

The urease method for estimation of blood urea was used in this study:

3.8.3.3.1. Principle:

Urea + H_2O Urease $2NH_4+$ + CO_2

NH_4+ + Salicylate + NaClO → Nitroprusside → indophenol.

3.8.3.3.2. Reagents:

- Reagent A_1: Sodium salicylate (62) mmol/l, Sodium nitroprusside (3.4) mmol/l phosphate buffer (20) mmol/l.

- Reagent$A_{2:}$ Urease (500) U/ml

- Reagent B: Sodium hypochloride (7) mmol/l, Sodium hydroxide (150) mmol/l.

3.8.3.4.: Serum Creatinine:

Jaffe reaction for estimation of serum Creatinine was used in this study.

3.8.3.4.1. Principle of the method used:

Creatinine reacts with alkaline picrate to produce reddish-orange color the intensity of which at (490) nm is directly proportional to the creatinine concentration.

3.8.3.4.2. Reagents:

Reagent A: Sodium hydroxide (0.4) mol/l

Reagent B: Picric acid (25) mmol/l

3.8.3.5.: Serum calcium:

Arsenazo III m**ethod** for estimation of serum Calcium was used in this study.

3.8.3.5.1. Principle of the method used:

Calcium + Arsenazo III $\xrightarrow{PH=7}$ a blue colored complex

By using 8-hydroxyquinoline-5-sulfonic acid to eliminate the interference of magnesium, calcium ions combine with Arsenazo III to produce a blue colored complex at a neutral solution. The absorbency increase is directly proportional to the concentration of calcium.

3.8.3.5.2. Reagents:

Reagent 1: phosphate buffer 50 mmol\L

8-hydroxyquinoline-5-sulfonic acid 5 mmol\L

Arsenazo III 0.12 mmol\L

3.8.3.6. Quality Control:

Mindray full automated chemistry analyzer was maintained and calibrated using multicalibrator daily. Biochemistry Control sera level (I) (Normal) and level (II) (Pathological) has been used to verify the performance of the measurement procedure each batch. Results of control sera level (I & II) were within the acceptable range. Biochemistry Control sera level I&II, were from BioSystem S.A. Costa Brava 30, Barcelona (Spain).

3.8. 4. Estimation of serum electrolyte using ion selective electrode:

3.8. 4.1.The Principle:

The ion-selective electrode (ISE) for sodium is often made of a lithium aluminum silicate or other composite silicon dioxide glass compound that selects for (Na^+) more readily than (K^+) or (H^+). The ISE for potassium typically contains a selective membrane containing valinomycin.

The valinomycin binds well with the potassium ions. The total plasma carbon dioxide (tCO_2) gas cell/electrode contains an acid to convert (HCO_3^-) to gas, which diffuses through a silicone membrane and reacts with a bicarbonate/carbonic acid buffer to produce (H^+) in proportion to the amount of (tCO_2) in the plasma. The (H^+) ions are detected by an ISE made of silicon dioxide/lithium and calcium oxide glass that selects for (H^+) in preference to (Na^+) and registers a change in potential versus the silver chloride reference electrode. Chloride can also be measured by an ISE of unique composition. A silver chloride membrane solid state electrode measures the activity of (Cl^-) and is highly accurate. [146]

3.8. 4.2. Procedure:

When the ion comes in contact with the electrode, there is a change in the potential compared to the reference electrode measured as a voltage change, due to the ionic activity. The specimen venous serum or lithium-heparinized plasma. Electrolytes may also be analyzed in body fluids such as urine, sweat, cerebrospinal fluid, and gastric fluids and are stable if maintained in closed containers and analyzed promptly.

3.8.5. hs C. reactive protein:

3.8.5.1. Principle

The test uses a sandwich immunodetection method, such that the detector antibody in buffer binds to CRP in sample and antigen-antibody complexes are captured to another CRP antibody that has been immobilized on test strip as sample mixture migrates nitrocellulose matrix. Thus the more CRP antigen in sample, the more antigen-antibody complexes accumulated on the test strip. Signal intensity of fluorescence on detector antibody reflects the amount of antigen captured and is processed by ichroma™ Reader to show CRP concentration in specimen. [162] [163]

3.8.5.2. Reference Range: < 10 mg/L

3.8.5.3. Components and reagents:

Ichroma™ CRP consists of a 'Test cartridge', an 'ID chip', a Blood Collecting Capillary, and a 'Detection buffer tube'

- The test cartridge contains a test strip; on the membrane of which, murine antibodies against CRP and rabbit IgG have been immobilized at the test line and the control line respectively.
- Each test cartridge is individually sealed in an aluminum foil pouch containing a desiccant. (25) sealed test cartridges are packed in a box which also contains an ID chip.
- The detection buffer pre-dispensed in a tube contains fluorochrome-labeled anti-CRP antibodies, fluorescent-labeled anti-rabbit IgG, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is dispensed in each detection buffer tube. (25) detection buffer tubes are packed in a separate box which is further

packed in a Styrofoam box provided with ice packs for the purpose of shipment.

- Blood collection capillary is used for picking up (10 μL) of whole blood, serum, plasma, or control solution. [164] [165]

3.8.5.4. Test procedure:

1. Puncture was made on the top of the detector tube by inserting an empty blood collection capillary.
2. Prick was made on a finger with a lancet. Draw whole blood with a blood collection capillary. (Serum or plasma or CRP control can be drawn with a blood collection capillary.)
3. The excess blood wipe out outside of the capillary with paper towel or Kimwipes.
4. Assembled the capillary and the tube into one.
5. The assembled tube was shaked (10 times) by inversion to take the blood out of capillary.
6. The cap off the top of tube was removed. Discard two drops of reagent onto the paper towel before applying to the cartridge.
7. Apply only two drops onto the sample well of a cartridge
8. To scan the sample-loaded test cartridge, insert it into the test cartridge holder of the ichroma™ Reader. Ensure proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder. An arrow has been marked on the test cartridge especially for this purpose.
9. Press 'Select' button on the ichroma™ Reader to start the scanning process.
10. ichroma™ Reader will start scanning the sample-loaded test cartridge after 3 minutes.
11. The test result was read on the display screen of the ichroma™ reader. [166] [167]

3.8.6. D.dimer:

3.8.6.1. Principle:

The test uses the sandwich immunodetection method, such that the detection antibody in buffer binds to D-Dimer in the plasma sample and antigen-antibody complexes are captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocelluose matrix. The more D-Dimer antigen in the plasma, the more antigen-antibody complexes are accumulated on test strip. Signal intensity of fluorescence on detection antibody reflects amount of antigen captured and is processed by ichroma™ Reader to show D-Dimer concentration in the specimen. The working range of ichroma™ D-Dimer test is (50 – 10,000 ng/ml). [174] [175]

3.8.6.2. Reference Value: 500 ng/ml.

3.8.6.3. Components and reagents:

Ichroma™ D-Dimer consists of Cartridge, an ID Chip, and Detection Buffers.

- The test cartridge contains a test strip; on the membrane of which, antibodies against D-Dimer and streptavidin have been immobilized at the test line and the control line respectively.

- Each test cartridge is individually sealed in an aluminum foil pouch containing a desiccant. (25) sealed test cartridges are packed in a box which also contains an ID chip.

- The detection buffer pre-dispensed in a tube contains fluorochrome-labeled anti-D-Dimer antibodies, fluorescent-labeled biotin-BSA, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.

- The detection buffer is dispensed in each detection buffer tube. (25) detection buffer tubes are packed in a separate pouch which is further packed in a Styrofoam box provided with ice packs for the purpose of shipment.

3.8.6.4. Test procedure:

1. (10µL) of serum/plasma/control sample was transfered using a transfer pipette to a tube containing the detection buffer.

2. The lid of the detection buffer tube closed and mixed the sample thoroughly by shaking it about (10 times). (The sample mixture must be used immediately.

3. Pipette out (75 µL) of a sample mixture and dispensed it into the sample well on the test cartridge.

4. The sample-loaded test cartridge was left at room temperature for (12 min).

5. For scanning, inserted into the test cartridge holder of the ichroma™ Reader. Ensure proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder. An arrow has been marked on the test cartridge especially for this purpose.

6. Press 'Select' button on the ichroma™ Reader to start the scanning process.

7. Ichroma™ Reader will start scanning the sample-loaded test cartridge immediately.

8. The test result was read on the display screen of the ichroma™ reader. (176)

3.9. Ethical consideration:

The consent of the selected individuals to the study was taken after being informed with all detailed objectives of the study and it is health emphasis in the future.

3.10. Data analysis:

The collected data code in master sheet and proceed for analysis using SPSS version 11.5 . (mean, standard deviation, standard error mean, P.value by using independent T.test).

Chapter Four
Results

4. Results

4.1: Demographic and clinical data:

A total of (100) blood sample collected from ischaemic heart disease patients and (100) samples collected as control from healthy individuals include frequency of sex was 32 males (32%) and 68 females (68%), frequency of age groups 40-80 years 95(95%). Frequency of weight (50-100) kg (97%) in the study group.

The average age of patients with ischaemic heart disease in the study was (61.44 ± 10.851), with a range of (40-80) years.

Furthermore, the majority of patients, 68(68%) from female, while the remaining 32(32%) from male.

Regarding to weight, the average weight of patients with ischaemic heart disease in the study was (68.08 ± 11.912), with a range of (50-100 kg). Table (4.1).

Table (4.1): Distribution of study population according to sex, age and weight:

Characteristic		Frequency	Percent %
Study groups	Case	100	50%
	Control	100	50%
Sex	Male	32	32%
	Female	68	68%
Age/yrs	<40 yrs	2	2%
	40-80 yrs	95	95%
	>80 yrs	3	3%
Weight/kg	Less than 50kg	2	2%
	50-100 kg	97	97%
	More than 100kg	1	1%

Participation to risk factors to ischaemic heart disease reflected that; 64 (64%) were HTN patients, while 36 (36%) were not. On the other hand, 32 (32%) were DM patients, while the remaining 68 (68%) were not.

Furthermore, 4 (4%) of the patients were smokers, while 96 (96%) of them were not. Concerning obesity, 24 (24%) of patients were obese, 76 (76%) with normal weight. Regarding family history, most of the patients 92 (92%) with no family history of ischemic heart disease and 8 (8%) were family history. table (4.2).

Table 4.2: Distribution of Study Population According to Risk Factors:

Characteristic		Frequency	Percent %
HTN	Yes	64	64%
	No	36	36%
DM	Yes	32	32%
	No	68	68%
Smoking	Yes	4	4%
	No	96	96%
Lipidaemia	Yes	24	24%
	No	76	76%
Family history	Yes	8	8%
	No	92	92%

Laboratory Data:

The mean values of Hb, PCV, RBCs, MCV, MCH, MCHC, RDW in case group were (12.3 g/dl), (37.7%), ($4.2x10^{12}$/l), (87.9 fl), (28.9 pg), (32.9 g/dl) and (16.5) respectively and in control group the mean values of Hb, PCV, RBCs, MCV, MCH, MCHC, RDW were (13.1 g/dl), (39.8%), ($4.4x10^{12}$/l), (89.0 fl), (29.2 pg), (32.8 g/dl) and (15.6) respectively. Table (4.3).

Table (4.3): Comparison between case and control in Hb, RBCs, RBCs indices and RDW:

Groups		Number	Mean	SD	P.value
Hb g/dl	Case	100	12.3	1.59	0.003
	Control	100	13.1	1.88	
RBCsx10^9	Case	100	4.23	.5190	0.001
	Control	100	4.48	.550	
PCV %	Case	100	37.7	4.20	0.002
	Control	100	39.8	5.13	
MCV fl	Case	100	87.9	6.71	0.199
	Control	100	89.0	4.53	
MCH pg	Case	100	28.9	2.63	0.444
	Control	100	29.2	1.78	
MCHC g/dl	Case	100	32.9	1.86	0.707
	Control	100	32.8	.760	
RDW	Case	100	16.5	2.53	0.000
	Control	100	15.6	.680	

The mean of TWBCs, Neutrophil, lymphocyte, monocyte, eosinophil, basophil, platelet count and MPV in IHD were (8.4 x 10^9 /l), (66.6%), (25%), (5.9%), (2.5%), (0%), (301.9 x 10^9 /l) and (8.4) respectively. the mean of TWBCs, Neutrophil, lymphocyte, monocyte, eosinophil, basophil, platelet count and MPV in control were (5.7 x 10^9 /l), (48.1%), (43.2%), (6.1%), (2.6%), (0%), (277.5 x 10^9 /l) and (9.1) respectively. table (4.4).

Table (4.4): Relationship between case and control in WBCs count and their subtype, platelet and MPV:

Group		Number	Mean	SD	P.value
WBCsx10^9	case	100	8.44	6.53	0.000
	control	100	5.78	1.34	
Neutrophil %	case	100	66.60	11.26	
	control	100	48.12	8.18	0.000
Lymphocyte %	case	100	25.00	10.24	
	control	100	43.28	7.55	0.000
Monocyte %	case	100	5.92	1.72	
	control	100	6.12	1.45	0.377
Eosinophil%	case	100	2.54	1.10	
	control	100	2.64	1.20	0.541
Plateletx10^9	case	100	301.92	117.52	
	control	100	277.52	64.67	0.070
MPV	case	100	8.412	.720	
	control	100	9.136	.700	0.000

The mean of hsCRP, D.dimer in case group were (5.896 mg/l), (1247.4 ng/ml), respectively. The mean of hsCRP, D.dimer in control group were (0.37 mg/l), (90.08 ng/ml), respectively. Table (4.5).

Table (4.5): Comparison between case and control in hsC-reactive protein and D.dimer:

	Group	Number	Mean	SD	P.value
hsCRP mg/dl	case	100	5.89	3.77	0.000
	control	100	.37	0.44	
D.dimer ng/dl	case	100	1247.44	2583.21	0.000
	control	100	90.08	25.60	

The mean of urea and Creatinine in case group were (57.68 mg/dl) and (1.55 mg/dl) respectively. The mean of urea and Creatinine in control were (30.00 mg/dl) and (1.18 mg/dl) respectively. Table (4.6)

Table (4.6): Comparison between case and control in urea and creatinine:

	Group	Number	Mean	SD	P.value
Urea mg/dl	case	100	57.68	42.742	0.000
	control	100	30.00	12.358	
Creatinine mg/dl	case	100	1.556	1.4612	0.020
	control	100	1.184	.61410	

The mean of (Na^+), (K^+) and (Ca^{2+}) in case group were (136.1 mmol/l), (3.87 mmol/l) and (9.73 mg/dl) respectively. The mean of (Na^+), (K^+) and (Ca^{2+}) in control group were (136.1 mmol/l), (3.87 mmol/l) and (9.73 mg/dl) respectively. table (4.7).

Table (4.7): Comparison between case and control in electrolytes:

	Group	Number	Mean	SD. Deviation	P.value
(Na^+) mmol/l	case	100	136.12	5.05	
	control	100	138.44	2.98	0.000
(K^+) mmol/l	case	100	3.876	0.5924	
	control	100	4.048	0.3037	0.010
(Ca^{2+}) mg/dl	case	100	9.736	0.5684	
	control	100	10.656	0.5916	0.000

Chapter Five
Discussion
Conclusion
Recommendations

5. Discussion, conclusion and Recommendations

5.1. Discussion

Ischaemic heart disease (IHD), is a group of diseases that includes stable angina, unstable angina, myocardial infarction, and sudden cardiac death. [177]

The results of this study denoted that the hypertensive patients were in high risk to IHD and showed an increased prevalence, followed by DM. The results according to type of IHD revealed that most of patients were non stem IHD and inferior, anterior ECG changes.

The results of this study obtained demonstrated that there was significant decrease in Hb, RBCs count and PCV compared to control. (P value >0.05). Results of this current study are different when compared to study done by Toshio team in Japan whom revealed that: high PCV and Hb were risk factors for IHD.

Several factors related to RBCs are associated with IHD including Hb levels, PCV and ESR but there are not enough data to suggest an association between the RBCs count and cardiovascular disease. [179]

The study findings prevailed a decrease in the mean of RBCs indices (MCV, MCH & MCHC) and there was no association when compared to control group (p value <0.05).

Finding of the parameters examined, reflected an increase in the mean of RDW compared to control group and there was strong significant statistical value depicted among study population; (P.value 0.000). This result agreed with the study conducted by Patel KV et al, that showed a significant association between RDW and IHD.[180] The RDW, a numerical measure of the variability of the size of circulating erythrocytes, is significantly associated with an increased risk of all-cause

death, and specifically with death secondary to CVD in cross-sectional studies of the population of the U.S. In addition, the RDW is an independent predictor of death in patients who have had previous MI or stroke and in men referred for coronary angiography.

The results of this research confirmed an increase in the mean of WBCs & neutrophils and a decrease in lymphocytes, eosinophils & monocytes means. There was strong significant statistical relationship found among study population; (P.value 0.000). Results of the present study were in agreement with a previous study done by Mohammad Madjid, and Omid Fatemi.2013, whom suggested that: leucocytosis can be considered as a marker of inflammatory changes in atherosclerotic lesions, because leucocytes play a role in initiation and progression of the disease. Leucocyte release cytokines, bringing about further macrophage recruitment and the proliferation of smooth muscle cells within the vascular wall.

The laboratory investigations done indicated an increase in the mean of platelet compared to control group. There was no significant statistical difference observed among study population; (P = 0.070). It appears that the role of platelets in the pathogenesis of IHD is due mainly to their functional properties and their interaction with plasma and tissue factors. The study estimated a decrease in MPV mean compared to control. There was strong significant statistical value demonstrated among study population; (P.value 0.000).

The results of the tests conducted showed an increase in the mean of hs-CRP compared to control group. There was strong significant statistical difference appeared among study population; (P = 0.000). The recent study showed strong association between hs-CRP and IHD. The results were in agreement with multiple other studies that presented an increase in CRP of IHD patients. One study denoted that: an increase in hs-CRP was associated with increased incidence of recurrent angina,coronary

revascularization and cardiovascular death. It has recently been suggested that hs-CRP is a marker of inflammation, along with serum cholesterol, may be critical component in the development and progression of atherosclerosis.[157] hs-CRP is emerging as the strongest and most independent predictive risk factor for CVD.

The outcome of the results obtained revealed an increase in the mean of D.dimer compared to control group. There was an effective significant statistical difference estimated among study population. This current study found that an increased D.dimer was associated with IHD. Inflammation and thrombosis are closely related. Platelets have clear roles in thrombosis and contributed to inflammation. A pathophysiological explanation for the association between high plasma level D. dimer and the increased risk of IHD thrombotic events in the fact that D.dimer is the part of the so –called inflammation –coagulation – axis. Results of the present study are relevant to the previous study done by (John Danesh el at, 2001).

The increased mean of urea & creatinine was observed in this study to be higher than control group. This study indicated an association between increased urea, creatinine and IHD.

This result was the same as the previos result adopted by (Kirtane et al, 2005),suggested renal dysfunction has been associated with adverse cardiovascular outcomes.

a signficiant reduction in this study in serum sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) levels among the IHD patients were detected, and compared to healthly indiviuals in the control group. This finding is in an accordance with the study carried out by (Choudhury MBK, *et al*, 2011) which depicted an effect in (K^+) level in IHD.

(Mg^{2+}) and (K^+) metabolism were closely linked. (Mg^{2+}) deficiency is closely associated with hypokalaemia by various mechanisms involving

(Na^+)-(K^+) ATPase, and renal outer medullary potassium (ROMK) channels in the kidney. Insufficient action of (Na^+)-(K^+) pump causing an intracellular sodium and depletion of intracellular potassium level.

5.2. Conclusion:

- Hb, PCV, red cells indices were lower in IHD patients when compared to healthly indiviuals in the control group.
- TWBCs, Neutrophil, platelet and MPV were higher in IHD patients when compared to healthly indiviuals in the control group.
- Serum hsC-reative protein was higher in IHD patients when compared to healthly indiviuals in the control group.
- Plasma D.dimer was higher in IHD patients when compared to healthly indiviuals in the control group.
- Renal function tests (urea & creatinine) were an increased in IHD patients when compared to healthly indiviuals in the control group.
- Serum sodium (Na^{+}), potassium (K^{+}) and calcium(Ca^{2+}) were lower in IHD patients when compared to healthy individuals in the control group.

5.3. Recommendations:

1-Haematological and biochemical tests should be checked regularly in ischaemic heart disease patients.

2-Health education, diet control and exercise are important factors in lowering the body weight especially in obese patients so as to achieve good control of ischaemic heart disease.

3-There is a need to improve the patients' with risk factors to IHD and general populations' awareness of IHD complications, and the importance of lifestyle modifications. This could be achieved by improving patients' counseling by primary care physicians, or through campaigns and media to aware general populations.

4-More investigations should be done for ischaemic heart disease patients, to determine which risk factors and thrombotic markers are important predictors of bleeding and thrombotic risk among ischaemic heart disease patients.

References

References

1. Maton, Anthea; Jean Hopkins, Charles William McLaughlin, Susan Johnson, Maryanna Quon Warner, David LaHart, Jill D. Wright. Human Biology and Health. Englewood Cliffs, New Jersey: Prentice Hall; 1993. ISBN 0-13-981176-1.
2. Bridget B. Kelly; Institute of Medicine; Fuster, Valentin. Promoting Cardiovascular Health in the Developing World: A Critical Challenge to Achieve Global Health. Washington, D.C: National Academies Press; 2010. ISBN 0-309-14774-3.
3. Dantas AP, Jimenez-Altayo F, Vila E. "Vascular aging: facts and factors". Frontiers in Vascular Physiology **3** (325): 1–2. doi:10.3389/fphys.2012.00325. PMID 22934073.
4. Bridget B. Kelly, editors ; Institute of Medicine of the National. Promoting cardiovascular health in the developing world : a critical challenge to achieve global health. Washington, D.C.: National Academies Press. pp. Chapter 2; 2010. ISBN 978-0-309-14774-3.
5. Mendis, S.; Puska, P.; Norrving, B. Global Atlas on cardiovascular disease prevention and control. 2011. ISBN 978-92-4-156437-3
6. Finegold JA; Asaria P; Francis, DP . "Mortality from ischaemic heart disease by country, region, and age: Statistics from World Health Organization and United Nations.". International journal of cardiology 168 (2): 934–945. doi:10.1016/j.ijcard.2012.10.046. PMID 23218570.
7. Gill HC, Mahan CA, Gidding SS . "Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study". March 2008.Circulation117(9):1216–27.

8. Onow RO, et al. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. 9th ed. Philadelphia, Pa.Saunders Elsevier; 2012.
9. Berra K, Fletcher, B. J. & Handberg, E. Antiplatelet therapy in acute coronary syndromes. 2011.
10. Clark, D. S. Acute Coronary Syndrome. American Academy of Family Physicians FP Essentials; 2010.
11. Devabhakthuni S. & Seybert, A. L. Oral antiplatelet therapy for the management of acute coronary syndromes: Defining the role of Prasugrel. 2011. Critical Care Nurse, 31, 51-63."
12. Howard BV; Wylie-Rosett, J. "Sugar and cardiovascular disease: A statement for healthcare professionals from the Committee on Nutrition of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association."Jul 23, 2002.Circulation 106 (4): 523–7. PMID 12135957.
13. Finks SW; Airee, A; Chow, SL; Macaulay, TE; Moranville, MP; Rogers, KC; Trujillo, TC. "Key articles of dietary interventions that influence cardiovascular mortality. April 2012. Pharmacotherapy 32 (4): e54–87.
14. Yusuf S, Hawken S, Ounpuu S, et al."Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries: case-control study".2004. Lancet 364 (9438): 937–52.
15. Estevez E, Baquero E, Mora-Rodriguez R. "Anaerobic performance when rehydrating with water or commercially available sports drinks during prolonged exercise in the heat". Applied Physiology, Nutrition and Metabolism.2008. **33** (2): 290–298.

16. Clayton J A, Rodgers S, J Blakey, A Avery, and P Hall. "Thiazide diuretic prescription and electrolyte abnormalities in primary care" Br J Clin Pharmacol. 2006 January; 61(1): 87–95.

17. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med, 2006, 3(11):e442.

18. Martí-Carvajal AJ, Solà I, Lathyris D, Salanti G. "Homocysteine lowering interventions for preventing cardiovascular events".2009. In Martí-Carvajal, Arturo J. Cochrane Database Syst Rev (4): CD006612.

19. Hung MJ, Cherng WJ. Comparison of white blood cell counts in acute myocardial infarction patients with significant versus insignificant coronary artery disease. Am J Cardiol 2003;91(11):1339–42.

20. http://www.national heart lung and blood institute diseases and conditions index.nih.gov/health//dci/diseases/bdt-expect.html

21. "Evolutionary adaptations of the circulatory system – Facilitating Exchange with Cells Throughout the Body". Boundless.com. Retrieved 2013-10-01.

22. "Heart". SeymourSimon.com.

23. Kumar; Abbas; Fausto (2005). Robbins and Cotran Pathologic Basis of Disease (7th ed.). Philadelphia: Elsevier Saunders. p. 556. ISBN 0-7216-0187-1.

24. Romer, Alfred Sherwood; Parsons, Thomas S. (1977). The Vertebrate Body. Philadelphia, PA: Holt-Saunders International. pp. 437–442. ISBN 0-03-910284-X.

25. MacDonald, Matthew (2009). Your Body: The Missing Manual. Sebastopol, CA: Pogue Press. ISBN 0-596-80174-2.
26. Cecie Starr; Christine Evers; Lisa Starr (2 January 2009). Biology: Today and Tomorrow With Physiology. Cengage Learning. pp. 422–. ISBN 978-0-495-56157-6. Retrieved 7 June 2012.
27. "the anatomy and applied anatomy of the mediastinal fascia". Retrieved 2013-02-27.
28. Pratiyogita Darpan (April 2009). Competition Science Vision. Pratiyogita Darpan. pp. 55–. Retrieved 7 June 2012.
29. "Heart". MedicaLook. Medicalook.com. Retrieved 2010-05-03.
30. "Pulmonary circuit". medical-dictionary.thefreedictionary.com. Retrieved 2011-05-10.
31. Emergency Medical Responder: A Skills Approach (3rd Canadian ed.). Toronto: Pearson. 2010. p. 131. ISBN 978-0-13-500485-2.
32. Marieb, Elaine Nicpon (2003). Human Anatomy & Physiology (6th ed.). Upper Saddle River: Pearson Education. ISBN 0-8053-5463-8.
33. "Gray's Anatomy of the Human Body – 6. Surface Markings of the Thorax". Bartleby.com. Retrieved 2010-10-18.
34. Crigg, Gordon; Johansen, Kjell (1987). "Cardiovascular Dynamics In Crocodylus Porosus Breathing Air And During Voluntary Aerobic Dives". Journal of Comparative Physiology B(Springer-Verlag)**157**(3): 381–392. doi:10.1007/BF00693365.

Archived from the original on July 3, 2012. Retrieved July 3, 2012.

35. Axelsson, Michael; Craig, Franklin; Löfman, Carl; Nilsson, Stefan; Crigg, Gordon (1996). "Dynamic Anatomical Study Of Cardiac Shunting In Crocodiles Using High-Resolution Angioscopy". The Journal of Experimental Biology (The Company of Biologists Limited) **199**: 359–365. PMID 9317958. Retrieved July 3, 2012.
36. Maton, Anthea; Jean Hopkins, Charles William McLaughlin, Susan Johnson, Maryanna Quon Warner, David LaHart, Jill D. Wright (1993). Human Biology and Health. Englewood Cliffs, New Jersey: Prentice Hall. ISBN 0-13-981176-1. OCLC 32308337.
37. Campbell; Reece (2005). Biology (7th ed.). San Francisco: Pearson. pp. 873–874. ISBN 0-8053-7171-0.
38. Guyton, A. C.; Hall, J. E. (2006). Textbook of Medical Physiology (11th ed.). Philadelphia: Elsevier Saunder. ISBN 0-7216-0240-1.
39. "Animal Tissues". Users.rcn.com. 2010-08-13. Retrieved 2010-10-17.
40. "Main Frame Heart Development". Meddean.luc.edu. Retrieved 2010-10-17.
41. DuBose, Miller , Moutos. "Embryonic Heart Rates Compared in Assisted and Non-Assisted Pregnancies". Obgyn.net. Retrieved 2010-10-18.
42. DuBose TJ, Cunyus JA, and Johnson L (1990). "Embryonic Heart Rate and Age". J Diagn Med Sonography **6** (3): 151–157. doi:10.1177/875647939000600306.
43. DuBose, TJ (1996) Fetal Sonography, pp. 263–274; Philadelphia: WB Saunders ISBN 0-7216-5432-0

44. Terry J. DuBose Sex, Heart Rate and Age
45. Vanhecke TE, Miller WM, Franklin BA, Weber JE, McCullough PA (Oct 2006). "Awareness, knowledge, and perception of heart disease among adolescents". Eur J Cardiovasc Prev. Rehabil. **13** (5): 718–23. doi:10.1097/01.hjr.0000214611.91490.5e. PMID 17001210.
46. Highlander P, Shaw GP (2010). "Current pharmacotherapeutic concepts for the treatment of cardiovascular disease in diabetics". Ther Adv Cardiovasc Dis. **4**: 43–54.
47. NPS Medicine wise (1 March 2011). "NPS Prescribing Practice Review 53: Managing lipids". Retrieved 1 August 2011.
48. Kvan E., Pettersen K.I., Sandvik L., Reikvam A. (2007). "High mortality in diabetic patient with acute myocardial infarction: cardiovascular co-morbidities contribute most to the high risk". Int J Cardiol **121**: 184–188.
49. Norhammar A., Malmberg K., Diderhol E., Lagerqvist B., Lindahl B., Ryde et al. (2004). "Diabetes mellitus: the major risk factor in unstable coronary artery disease even after consideration of the extent of coronary artery disease and benefits of revascularization. J". Am Coll Cardiol **43**: 585–591.
50. DECODE , European Diabetes Epidemiology Group (1999). "Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria". Lancet 354: 617–621. doi:10.1016/S0140-6736(98)12131-1. PMID 10466661.
51. Moyer, VA; U.S. Preventive Services Task, Force (Oct 2, 2012). "Screening for coronary heart disease with electrocardiography: U.S. Preventive Services Task Force recommendation statement.". Annals of Internal Medicine **157**

(7): 512–8. doi:10.7326/0003-4819-157-7-201210020-00514. PMID 22847227.

52. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Selhub J, Robins SJ, Benjamin EJ, D'Agostino RB, Vasan RS (2006). "Multiple biomarkers for the prediction of first major cardiovascular events and death". N. Engl. J. Med. **355** (25): 2631–billy bob joe9. doi:10.1056/NEJMoa055373. PMID 17182988.
53. Spence JD (2006). "Technology Insight: ultrasound measurement of carotid plaque--patient management, genetic research, and therapy evaluation". Nat Clin Pract Neurol **2** (11): 611–9. doi:10.1038/ncpneuro0324. PMID 17057748.
54. Bertazzo, S. et al. Nano-analytical electron microscopy reveals fundamental insights into human cardiovascular tissue calcification. Nature Materials **12**, 576-583 (2013).
55. Inaba, Y; Chen, JA; Bergmann, SR (January 2012). "Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis.". Atherosclerosis **220** (1): 128–33. doi:10.1016/j.atherosclerosis.2011.06.044. PMID 21764060.
56. Clin Lipidol. 2007 Dec;1(6) 583-92. doi: 10.1016/j.jacl.2007.10.001. LDL Particle Number and Risk of Future Cardiovascular Disease in the Framingham Offspring Study - Implications for LDL Management.
57. Wang TJ, Larson MG, Levy D, et al. (Feb 2004). "Plasma natriuretic peptide levels and the risk of cardiovascular events and death". N Engl J Med. **350** (7): 655–63. doi:10.1056/NEJMoa031994. PMID 14960742.
58. Ignarro, LJ; Balestrieri, ML, Napoli, C (Jan 15, 2007). "Nutrition, physical activity, and cardiovascular disease: an

update.". Cardiovascular research **73** (2): 326–40. doi:10.1016/j.cardiores.2006.06.030. PMID 16945357.

59. World Heart Federation (5 October 2011). "World Heart Federation: Cardiovascular disease risk factors". Retrieved 5 October 2011.
60. The National Heart, Lung, and Blood Institute (NHLBI) (5 October 2011). "How To Prevent and Control Coronary Heart Disease Risk Factors - NHLBI, NIH". Retrieved 5 October 2011.
61. Klatsky AL (May 2009). "Alcohol and cardiovascular diseases". Expert Rev Cardiovasc Ther **7** (5): 499–506. doi:10.1586/erc.09.22. PMID 19419257.
62. McTigue KM, Hess R, Ziouras J (September 2006). "Obesity in older adults: a systematic review of the evidence for diagnosis and treatment". Obesity (Silver Spring) 14 (9): 1485–97. doi:10.1038/oby.2006.171. PMID 17030958.
63. Linden W, Stossel C, Maurice J (April 1996). "Psychosocial interventions for patients with coronary artery disease: a meta-analysis". Arch. Intern. Med. 156 (7): 745–52. doi:10.1001/archinte.1996.00440070065008. PMID 8615707.
64. Marshall, IJ; Wolfe, CD; McKevitt, C (Jul 9, 2012). "Lay perspectives on hypertension and drug adherence: systematic review of qualitative research.". BMJ (Clinical research ed.) 345: e3953. PMC 3392078. PMID 22777025.
65. Dickinson, HO; Mason, JM; Nicolson, DJ; Campbell, F; Beyer, FR; Cook, JV; Williams, B; Ford, GA (February 2006). "Lifestyle interventions to reduce raised blood pressure: a systematic review of randomized controlled trials.". Journal of hypertension 24 (2): 215–33.

66. Moyer, VA; U.S. Preventive Services Task, Force (Sep 4, 2012). "Behavioral counseling interventions to promote a healthful diet and physical activity for cardiovascular disease prevention in adults: U.S. Preventive Services Task Force recommendation statement.". Annals of Internal Medicine 157 (5): 367–71. doi:10.7326/0003-4819-157-5-201209040-00486. PMID 22733153
67. Walker C, Reamy BV (April 2009). "Diets for cardiovascular disease prevention: what is the evidence?". Am Fam Physician 79 (7): 571–8. PMID 19378874.
68. Estruch, Ramón et al (February 25, 2013). "Primary Prevention of Cardiovascular Disease with a Mediterranean Diet". New England Journal of Medicine. doi:10.1056/NEJMoa1200303. Retrieved February 25, 2013.
69. Nordmann, AJ; Suter-Zimmermann, K; Bucher, HC; Shai, I; Tuttle, KR; Estruch, R; Briel, M (September 2011). "Meta-analysis comparing Mediterranean to low-fat diets for modification of cardiovascular risk factors.". The American Journal of Medicine 124 (9): 841–51.e2.
70. Sacks FM, Svetkey LP, Vollmer WM, et al. (January 2001). "Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group". N. Engl. J. Med. 344 (1): 3–10.
71. Obarzanek E, Sacks FM, Vollmer WM, et al. (July 2001). "Effects on blood lipids of a blood pressure-lowering diet: the Dietary Approaches to Stop Hypertension (DASH) Trial". Am. J. Clin. Nutr. **74** (1): 80–9. PMID 11451721.
72. Azadbakht L, Mirmiran P, Esmaillzadeh A, Azizi T, Azizi F (December 2005). "Beneficial effects of a Dietary Approaches

to Stop Hypertension eating plan on features of the metabolic syndrome". Diabetes Care **28** (12): 2823–31. PMID 16306540.

73. Logan AG (March 2007). "DASH Diet: time for a critical appraisal?". Am. J. Hypertens. **20** (3): 223–4. doi:10.1016/j.amjhyper.2006.10.006. PMID 17324730.
74. Threapleton, D. E.; Greenwood, D. C.; Evans, C. E. L.; Cleghorn, C. L.; Nykjaer, C.; Woodhead, C.; Cade, J. E.; Gale, C. P.; Burley, V. J. (19 December 2013). "Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis". BMJ 347 (dec19 2): f6879–f6879. doi:10.1136/bmj.f6879.
75. Willett, WC (July 2012). "Dietary fats and coronary heart disease.". Journal of internal medicine 272 (1): 13–24. doi:10.1111/j.1365-2796.2012.02553.x. PMID 22583051
76. "Coronary heart disease - causes, symptoms, prevention". Southern Cross Healthcare Group. Retrieved 15 September 2013.
77. Faxon, D. P. (1 June 2004). "Atherosclerotic Vascular Disease Conference: Executive Summary: Atherosclerotic Vascular Disease Conference Proceeding for Healthcare Professionals From a Special Writing Group of the American Heart Association". Circulation 109 (21): 2595–2604. doi:10.1161/01.CIR.0000128517.52533.DB.
78. "Coronary heart disease". NIH. Retrieved 15 September 2013.
79. Bhatia, Sujata K. (2010). Biomaterials for clinical applications (Online-Ausg. ed.). New York: Springer. p. 23. ISBN 9781441969200.
80. Wong, ND (May 2014). "Epidemiological studies of CHD and the evolution of preventive cardiology.". Nature reviews.

Cardiology 11 (5): 276–89. doi:10.1038/nrcardio.2014.26. PMID 24663092.

81. GBD 2013 Mortality and Causes of Death, Collaborators (17 December 2014). "Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013.". Lancet 385: 117–171. doi:10.1016/S0140-6736(14)61682-2. PMC 4340604. PMID 25530442.
82. "What Are the Signs and Symptoms of Coronary Heart Disease?". http://www.nhlbi.nih.gov/. 29 September 2014. Retrieved 23 February 2015.
83. "Coronary Artery Disease (CAD)". cdc.gov. 12 March 2013. Retrieved 23 February 2015
84. Mehta, PK; Wei, J; Wenger, NK (16 October 2014). "Ischemic heart disease in women: A focus on risk factors.". Trends in Cardiovascular Medicine 25: 140–151. doi:10.1016/j.tcm.2014.10.005. PMID 25453985.
85. Mendis, Shanthi; Puska, Pekka; Norrving, Bo (2011). Global atlas on cardiovascular disease prevention and control (PDF) (1st ed.). Geneva: World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization. pp. 3–18. ISBN 9789241564373.
86. Charlson, FJ; Moran, AE; Freedman, G; Norman, RE; Stapelberg, NJ; Baxter, AJ; Vos, T; Whiteford, HA (26 November 2013). "The contribution of major depression to the global burden of ischemic heart disease: a comparative risk assessment". BMC medicine **11**: 250. doi:10.1186/1741-7015-11-250. PMID 24274053.

87. http://www.nhlbi.nih.gov/. 29 September 2014. Retrieved 25 February 2015. "How is Coronary Heart Disease Diagnosed?".
88. http://www.nhlbi.nih.gov. Retrieved 25 February 2015. "How Can Coronary Heart Disease Be Prevented or Delayed?".
89. Desai, CS; Blumenthal, RS; Greenland, P (April 2014). "Screening low-risk individuals for coronary artery disease.". Current atherosclerosis reports 16 (4): 402. doi:10.1007/s11883-014-0402-8. PMID 24522859
90. Boden WE; Franklin, B; Berra, K; Haskell, WL; Calfas, KJ; Zimmerman, FH; Wenger, NK (October 2014). "Exercise as a therapeutic intervention in patients with stable ischemic heart disease: an underfilled prescription.". The American Journal of Medicine 127 (10): 905–11.
91.. http://www.nhlbi.nih.gov/. 29 September 2014. Retrieved 25 February 2015."How is Coronary Heart Disease Treated?"
92. Deb, S; Wijeysundera, HC; Ko, DT; Tsubota, H; Hill, S; Fremes, SE (20 November 2013). "Coronary artery bypass graft surgery vs. percutaneous interventions in coronary revascularization: a systematic review.". JAMA 310 (19): 2086–95
93. Rezende, PC; Scudeler, TL; da Costa, LM; Hueb, W (16 February 2015). "Conservative strategy for treatment of stable coronary artery disease". World journal of clinical cases **3** (2): 163–70. doi:10.12998/wjcc.v3.i2.163. PMID 25685763.
94. Moran, AE; Forouzanfar, MH; Roth, GA; Mensah, GA; Ezzati, M; Murray, CJ; Naghavi, M (8 April 2014). "Temporal trends in ischemic heart disease mortality in 21 world regions, 1980 to 2010: the Global Burden of Disease 2010 study". Circulation 129 (14): 1483–92.

95. Moran, AE; Forouzanfar, MH; Roth, GA; Mensah, GA; Ezzati, M; Flaxman, A; Murray, CJ; Naghavi, M (8 April 2014). "The global burden of ischemic heart disease in 1990 and 2010: the Global Burden of 129 Disease 2010 study". Circulation (14): 1493–501.
96. Centers for Disease Control and Prevention, (CDC) (14 October 2011). "Prevalence of coronary heart disease--United States, 2006-2010.". MMWR. Morbidity and mortality weekly report 60 (40): 1377–81. PMID 21993341
97. Kontos, MC; Diercks, DB; Kirk, JD (Mar 2010). "Emergency department and office-based evaluation of patients with chest pain". Mayo Clinic Proceedings 85 (3): 284–99. doi:10.4065/mcp.2009.0560. PMID 20194155.
98. Calderon R, Jr; Schneider, RH; Alexander, CN; Myers, HF; Nidich, SI; Haney, C (1999). "Stress, stress reduction and hypercholesterolemia in African Americans: a review.". Ethnicity & disease 9 (3): 451–62. PMID 10600068.
99. "Causes". Coronary artery disease. Mayo Foundation for Medical Education and Research. 29 June 2012. DS00064.
100. Kivimäki M, Nyberg ST, Batty GD, Fransson EI, el at, (October 2012). "Job strain as a risk factor for coronary heart disease: a collaborative meta-analysis of individual participant data". Lancet 380 (9852): 1491–97. doi:10.1016/S0140-6736(12)60994-5. PMC 3486012. PMID 22981903.
101. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT (July 2012). "Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy". Lancet **380** (9838): 219–29. doi:10.1016/S0140-6736(12)61031-9. PMC 3645500. PMID 22818936.

102. "Agent Orange: Diseases Associated with Agent Orange Exposure". Department of Veterans Affairs Office of Public Health and Environmental Hazards. 25 March 2010. Archived from the original on 9 May 2010. Retrieved 4 May 2010.

103. Esdaile, JM; Abrahamowicz, M; Grodzicky, T; Li, Y; Panaritis, C; du Berger, R; Côte, R; Grover, SA; Fortin, PR; Clarke, AE; Senécal, JL (October 2001). "Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus.". Arthritis and rheumatism 44 (10): 2331–7. PMID 11665973.

104. Kerola, AM; Kauppi, MJ; Kerola, T; Nieminen, TV (October 2012). "How early in the course of rheumatoid arthritis does the excess cardiovascular risk appear?". Annals of the rheumatic diseases **71** (10): 1606–15. PMID 22736093.

105. Wang HX, Leineweber C, Kirkeeide R, Svane B, Schenck-Gustafsson K, Theorell T, Orth-Gomér K (March 2007). "Psychosocial stress and atherosclerosis: family and work stress accelerate progression of coronary disease in women. The Stockholm Female Coronary Angiography Study". J. Intern. Med. 261 (3): 245–54. doi:10.1111/j.1365-2796.2006.01759.x. PMID 17305647.

106. Munker.R, Hiller.E, Glass.J, et al(2007). Biology and Clinical Management in: Modern Hematology, Second Edition.

107. Pirro M[1], Bergeron J, Dagenais GR, Bernard PM, Cantin B, Després JP, Lamarche B. Age and duration of follow-up as modulators of the risk for ischemic heart disease associated with high plasma C-reactive protein levels in men. Arch Intern Med. 2001 Nov 12;161(20):2474-80.

108. Mohammad Madjid, and Omid Fatemi Components of the Complete Blood Count as Risk Predictors for Coronary Heart Disease Tex Heart Inst J. 2013; 40(1): 17–29

109.Onat A, Sansoy V, Yildirim B, Keleş I, Uysal O, Hergenç G.C-reactive protein and coronary heart disease in western Turkey. Am J Cardiol. 2001 Sep 15;88(6):601-7.

110. Buckley DI, Fu R, Freeman M, Rogers K, Helfand M.C-reactive protein as a risk factor for coronary heart disease: a systematic review and meta-analyses for the U.S. Preventive Services Task Force. Ann Intern Med. 2009 Oct 6;151(7):483-95.

111.Lowe GD, Yarnell JW, Rumley A, Bainton D, Sweetnam PM.C-reactive protein, fibrin D-dimer, and incident ischemic heart disease in the Speedwell study: are inflammation and fibrin turnover linked in pathogenesis. Arterioscler Thromb Vasc Biol. 2001 Apr;21(4):603-10.

112.Lowe GD, Sweetnam PM, Yarnell JW, Rumley A, Rumley C, Bainton D, Ben-Shlomo Y.C-reactive protein, fibrin D-dimer, and risk of ischemic heart disease: the Caerphilly and Speedwell studies. Arterioscler Thromb Vasc Biol. 2004 Oct;24(10):1957-62. Epub 2004 Aug 12.

113.John Danesh, Peter Whincup, Mary Walker, Lucy Lennon, Andrew Thomson, Paul Appleby, Ann Rumley, Gordon D.O. Low Fibrin D-Dimer and Coronary Heart Disease. https://doi.org/10.1161/01.CIR.103.19.2323.

114.Yarnell JW[1], Baker IA, Sweetnam PM, Bainton D, O'Brien JR, Whitehead PJ, Elwood PC. Fibrinogen, viscosity, and white blood

cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies. Circulation. 1991 Mar;83(3):836-44.

115. Choudhury MBK, Rahman MS ,Hassan MM, Begum R, Hoque N, Akhtaruzzaman M, Choudhury AN.Comparison of Serum Magnesium and Potassium in Acute Myocardial Infarction and Chronic Ischemic Heart Disease J. Dhaka National Med. Coll. Hos. 2011; 17 (01): 33-36

116. Toshio KOBAYASHI, Yuji MIYOSHI, Kazue YAMAOKA and Eiji YANO. Relationship between Hematological Parameters and Incidence of Ischemic Heart Diseases among Japanese White-Collar Male Workers. Occup Health 2001; 43: 85–89.

117. Swap CJ, Nagurney JT. Value and limitations of chest pain history in the evaluation of patients with suspected acute coronary syndromes. JAMA. 2005; 294: 2623–2629.

118. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001; 69: 89–95.

119.Fox N, Growdon JH. Biomarkers and surrogates. Neuro Rx. 2004; 1: 181.

120. Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. Stat Med. 1989; 8: 431–440.

121.Colburn WA. Optimizing the use of biomarkers, surrogate endpoints, and clinical endpoints for more efficient drug development. J Clin Pharmacol. 2000; 40: 1419–1427.

122. De Gruttola VG, Clax P, DeMets DL, Downing GJ, Ellenberg SS, Friedman L, Gail MH, Prentice R, Wittes J, Zeger SL. Considerations in the evaluation of surrogate endpoints in clinical trials: summary of a National Institutes of Health workshop. Control Clin Trials. 2001; 22: 485–502.

123.Freedman LS, Graubard BI, Schatzkin A. Statistical validation of intermediate endpoints for chronic diseases. Stat Med. 1992; 11: 167–178.

124. Manolio T. Novel risk markers and clinical practice. N Engl J Med. 2003; 349: 1587–1589.

125. Morrow DA, de Lemos JA, Sabatine MS, Antman EM. The search for a biomarker of cardiac ischemia. Clin Chem. 2003; 49: 537–539.

126.Vitzthum F, Behrens F, Anderson NL, Shaw JH. Proteomics: from basic research to diagnostic application: a review of requirements & needs. J Proteome Res. 2005; 4: 1086–1097.

127.Zolg JW, Langen H. How industry is approaching the search for new diagnostic markers and biomarkers. Mol Cell Proteomics. 2004; 3: 345–354.

128. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, Lijmer JG, Moher D, Rennie D, de Vet HCW. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Clin Chem. 2003; 49: 1–6.

129. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies. J Clin Oncol. 2005; 23: 9067–9072.

130.Sackett DL, Haynes RB, Guyatt GH, Tugwell P. The Interpretation of Diagnostic Data: Clinical Epidemiology, a Basic Science for Clinical Medicine. Boston, Mass: Little, Brown; 1991: 69–152.

131.Batstone G. Practicing by the evidence: the role of pathology. J Clin Pathol. 1997; 50: 447–448.

132. Rembold CM. Number needed to screen: development of a statistic for disease screening. BMJ. 1998; 317: 307–312.

133. LaBaer J. So, you want to look for biomarkers. J Proteome Res. 2005; 4: 1053–1059.

134. Hlatky MA. Exercise testing to predict outcome in patients with angina. J Gen Intern Med. 1999; 14: 63–65.

135. Mushlin AI, Kern LM, Paris M, Lambert DR, Williams G. The value of diagnostic information to patients with chest pain suggestive of coronary artery disease. Med Decis Making. 2005; 25: 149–157.

136. Rowley PT. Inherited susceptibility to colorectal cancer. Ann Rev Med. 2005; 56: 539–554.

137. Marteau TM, Roberts S, LaRusse S, Green RC. Predictive genetic testing for Alzheimer's disease: impact upon risk perception. Risk Anal. 2005; 25: 397–404.

138. Fortmann SP, Ford E, Criqui MH, Folsom AR, Harris TB, Hong Y, Pearson TA, Siscovick D, Vinicor F, Wilson PF. CDC/AHA workshop on markers of inflammation and cardiovascular disease: application to clinical and public health

practice: report from the Population Science Discussion Group. Circulation. 2004; 110: e554–e559.

139. Solberg HE. Approved recommendations (1986) on the theory of reference values, I: the concept of reference values. J Clin Chem Clin Biochem. 1987; 25: 337–342.

140.Solberg HE. Approved recommendations (1987) on the theory of reference values, II: selection of individuals for the production of reference values. J Clin Chem Clin Biochem. 1987; 25: 639–644.

141. Solberg HE. Approved recommendations (1987) on the theory of reference values, V: statistical treatment of collected reference values: determination of reference limits. J Clin Chem Clin Biochem. 1987; 25: 645–656.

142. Lott JA, Mitchell LC, Moeschberger ML, Sutherland DE. Estimation of reference ranges: how many subjects are needed? Clin Chem. 1992; 38: 648–650.

143. Antman E, Bassand JP, Klein W, Ohman M, Lopez Sendon JL, Ryden L, Simoons M, Tendera M, for the Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial infarction redefined: a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol. 2000; 36: 959–969.

144. Apple FS, Parvin CA, Buechler KF, Christenson RH, Wu AHB, Jaffe AS. Validation of the 99th percentile cutoff independent of assay imprecision (CV) for cardiac troponin

monitoring for ruling out myocardial infarction. Clin Chem. 2005; 51: 2198–2200.

145. Sunderman FW Jr. Current concepts of “normal values,” “reference values,” and “discrimination values” in clinical chemistry. Clin Chem. 1975; 21: 1873–1877.

146. Dao Q, Krishnaswamy P, Kazanegra R, Harrison A, Amirnovin R, Lenert L, Clopton P, Alberto J, Hlavin P, Maisel AS. Utility of B-type natriuretic peptide in the diagnosis of congestive heart failure in an urgent-care setting. J Am Coll Cardiol. 2001; 37: 379–385.

147. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett J. Plasma brain natriuretic peptide concentration: impact of age and gender. J Am Coll Cardiol. 2002; 40: 976–982.

148. Shapiro BP, Chen HH, Burnett JC Jr, Redfield MM. Use of plasma brain natriuretic peptide concentration to aid in the diagnosis of heart failure. Mayo Clin Proc. 2003; 78: 481–486.

149. J, Estevez E, Baquero E, Mora-Rodriguez R (2008). "Anaerobic performance when rehydrating with water or commercially available sports drinks during prolonged exercise in the heat". Applied Physiology, Nutrition and Metabolism **33** (2): 290–298. doi:10.1139/H07-188. PMID 18347684.

150. "Rehydration drinks". Webmd.com. 2008-04-28. Retrieved 2010-08-20.

151.Kamil Perzyna, Regina Borkowska, Jaroslaw Syzdek, Aldona Zalewska, Wladyslaw Wieczorek (2011). "The effect of additive of

Lewis acid type on lithium–gel electrolyte characteristics". Electrochimica Acta **57**: 58–65. doi:10.1016/j.electacta.2011.06.014.

152."The Roll-to-Roll Battery Revolution". Ev World. Retrieved 2010-08-20.

153. Syzdek, Jaroslaw, et al., Journal of Power Sources, 173, 2007, p712-720 doi:10.1016/j.jpowsour.2007.05.061

154. Syzdek, Jaroslaw, et al., Electrochimica Acta, 55, 2010, p1314-1322, doi:10.1016/j.electacta.2009.04.025

155. Syzdek, Jaroslaw, et al., Journal of Power Sources, 194, 2009, p66-72, doi:10.1016/j.jpowsour.2009.01.070

156. Pepys MB and Hirschfield GM. C-reactive protein: a critical update. J Clin. Invest 2003; 111:1805-1812.

157. Volanakis JE. Human C-reactive protein: expression, structure, and function. Mol Immunol 2001;38:189-197. 3. Koenig W, Sund M, Frohlich M, et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially.

158.healthy middle-aged men. Circulation 1999; 99:237-242.

159.Rifai N, Ridker PM. Proposed Cardiovascular Risk Assessment Algorithm Using High-Sensitivity C-reactive protein and Lipid Screening. Clin. Chem. 2001; 47:28-30.

160.Rifai N and Ridker PM. High-Sensitivity C-Reactive Protein: A novel and Promising Marker of Coronary Heart Disease. Clin. Chem. 2001; 47(3): 403-411.

161.Biasucci LM, Liuzzo G, Grillo RL, et al. Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability. Circulation 1999; 99:855-860.

162. Taubes G. Does inflammation cut to the heart of the matter? Science 2002; 296:242-245.

163.Ridker PM, Hennekens CH, Buring JE, and Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000:342(12): 836-843.

164. Brooks DE, Devine DV, Harris PC, et al. RAMP(TM): A rapid, quantitative whole blood immunochromatographic platform for point-of-care testing. Clin Chem 1999; 45:1676-1678.

165.Oh SW, Moon JD, Park SY, et al. Evaluation of fluorescence hs-CRP immunoassay for point-of –care testing. Clin Chim Acta 2005; 356:172-177.

166.Claus DR, Osmond AP, Gewurz H. Radioimmunoassay of human C-reactive protein and levels in normal sera. J. Lab. Clin Med 1976;87:120-128

167.Kindmark CO. The concentration of C-reactive protein in sera from healthy individuals. Scand J Clin Lan Invest 1972;29:407-411

168.Performance of two relatively new quantitative D-dimer assays (Innovance D-dimer and AxSYM D-dimer) for the exclusion of

deep vein thrombosis J.L. Elf K. Strandberg b, P.J. Svensson b J.L. Elf et al. / Thrombosis Research 124 (2009) 701–705.

169.Rowbotham BJ, Caroll P, Whitaker AN, Bunce IH, Cobcroft RG, Elms MJ, et al. Measurement of crosslinked fibrin derivates-use in the diagnosis of venous thrombosis. Thromb Haemost 1987;57:59–61.

170. Stein PD, Hull RD. D-dimer for the exclusion of acute deep vein thrombosis and pulmonary embolism: A systematic review. Ann Intern Med 2004;140(8):589–602. [4] Wells PS, Anderson DR, Bormanis J, Guy F, Mitchell M, Gray L, et al. Value of assessment of pretest probability of deep-vein thrombosis in clinical management. Lancet 1997;350:1795–8.

171. Comparison of an immuno-turbidometric method (STalia_R D-DI) with an established enzyme linked fluorescent assay (VIDAS_R) D-dimer for the exclusion of venous thromboembolism Journal compilation _ 2007 Blackwell Publishing Ltd, Int. Jnl. Lab. Hem. 2008, 30, 200–204

172. Different cut-off values of quantitative D-dimer methods to predict the risk of venous thromboembolism recurrence: a post-hoc analysis of the PROLONG study haematologica | 2008; 93(6) | 901
173.Performance characteristics of the AxSYM D-dimer assay Sonia L. La'ulu a, Camille M. Dominguez b, William L. Roberts c, S.L. La'ulu et al. / Clinica Chimica Acta 390 (2008) 148–151

174. Analytical performances of the D-dimer assay for the Immulite 2000 automated immunoassay analyser G. LIPPI, G. L. SALVAGNO, L. ROSSI, M. MONTAGNANA, M. FRANCHINI,

G. C. GUIDI Journal compilation _ 2007 Blackwell Publishing Ltd, Int. Jnl. Lab. Hem. 2007, 29, 415–420.

175. Diagnostic accuracy of the Triage® D-dimer test for exclusion of venous thromboembolism in outpatients Timothy Ghys, Wim Achtergael, Inge Verschraegen, Jochmans Thrombosis Research (2008) 121, 735–741

176.Kyrle PA, Eichinger S. Deep vein thrombosis. Lancet 2005;365:1163–74. Bhatia, Sujata K. (2010). Biomaterials for clinical applications (Online-Ausg. ed.). New York: Springer. p. 23. ISBN 9781441969200.

177. Wong, ND. "Epidemiological studies of CHD and the evolution of preventive cardiology. (May 2014)". Nature reviews. Cardiology 11 (5): 276–89. doi:10.1038/nrcardio.2014.26. PMID 24663092.

178. GBD 2013 Mortality and Causes of Death, Collaborators (17 December 2014). "Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013.". Lancet 385: 117–171. doi:10.1016/S0140-6736(14)61682-2.

179.Puddu PE, Lanti M, Menotti A, Mancini M, Zanchetti A, Cirillo M, et al. Red blood cell count in short –term prediction of cardiovascular disease incidence in the Gubbio population study . Acta cardiol 2002;57(3):177-85.

180.Patel KV, Ferrucci L , Eeshler WB, Longo DL, Guralnik JM. Red blood cell distribution width and risk of death in middle – aged and older adults.2009;169(5):515-23.

181. Stevens LA, Coresh J, Greene T, Levey AS (June 2006). "Assessing kidney function--measured and estimated glomerular filtration rate". The New England Journal of Medicine 354 (23): 2473–83.

Appendices

Appendix I

Evaluation of Selected Haematological and Biochemical Predictors for Ischaemic Heart Disease in Shendi Locality River Nile State, Sudan

Questionnaire

Demographic and Clinical Features of Included Patients

- *No. of Case* ☐
- *Age* ☐
- *Occupation* ☐
- *Address* ☐
- *WT* ☐

- *Gender*

Female ☐
Male ☐

- *Risk factor for IHD*

HTN ☐
DM ☐
Smoker ☐
Hyperlipidaemia ☐
Alcoholic ☐
FH ☐

- *Type of IHD*

STEMI ☐
Non STEMI ☐
Unstable angina ☐

- *ECG finding lead s involved*

Anterior ☐
Inferior ☐
Posterior ☐

Anteroseptal ☐

Lateral ☐

Anterolateral ☐

Left BBB ☐

Results

- *CBC finding*

Hb g/dl ☐

RBCS count ☐

TWBCS ☐

PCV ☐

MCV ☐

MCH ☐

MCHC ☐

RDW ☐

Platelet count ☐

MPV ☐

Differential WBCs N…… L …… M …… E ….. B …… ☐

CRP ☐

D.dimer ☐

- *RFT*

Blood urea ☐

S .creatinine ☐

- *Electrolytes*

S Na^{+} ☐

S k^{+} ☐

S Ca^{++} ☐

Appendix II

إقـــرار بالموافقـــة

الاسم :----------

العمر :---------- العنوان :----------

أوافق بمحض ارادتى بالمشاركة فى البحث العلمى المتعلق بدراسة التغييرات الدموية و البيوكيميائية لدي مرضي امراض القلب الاحتشائية فى مستشفى الملك نمر الجامعى .

أ.حمزة أحمد حسن محمد التوم

بعد أن شرح لى بأنه لا يترتب عليه أى أذى جسدى او نفسى واعلم أن المشاركة في هذا البحث لن تؤثر بأي حال من الأحوال في الرعاية الطبية التي أتلقاها كما أنه يحق لي بدون ابداء أسباب الإنسحاب من هذا البحث في أي مرحلة من مراحله.

البحث بإشراف :

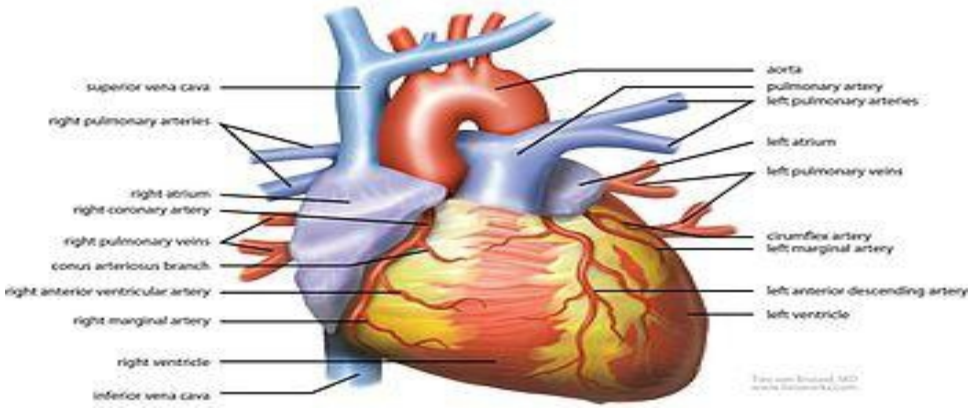
أ.د. سناء الطاهر عبدالله

مساعد مشرف:

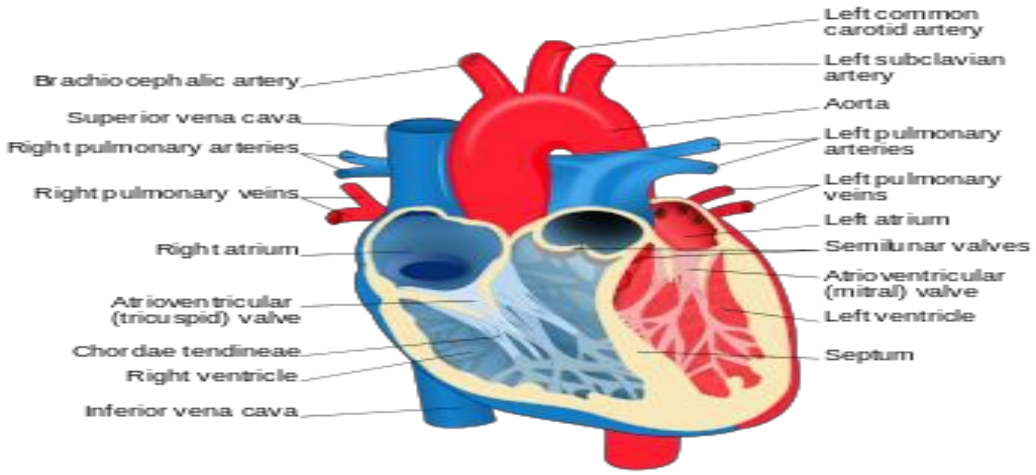
د. اسامه خضر احمد

التوقيع : ---------- التاريخ :----------

Appendix III

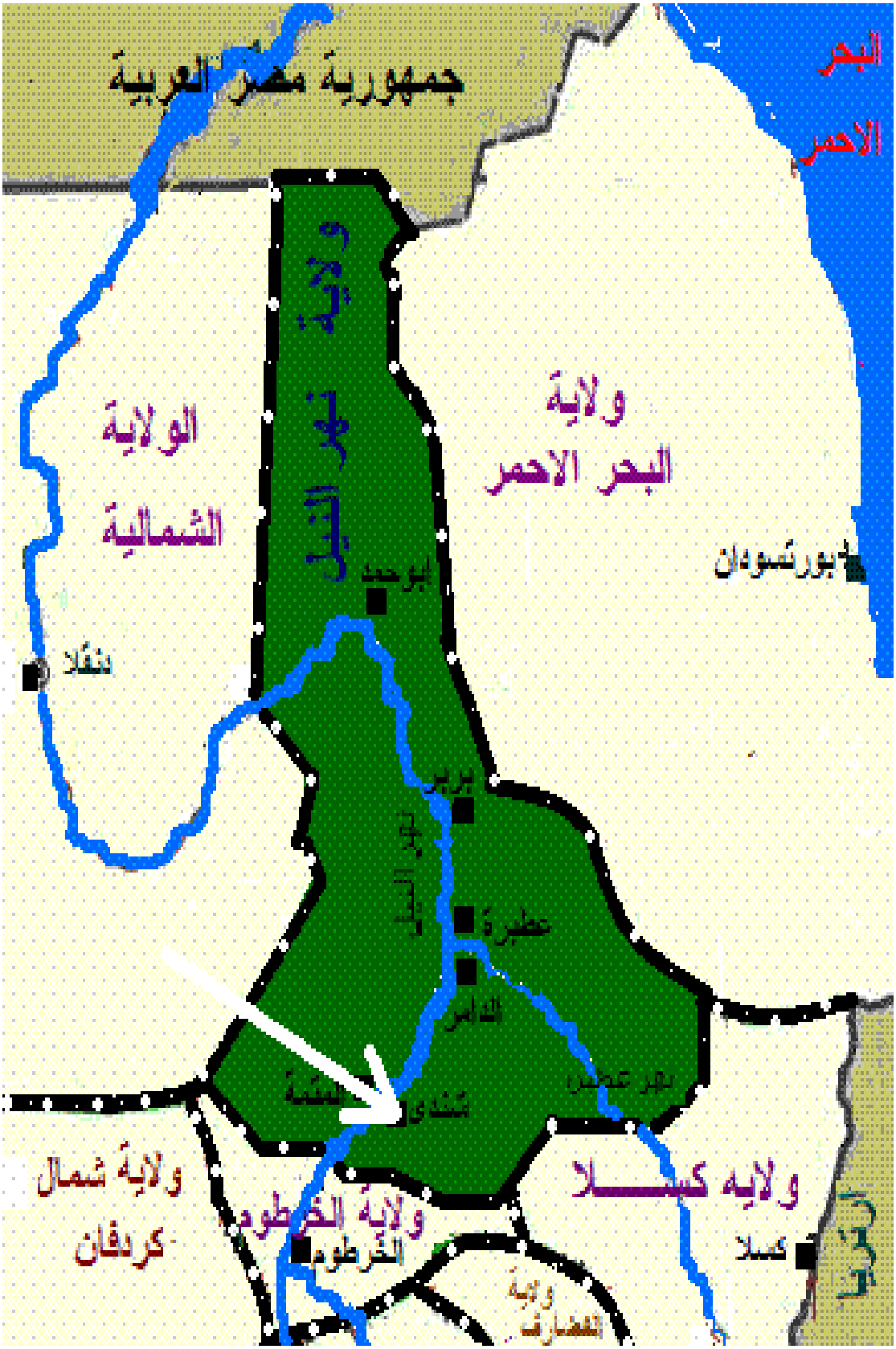


The human heart



Structure diagram of the human heart from an anterior view. Blue components indicate deoxygenated blood pathways and red components indicate oxygenated pathways

Appendix IV


جمهورية مصر العربية
البحر الاحمر
ولاية البحر الاحمر
الولاية الشمالية
بورتسودان
ابو حمد
نقلا
بربر
نهر النيل
عطبرة
الدامر
شندى
نهر عطبرة
ولاية شمال كردفان
ولاية الخرطوم
الخرطوم
ولاية القضارف
ولايه كسـلا
كسلا