Sudan University of Science and Technology

College of Graduate Studies

Determination of Red Cell Distribution Width (RDW) in Patients with Congestive Heart Failure

قياس توزيع عرض الخلية الحمراء لدى المرضى المصابين بفشل القلب الاحتقاني

A dissertation Submitted in Partial Fulfillment for the Master Degree in Medical Laboratory Science Hematology and Immunohematology (SUST)

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إقرار

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قياس توزيع عرض الخلية الحمراء لدى المرضى

المصابين بفشل القلب الاحتقاني

وهى منتج فكري أصيل . وباختياري أعطى حقوق طبع ونشر هذا العمل لكلية الدراسات العليا - جامعه السودان للعلوم والتكنولوجيا، عليه يحق للجامعه نشر هذا العمل للأغراض العلمية .

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الآية

بسم الله الرحمن الرحيم

قال تعالى

قُل لَّوْ كَانَ الْبَحْرُ مِدَادًا لِّكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَن تَنفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا

صدق الله العظيم

سورة الكهف

(الآية 109)

Dedication

To my parents who encourage me to this study and supported me through my live.

To my brothers and sisters.

To my teachers and supervisor who supported me and gave me the chance to prove myself.

To all the above mention and all whom help me to make this work I dedicate this work

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Abstract

This is a case control study conducted at Alshaab Teaching Hospital in Khartoum state during September 2014 to January 2015. The study was designed to determine the red cell distribution width in patients with congestive heart failure. The study population was 100 patients with congestive heart failure and 50 healthy subjects as control. The red cell distribution width was determined using Hematological analyzer (Sysmex KX-21). Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 16.0 software.

The RDW-SD values were significantly higher ($P < 0.05$) in the congestive heart failure patients (57.30±.729 fL) compared with the control group (42.30±.378 fL). Also the RDW-CV values were found to be significantly higher ($P < 0.05$) in the congestive heart failure patients (18.28±.267%) compared with the control group (13.85±.613%). No variation was observed in either RDW-SD or RDW-CV values with gender. The results also showed that the RDW-SD was significantly higher ($P < 0.05$) in age group between 66-85 years (58.75±1.039 fL) than the other groups. While the RDW-CV was not affected in all age group. These results concluded that RDW parameters may be a good markers in patients with congestive heart failure.

مستخلص البحث

هذه دراسة لمجموعة حالات و عينات ضابطة أجريت في مستشفى الشعب التعليمي في الخرطوم في الفترة من شهر ديسمبر ٢٠١٤ الي شهر يناير ٢٠١٥ . صممت هذه الدراسة لقياس توزيع عرض الخلية الحمراء لدى المرضى المصابين بفشل القلب الاحتقاني . وكان مجتمع الدراسة ١٠٠ عينة لمرضى مصابين بفشل القلب الاحتقاني و ٥٠ عينة ضابطة .واستخدم جهاز تحليل الدم الآلي لقياس عرض توزيع الخلية الحمراء. وتم تحليل النتائج باستخدام برنامج الحزم الإحصائية للعلوم الاجتماعية إصدار ١٦,٠.

وجد ان قيمة RDW-SD ذات دلالة احصائية (ق < ,٠٥) في مجموعة حالات فشل القلب الاحتقاني (٥٧,٣٠ ± ,٧٢٩ fL) مقارنة بمجموعة العينات الضابطة (٤٢,٣٠ ± ,٣٧٨ fL). وايضا وجد ان قيمة RDW-CV ذات دلالة احصائية (ق < ,٠٥) في مجموعة حالات القلب الاحتقاني (١٨,٢٨ ± ,٢٦٧ %) مقارنة بمجموعة العينات الضابطة (١٣,٨٥ ± ,٦١٣ %) . ولا يوجد اختلاف في قيمة RDW-SD اوRDW-CV لكل من الرجال و النساء. ووجد ان قيمة RDW-SD ذات دلالة احصائية (ق < ,٠٥) في الفئة العمرية ما بين ٦٦-٨٥ بالنسبة لعينات المرضى المصابين بفشل القلب الاحتقاني مقارنة بالفئات العمرية الصغيرة (٥٨,٧٥ ± ١,٠٣٩ fL) اما بالنسبة لقيمة RDW-CV فانه ذات دلالة غير احصائية لكل الفئات العمرية.واستنتجت من هذه النتائج ان RDW قد يكون علامة جيدة في المرضى الذين يعانون من فشل القلب الاحتقاني.

List of contents

Subject		Page
الايه		I
Dedication		II
Acknowledgment		III
Abstract (English)		IV
مستخلص البحث		V
List of contents		VI
List of tables		IX
List of abbreviations		X
List of Figure		XI
Chapter One Introduction and literature review		
1.1	Introduction	1
1.2.	literature review	2
1.2.1	The Heart	2
1.2.1.1	Understanding blood flow in the heart and body	2
1.2.2	Congestive heart failure	3
1.2.2.1	Epidemiology	4
1.2.2.2	Risk Factors of congestive heart failure	5
1.2.2.2.1	Medical Conditions	5
1.2.2.2.2	Specific Lifestyle Factor	5
1.2.2.2.3	Age	5
1.2.2.2.4	Gender	5

1.2.2.3	Pathophysiology of Congestive Heart Failure	5
1.2.2.4	Causes of congestive heart failure	11
1.2.2.5	Sign and symptom of Congestive Heart Failure	12
1.2.2.6	Diagnosis	13
1.2.2.7	Congestive Heart Failure Stage	14
1.2.2.8	Treatment	15
1.2.2.9	Management goals of CHF of therapy	15
1.2.2.10	Prognosis	16
1.2.3	Complete blood count	16
1.2.4	Red blood cells (RBCs)	18
1.2.5	Mean corpuscular volume (MCV)	18
1.2.6	Red cell distribution width (RDW)	19
1.2.6.1	Interpretation	20
1.2.7	Hematological analyzer (The Sysmex KX-21)	23
1.2.7.1	Principle of DC Detection Method	23
1.3	Justification	24
1.4	Objectives	25
1.4.1	General objective	25
1.4.2	Specific objectives	25
Chapter Two Material and Methods		
2.1	Study design	26
2.2	Study population	26

2.3	Sample collection	26
2.3.1	Requirements	26
2.3.2	Procedure of collection	26
2.4	Method of analytic	27
2.4.1	Procedure	27
2.5	Ethical consideration	27
2.6	Statistical analysis	27

Chapter Three Result	
Result	28- 30
Discussion	
Discussion, Conclusion and Recommendation	31- 32
References	
References	33- 37
Appendixes	
Appendix (1): Questionnaire	38
Appendix (2): اقرار الموافقة	39
Appendix (3): براءة اخلاقية	

List of tables

Table No	Title	Page
3-1	Distribution of study subjects according to gender	29
3-2	Distribution of study subjects according to age group	29
3-3	Results of Red cell distribution width (RDW-SD and RDW-CV) related to Subject	29
3-4	Effect of gender related to red cell distribution width (RDW-SD and RDW-CV) in congestive heart failure	30
3-5	Effect of age group related to red cell distribution width (RDW-SD and RDW-CV) in congestive heart failure	30

List of abbreviations

CHF	Congestive Heart Failure
CHD	Congenital Heart Diseases
ECG	Electrocardiogram
EMB	Endomyocardial biopsy
HF	Heart failure
K_3EDTA	Ethylene diamine tetra acetic acid
LV	Left Ventricle
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MPV	Mean platelet volume
MRI	Magnetic Resonance Imaging
NT-pro- BNP	N-Terminal pro-brain Natriuretic Peptide
NYHA	New York Heart Association
PCV	packed cell volume
RBCs	Red blood cells
RDW	Red cell distribution width
RV	Right Ventricle
TNF-alpha	Tumor Necrosis Factor Alpha
TSH	Thyroid stimulating hormone
WBCs	White Blood Cells

List of Figures

Figure No	Title	Page
1	Determinants of cardiac output	6
2	The Frank-Starling law	7
3	Frank-Starling curves	8

Chapter One

CHAPTER I

INTRODUTION AND LITERATURE REVIEW

1.1 Introduction

Congestive heart failure (CHF) occurs when the heart is unable to pump sufficiently to maintain blood flow to meet the body's needs. (McDonagh and Theresa, 2011).

Red blood cell distribution width is a numerical measure of the variability in size of circulating erythrocytes. This parameter is routinely reported as part of the complete blood count, but its use is generally restricted to narrowing the differential diagnosis of anemia. (Perkins, 2003)

Anemia is a common feature of patients with CHF and it has important implications for the prognosis and treatment of CHF. A cause and effect relationship between anemia and CHF has not been demonstrated **(**Ghali, 2009).

RDW has been recently discovered as a new marker in heart failure. It was found that increased RDW was associated with increased morbidity and mortality in chronic heart failure there is no definite pathophysiology explaining this association .Several factors may be proposed including inflammation, nutritional deficiencies, and inadequate production of erythropoietin (Felker, *etal,* 2007)

1.2 Literature Review

1.2.1 The Heart

The heart is a muscular organ in both humans and other animals, which pumps blood through the blood vessels of the system. Blood provides the body with oxygen and nutrients, and also assists in the removal of wastes. The heart is located in the middle compartment of the mediastinum in the chest (Hall and John, 2011).

In humans, other mammals and birds the heart is divided into four chambers: upper left and right atria; and lower left and right ventricles. Commonly the right atrium and ventricle are referred together as the right heart and their left counterparts as the left heart. In a healthy heart blood flows one way through the heart due to heart valves, which prevent back flow. The heart is enclosed in a protective sac, the pericardium, which also contains a small amount of fluid. The wall of the heart is made up of three layers: epicardium; myocardium; and endocardium (Phibbs and Brendan, 2007).

The right atrium and right ventricle receive blood from the body through the veins and then pump the blood to the lungs. The left atrium and left ventricle receive blood from the lungs and pump it out through the aorta into the arteries, which feed all organs and tissues of the body with oxygenated blood. Because the left ventricle has to pump blood to the entire body, it is a stronger pump than the right ventricle (Hall and John, 2011).

1.2.1.1 Understanding blood flow in the heart and body

The heart pumps blood through both circulatory systems. Blood low in oxygen from the systemic circulation enters the right atrium from the superior and inferior vena cavae and passes to the right ventricle. From here

it is pumped into the pulmonary circulation, through the lungs where it receives oxygen and gives off carbon dioxide. Oxygenated blood then returns to the left atrium, passes through the left ventricle and is pumped out

Through the aorta to the systemic circulation where the oxygen is used and metabolized to dioxide. In addition the blood carries nutrients from the liver and gastrointestinal tract to various organs of the body, while transporting waste to the liver and kidneys. Normally with each heartbeat, the right ventricle pumps the same amount of blood into the lungs as the left ventricle pumps out into the body. Veins transport blood to the heart, while arteries transport blood away from the heart. Veins normally have lower pressures than arteries. The heart contracts at a rate of around 72 beats per minute, at rest. Exercise temporarily increases this rate, but lowers resting heart rate in the long term, and is good for heart health (Hall and John, 2011).

1.2.2 Congestive heart failure

Congestive heart failure (CHF) is a clinical syndrome in which the heart fails to pump blood at the rate required by the metabolizing tissues or in which the heart can do so only with an elevation in filling pressure.

The heart's inability to pump a sufficient amount of blood to meet the needs of the body's tissues may be a result of insufficient or defective cardiac filling and/or impaired contraction and emptying. Compensatory mechanisms increase blood volume, as well as the cardiac filling pressure, heart rate, and cardiac muscle mass, to maintain the pumping function of the heart and to cause a redistribution of blood flow. Despite these compensatory mechanisms, the ability of the heart to contract and relax declines progressively and heart failure (HF) worsens (Plein, *et al*, 2012).

The clinical manifestations of HF vary enormously and depend on a variety of factors, including the age of the patient, the extent and rate at which cardiac performance becomes impaired, and which ventricle is initially involved in the disease process. A broad spectrum of severity of impairment of cardiac function is ordinarily included in the definition of HF. These impairments range from the mildest forms, which are manifest clinically only during stress, to the most advanced forms, in which cardiac pump function is unable to sustain life without external support (Plein, *et al*, 2012).

1.2.2.1 Epidemiology

Heart failure is the leading cause of hospitalization in people older than 65.In developed countries; the mean age of patients with heart failure is 75 years old. In developing countries, two to three percent of the populations have heart failure, but in those 70 to 80 years old, it occurs in 20–30 percent. More than 20 million people have heart failure worldwide (Bui, *et al*, 2011). The prevalence and incidence of heart failure are increasing, mostly because of increasing life span, but also because of increased prevalence of risk factors (hypertension, diabetes, dyslipidemia, and obesity) and improved survival rates from other types of cardiovascular disease (myocardial infarction, valvular disease, and arrhythmias) (Mann and Chakinala, 2012). In the United States, heart failure affects 5.8 million people, and each year 550,000 new cases are diagnosed (Bui, *et al*, 2011). In 2011, congestive heart failure was the most common reason for hospitalization for adults aged 85 years and older, and the second most common for adults aged 65–84 years (Pfuntner, *et al*, 2011). In Sudan especially in Khartoum state had about five hospitals especial for heart diseases. At Alshaab Teaching Hospital, the patients were diagnosed with congestive heart failure in 2013

about 4,329 and in 2014 about 4,344; these obtained from statistical department of hospital.

1.2.2.2 Risk Factors of congestive heart failure

Risk factors for congestive heart failure (CHF) include:

1.2.2.2.1 Medical Conditions

- Hypertension (high blood pressure)
- Coronary artery disease
- Diabetes
- Obesity
- Hyperthyroidism
- Severe emphysema
- Previous history of heart disease
- Valvular heart disease (Eugene, *etal,* 2001).

1.2.2.2.2 Specific Lifestyle Factor

- Excessive alcohol consumption
- Smoking
- Long-term use of anabolic steroids (Eugene, *etal,* 2001).

1.2.2.2.3 Age

CHF is most common in people who are older; most people who have CHF are age 65 or older. CHF is the leading cause of hospital admission in patients older than 65 (Eugene, *etal,* 2001).

1.2.2.2.4 Gender

Both men and women can develop CHF. However, men are at a slightly higher risk of developing CHF (Eugene, *etal,* 2001).

1.2.2.3 Pathophysiology of Congestive Heart Failure

The syndrome of CHF arises as a consequence of an abnormality in cardiac structure, function, rhythm, or conduction. In developed countries,

ventricular dysfunction accounts for the majority of cases and results mainly from myocardial infarction (systolic dysfunction), hypertension (diastolic and systolic dysfunction), or in many cases both. Degenerative valve disease, idiopathic cardiomyopathy, and alcoholic cardiomyopathy are also major causes of heart failure. Heart failure often occurs in elderly patients who have multiple comorbid conditions (e.g. angina ,hypertension, diabetes, and chronic lung disease).Some common co morbidities such as renal dysfunction are multi factorial (decreased perfusion or volume depletion from over diuresis), whereas others (e.g. anemia, depression, disorders of breathing, and cachexia) are poorly understood (McMurray and Pfeffer, 2005). CHF indicates not only an inability of the heart to maintain adequate oxygen delivery; it is also a systemic response attempting to compensate for the inadequacy. The determinants of cardiac output include heart rate and stroke volume (Fig. 1).

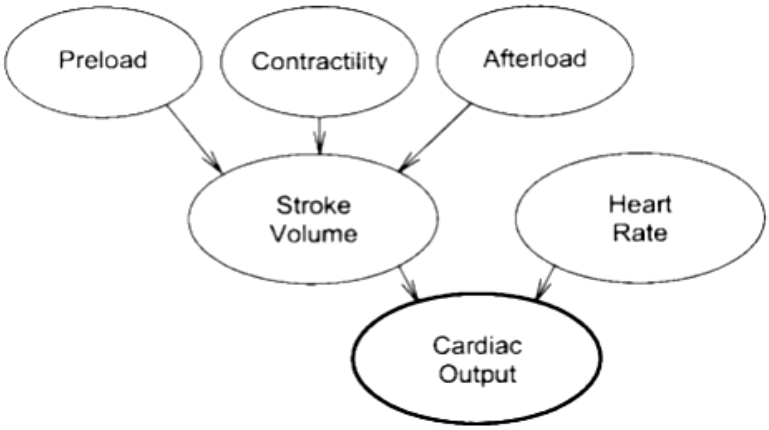

Figure 1: Determinants of cardiac output

The stroke volume is further determined by the preload (the volume that enters the left ventricle), contractility, and after load (the impedance of the flow from the left ventricle). These variables are important in understanding

the pathophysiologic consequences of heart failure and the potential treatments. Furthermore, an appreciation of cardiopulmonary interactions is important in our understanding of heart failure. In the simplest terms, the heart can be viewed as a dynamic pump. It is not only dependent on its inherent properties, but also on what is pumped in and what it must pump against. The preload characterizes the volume that the pump is given to send forward, the contractility characterizes the pump, and the after load determines what the heart must work against. The preload is often expressed as the end diastolic pressure volume of the left ventricle and is clinically assessed by measuring the right atrial pressure. However, the preload is not only dependent on intravascular volume; it is also influenced by any restriction to ventricular filling. Since the heart resides in the thoracic cavity, an increased positive pleural pressure (as seen with dynamic hyper inflation in chronic obstructive pulmonary disease or asthma) can reduce right atrial pressure (which equals central venous pressure minus pleural pressure) and thus reduce ventricular filling. The cardiac pump is a muscle and will respond to the volume it is given with a determined output. If volume increases, so will the amount pumped out in a normal physiologic state, to a determined plateau; this relationship is described by the Frank Starling law (Figs. 2 and 3)(Brausnwald, 1998) .

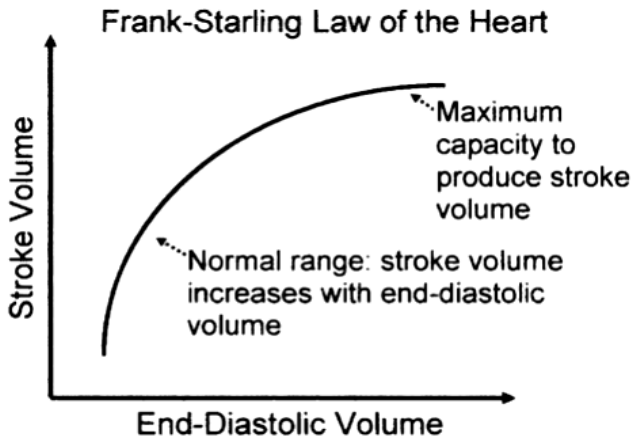

Figure 2: The Frank-Starling law of the heart states that as the ventricular volume increases and stretches the myocardial muscle fibers, the stroke volume increases, up to its maximum capacity. After that point, increasing volume increases pulmonary capillary pressure (and pulmonary congestion), without increasing the stroke volume or cardiac output. The mechanism is the length force relationships of muscle cont (Brausnwald, 1998).

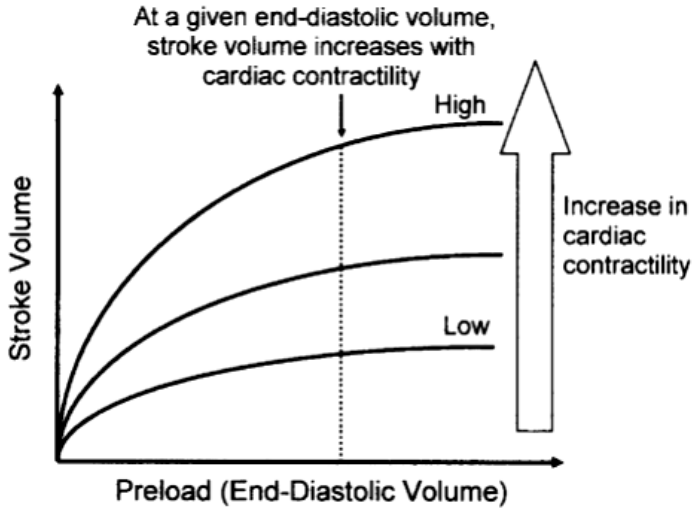

Figure 3: This series of Frank-Starling curves demonstrates that at any given preload (end-diastolic volume), increases in contractility will increase stroke volume (volume of blood ejected from the ventricle with each beat). (Brausnwald, 1998).

A concept that is often poorly understood is the diastolic function of the heart. Diastolic function is determined by 2 factors: the elasticity or distensibility of the left ventricle, which is a passive phenomenon, and the process of myocardial relaxation, which is an active process that requires metabolic energy (Aurigemma and Gaasch, 2004). Relaxation of the myocardium occurs in early diastole, and the "untwisting" of the left ventricle is an active process that produces a suction effect that augments left ventricular filling. Loss of normal left ventricular distensibility or relaxation by either structural changes (e.g. left ventricular hypertrophy) or functional changes (e.g. ischemia) impairs ventricular filling (preload). The exercise intolerance seen with diastolic dysfunction largely results from the impairment of ventricular filling, which elevates left atrial pressure and pulmonary venous pressure and causes pulmonary congestion (Kitzman, 2005). Additionally, inadequate cardiac output during exercise results in poor perfusion of skeletal muscles, especially the leg muscles and the accessory muscles of respiration (Mancini, 1995). The second variable of stroke volume is cardiac contractility, which represents the muscular pumping of the heart and is commonly expressed as the ejection fraction. Based on autonomic input, the heart will respond to the same preload with different stroke volumes, depending on inherent characteristics of the heart. A heart with normal systolic function will maintain an ejection fraction of over 50–55%. A previous myocardial infarction may result in nonfunctioning myocardium that will impair contractility. A recent concept is that ischemic myocardial tissue can be nonfunctioning (hibernating) but revitalized by surgical or medical therapy directed at ischemic heart disease (Choudhury, *et al*, 2002). Other depressants of myocardial systolic function include pharmacologic agents (calcium channel blockers), hypoxemia, and

severe acidosis. The final determinant of stroke volume is after load. In basic terms, after load is the load that the pump has to work against, which is usually clinically, estimated by the mean arterial pressure. The normal cardiac output is relatively insensitive to after load up to 140 mm Hg. However, the after load represents not only the vascular resistance but also the wall tension and intrathoracic pressure that the myocardium must work against. Together, these 3 variables are impaired in the patient with CHF.The failing heart in CHF can be best evaluated with the above variables considered together. If cardiac output falls, either the heart rate or stroke volume must change in order to maintain perfusion. If stroke volume cannot be maintained, then heart rate must increase to maintain cardiac output. However, the pathophysiology behind CHF includes not only a structural abnormality; it also includes the cardiovascular response to poor perfusion with the activation of the neurohumoral system (Jessup and Brozena, 2003). Activation of the rennin angiotensin system attempts to increase preload by stimulating retention of salt and water, increasing vasoconstriction (and, thus, after load), and augmenting cardiac contractility. Initially, this response will suffice, but prolonged activation results in loss of myocytes and maladaptive changes in the surviving myocytes and the extracellular matrix. The stressed myocardium undergoes remodeling and dilation in response to the insult (Eichhorn and Bristow 1996). This process also has detrimental effects on the functioning of the lungs, kidneys, muscles, blood vessels, and probably other organs. Remodeling also results in additional cardiac decompensation from complications, including mitral regurgitation from valvular annulus stretching, and cardiac arrhythmias from atrial remodeling (Jessup and Brozena, 2003). The respiratory care provider often becomes involved with the CHF patient as the elevated end diastolic pressure leads to

pulmonary edema and dyspnea. Patients' presentation can greatly differ, depending on the chronicity of the disease. For instance, most patients experience dyspnea when pulmonary artery occlusion pressure exceeds 25 mm Hg. However, the patient with longstanding CHF can tolerate filling pressure up to 40 mm Hg. The lung provides multiple mechanisms to avoid the consequences of pulmonary edema. Initially, as pressure increases, pulmonary capillaries are recruited and increase capacitance to deal with the added volume (Gehlbach and Geppert, 2004). As pressure continues to increase volume can be diverted from the alveoli to the interstitium. At this point by action of pressure gradients, fluid will form in the interlobular septae and the perihilar region. As noted above, chronic heart failure is associated with increased venous capacitance and lymphatic drainage of the lung. As a result, crackles are often absent, even in the setting of elevated pulmonary capillary pressure. Continued sodium retention preferentially results in peripheral edema and, ultimately in the development of pleural effusions (Malik, *et al*, 2000). With acute decompensationn, the pulmonary capillary membrane may succumb to increased pressure, with shearing of the capillary and release of fluid, protein, and occasionally red blood cells into the alveoli. The lungs' response will include cough, to expel the fluid in the alveoli. The long term response to elevated pulmonary venous pressure includes interstitial fibrosis with thickening of the alveolar membrane (Gehlbach and Geppert, 2004). Thus, severe chronic heart failure can result in interstitial fibrosis and a restrictive lung disease.

1.2.2.4 Causes of congestive heart failure

Damage to the mechanisms that control the input to and output of blood from the heart is usually the last stage of one of several heart or circulatory

diseases. Heart failure can be a direct result of one of these diseases or it can occur over time as the heart tries to compensate for abnormalities caused by these conditions (Robert, 2002).

1- Coronary Artery Disease
2- Damage after a Heart Attack
3- High Blood Pressure
4- Diabetes
5- Valvular Heart Disease
6- Cardiomyopathies
7-Congenital heart disease (condition you are born with)
8- Other Causes

- Alcoholism
- Severe emphysema is a major cause of right sided congestive heart failure.
- Other less common causes of heart failure include excessive salt consumption, hyperthyroidism, thiamin deficiency, pneumonia, high fever, and failure of the liver or kidneys.
- Rarely, certain viral illnesses cause an infection of the heart muscle known as acute myocarditis
- Long term use of anabolic steroids (Robert, 2002).

1.2.2.5 Signs and symptoms

Signs and symptoms of heart failure include the following:

1- Exertional dyspnea and/or dyspnea at rest.
2- Orthopnea.
3-Acute pulmonary edema.
4- Chest pain/pressure and palpitations.
5- Tachycardia.

6- Fatigue and weakness.

7- Nocturia and oliguria.

8- Anorexia, weight loss, nausea.

9- Exophthalmos and/or visible pulsation of eyes.

10- Distention of neck veins.

11- Weak, rapid, and thready pulse.

12- Rales, wheezing.

13- S_3 gallop and/or pulsus alternans.

14- Increased intensity of P_2 heart sound.

15- Hepatojugular reflux.

16- Ascites, hepatomegaly, and/or anasarca.

17- Central or peripheral cyanosis, pallor (Hunt, *et al*, 2005).

1.2.2.6 Diagnosis

The diagnosis of congestive heart failure is mainly clinical but various investigations help us to understand the underlying cause and assessment of severity of CHF. The following tests may be useful in the initial evaluation for suspected congestive heart failure:

- Complete blood count (CBC)
- Urinalysis
- Electrolyte levels
- Renal and liver function studies
- Fasting blood glucose levels
- Lipid profile
- Thyroid stimulating hormone (TSH) levels
- B-type natriuretic peptide levels
- N-terminal pro-B-type natriuretic peptide
- Electrocardiography(ECG)
- Magnetic Resonance Imaging(MRI)
- Chest radiography
- 2-dimensional (2-D) echocardiography

- Nuclear imaging
- Endomyocardial biopsy(EMB)
- Maximal exercise testing
- Pulse oximetry or arterial blood gas (Dickstein, *etal*, 2008).

1.2.2.7 Congestive Heart Failure Stages

Once a diagnosis of heart failure is established, evaluation of heart failure is important. Providing a complete and accurate history of symptoms is essential. Two major groups have established various stages of congestive heart failure. The American College of Cardiology/American Heart Association stages patients according to the progression of their heart failure. The stages are as follows: (Hunt, *et al*, 2005).

- **Stage A:** High risk of heart failure but no structural heart disease or symptoms of heart failure
- **Stage B:** Structural heart disease but no symptoms of heart failure
- **Stage C:** Structural heart disease and symptoms of heart failure
- **Stage D:** Refractory heart failure requiring specialized interventions

A classification system was developed by the New York Heart Association to grade congestive heart failure by severity of symptoms. These classifications help physicians determine treatment options. (Raphael, *et al*, 2007).

- **Class I:** No limitation of physical activity
- **Class II:** Slight limitation of physical activity
- **Class III:** Marked limitation of physical activity
- **Class IV:** Symptoms occur even at rest; discomfort with any physical activity

1.2.2.8 Treatment

Treatment includes the following:

- Nonpharmacologic therapy: Oxygen and noninvasive positive pressure ventilation, dietary sodium and fluid restriction, physical activity as appropriate, and attention to weight gain
- Pharmacotherapy: Diuretics, vasodilators, inotropic agents, anticoagulants, beta blockers, and digoxin (Robert, 2002)

Surgical options

Surgical treatment options include the following:

- Electrophysiologic intervention
- Revascularization procedures
- Valve replacement/repair
- Ventricular restoration
- Extracorporeal membrane oxygenation
- Ventricular assist devices
- Heart transplantation
- Total artificial heart (Robert, 2002).

1.2.2. 9 Management goals of CHF of therapy are

1. Prevent CHF in people at risk.
2. Detect asymptomatic LV dysfunction early.
3. Relieve symptoms and improve quality of life.
4. Slow disease progress and prolong survival.
5. Improve physical activity tolerance.
6. Reduce hospital admissions (Hunt, *et al*, 2005).

1.2.2.10 Prognosis

The prognosis for a specific person with heart failure depends to a large degree on effects of the disease, such as the level of blood output of the left ventricle, or his or her ability to exercise, as well as other factors, including age, overall health, and other medical conditions. The sooner heart failure is diagnosed and action is taken to control the problem, the better. In many cases, heart failure can be effectively treated to prevent or slow the progression of the disease and to alleviate its symptoms. Therapy can achieve several goals: It can improve the performance of the left ventricle, prevent further deterioration of heart function, improve a patient's ability to exercise, and improve quality of life. In addition, it is possible that in selected instances, early, effective treatment may increase a person's likelihood of improved survival (Robert, 2002).

1.2.3 Complete blood count

A complete blood count (CBC) is a blood panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood, such as the cell count for each cell type and the concentrations of various proteins and minerals. Blood counts of various types have been used for clinical purposes since the 19th century. Automated equipment to carry out complete blood counts was developed in the 1950s and 1960s. (Verso, 1962)

A complete blood count will normally include:

1-White cells

White Blood Cell Count is the number of leukocytes measured directly, multiplied by the calibration constant, and expressed as n x 103 cells/μL.

The major types of white blood cells are neutrophils, lymphocytes,

monocytes, eosinophils, and basophils (David and Dugdale, 2012).

2-Red cells

Total red blood cells: The number of red cells is given as an absolute number per liter (David and Dugdale, 2012).

3-Hemoglobin

Hemoglobin: The amount of hemoglobin in the blood, expressed in grams per deciliter (David and Dugdale, 2012).

4-Hematocrit

Hematocrit or packed cell volume (PCV): This is the fraction of whole blood volume that consists of red blood cells (David and Dugdale, 2012).

5-Red blood cell indices

(I) MCV

Mean corpuscular volume (MCV): the average volume of the red cells, measured in femtolitres.

(II) MCH

Mean corpuscular hemoglobin (MCH): the average amount of hemoglobin per red blood cell, in pictograms.

(III) MCHC

Mean corpuscular hemoglobin concentration (MCHC): the average concentration of hemoglobin in the cells.

(IV) RDW

Red blood cell distribution width (RDW): the variation in cellular volume of the RBC population (David and Dugdale, 2012).

6-Platelets

Platelet numbers are given, as well as information about their size and the range of sizes in the blood (David and Dugdale, 2012).

7-MPV

Mean platelet volume (MPV): The average volume of individual platelets (David and Dugdale, 2012).

1.2.4 Red blood cells (RBCs)

Red blood cells (RBCs), also called erythrocytes, are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (O_2) to the body tissues via blood flow through the circulatory system. RBCs take up oxygen in the lungs or gills and release it into tissues while squeezing through the body's capillaries. The cytoplasm of erythrocytes is rich in hemoglobin, an iron containing biomolecule that can bind oxygen and is responsible for the red color of the cells. The cell membrane is composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deformability and stability while traversing the circulatory system and specifically the capillary network. In humans, mature red blood cells are flexible and oval biconcave disks. They lack a cell nucleus and most organelles, in order to accommodate maximum space for hemoglobin. Approximately 2.4 million new erythrocytes are produced per second in human adults **(**Erich, 1995). The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells (Pierige, *etal*, 2008).

1.2.5 Mean corpuscular volume (MCV)

Mean corpuscular volume (MCV) is the average volume of red cells in a specimen. MCV is elevated or decreased in accordance with average red cell

size; i.e., low MCV indicates microcytic (small average RBC size), normal MCV indicates normocytic (normal average RBC size), and high MCV indicates macrocytic (large average RBC size). It can be directly measured by automated hematology analyzer or it can be calculated from hematocrit (Hct) and the red blood cell count (RBC) as follows:
MCV in fl = (Hct [in L/L] / RBC [in x10 ^{12}/L]) x 1000
The reference range for MCV is 80-96 fL/red cell in adult. Reference ranges may vary depending on the individual laboratory and patient's age (Vajpayee, *et al,* 2011). MCV along with mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), is a part of RBC indices (erythrocyte indices), which are measurements and/or calculations for determining the size, content, and hemoglobin concentration. More recently, red cell distribution width (RDW) has also been included as a part of RBC indices. The indices are useful in the morphologic characterization of anemia (Ryan, *etal,* 2010).

1.2.6 Red cell distribution width (RDW):

Red cell distribution width (RDW) is a parameter that measures variation in red blood cell size or red blood cell volume. RDW is elevated in accordance with variation in red cell size (anisocytosis), i.e. when elevated RDW is reported on complete blood count; marked anisocytosis (increased variation in red cell size) is expected on peripheral blood smear review. The reference range for RDW is as follows:

- RDW-SD 39-46 fL
- RDW-CV 11.6-14.6% in adult. (Briggs, *et al*, 2012)

Reference range may vary depending on the individual laboratories and patient's age. RDW stability in blood specimens RDW % (RDW-SD) is driven by MCV. Because MCV is unstable in blood samples, RDW % would be expected to have the same drawback (Briggs, *et al*, 2012). Laboratory experts have reported that RDW is unstable at room temperature and that significant changes occur between 6 and 24 hours after specimen collection (Martina, 2012).

1.2.6.1 Interpretation

Red cell distribution width (RDW) is a red blood cell parameter that measures variability of red cell volume/size (anisocytosis). Depending on the types of hematology analyzer instruments, RDW can be reported statistically as coefficient of variation (CV) and/or standard deviation (SD), RDW-CV and/or RDW-SD, respectively. RDW-SD (express in fL) is an actual measurement of the width of the RBC size distribution histogram and is measured by calculating the width (in fL) at the 20% height level of the RBC size distribution histogram. RDW-SD is therefore not influenced by the average RBC size (mean corpuscular volume, MCV). RDW-CV (express in %) is calculated from standard deviation and MCV as follows :

- RDW-CV (%) = (Standard deviation of MCV ÷ mean MCV) x 100%

RDW is often elevated in clinical practice by nutrient deficiencies, which lead to heterogeneous red cell populations. A large number of reticulocytes can also increase the RDW, as can lab errors from red cell clumping or counting of large platelets or schistocytes (Harmening, *et al*, 2009).

RDW is useful in the following conditions

- Elevated RDW helps provide a clue for a diagnosis of early nutritional deficiency such as iron, folate, or vitamin B12 deficency as it becomes elevated earlier than other red blood cell parameters.
- It can also help distinguish between megaloblastic anemia such as folate or vitamin B12 deficiency anemia (elevated RDW) and other causes of macrocytosis (often normal RDW).
- RDW can be used as guidance for flagging samples that may need manual peripheral blood smear examination, since elevated RDW may indicate red cell fragmentation, agglutination, or dimorphic red blood cell populations (Briggs, *et al*, 2012).
- It aids in distinguishing between uncomplicated iron deficiency anemia (elevated RDW, normal to low MCV) and uncomplicated heterozygous thalassemia (normal RDW, low MCV); however, definitive tests are required (Vaya, *et al*, 2014).

A low RDW (below 10.2%)

Means that the red blood cells vary very little in size. One reason for a low RDW level is macrocytic anemia. Macrocytic anemia is a blood disorder in which not enough red blood cells are produced, but the ones that are present are large. Another cause of a low RDW level is microcytic anemia. Microcytic anemia is a condition in which abnormally small red blood cells are present. In these two disorders the red blood cells do not vary much in size because they are either all small or all large. This is what causes the RDW level to be low (Mosby, 2012).

RDW along with mean corpuscular volume (MCV) is helpful in narrowing the cause of anemia

Normal RDW and low MCV is associated with the following conditions

- Anemia of chronic disease.
- Heterozygous thalassemia.
- Hemoglobin E trait. (Marks, *et al*, 2009)

Elevated RDW and low MCV is associated with the following conditions

- Iron deficiency.
- Sickle cell-β-thalassemia. (Marks, *et al*, 2009)

Normal RDW and high MCV is associated with the following conditions

- Aplastic anemia.
- Chronic liver disease.
- Chemotherapy/antiviral/alcohol. (Marks, *et al*, 2009)

Elevated RDW and high MCV is associated with the follow in conditions

- Folate or vitamin B12 deficiency.
- Immune hemolytic anemia.
- Cytoxic chemotherapy.
- Chronic liver disease.
- Myelodysplastic syndrome. (Marks, *et al*, 2009)

Normal RDW and normal MCV is associated with the following conditions

- Anemia of chronic disease.
- Acute blood loss or hemolysis.
- Anemia of renal disease. (Marks, *et al*, 2009)

Elevated RDW and normal MCV is associated with the following conditions

- Early iron, vitamin B12, or folate deficiency.
- Dimorphic anemia (for example, iron and folate deficiency).

- Sickle cell disease.
- Chronic liver disease.
- Myelodysplastic syndrome (Marks, *et al*, 2009).

1.2.7 Hematological analyzer (The Sysmex KX-21)

The Sysmex KX-21 is an automatic multi parameter blood cell counter for in vitro diagnostic use in clinical laboratories. It is processes approximately sixty samples an hour and displays on the LCD screen the particle distribution curves of WBC, RBC, and platelets, along with data of 18 parameters, as the analysis results. (Stiene, *etal*, 1998)

1.2.7.1 Principle of DC Detection Method

Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture, there are the electrodes between which a direct current flows. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data. (Stiene, *etal*, 1998)

1.3 Rationale

Red cell distribution width is a part of routine haematological laboratory tests and is a useful indicator for differentiation and classification of anaemia. Anemia is a common feature of patients with CHF and it has important implications for the prognosis and treatment of CHF. Recent studies, RDW measurements are requested for a wider spectrum of indications. RDW as an applicable parameter in the prediction of risk and determination of prognosis in cardiovascular diseases including heart failure, diseases of peripheral arteries and lungs, myocardial infarction, and angina pectoris . Felker *et al.* first reported higher RDW as a novel predictor of morbidity and mortality among congestive heart failure patients in a large clinical trial. Subsequent studies have validated this observation and shown association with worse long term outcome.

The present study conducted to explore the possible relationships between the red cell distribution width and congestive heart failure in Khartoum State.

1.4 Objectives

1.4.1 General objective

To determine the value of the red cell distribution width as a biomarker for congestive heart failure.

1.4.2 Specific objectives

1- To determine RDW-SD and RDW-CV in patients with congestive heart failure.

2- To investigate the red cell distribution width in regard to age of the patients.

3- To compare the values of the red cell distribution width of male patients with females.

Chapter Two

CHAPTER Two

MATERIAL and METHODS

2.1 Study design

This is a case control study conducted at Alshaab Teaching Hospital in Khartoum State in the period from September 2014 to January 2015. This study designed to determine red cell distribution width in patients with congestive heart failure.

2.2 Study population

The study includes 100 patients diagnosed with congestive heart failure and 50 samples from healthy subjects.

2.3 Sample collection

2.3.1 Requirements

1. Plastic K_3EDTA containers.
2. Sterile cotton.
3. Alcohol (70%).
4. Disposable syringes.
5. Tourniquet.

2.3.2 Procedure

Patient was sitting comfortable; a tourniquet applied above elbow, and superficial antecubital from vein was identified .The skin steriled with 70% ethanol and allowed to dry. Syringe needle inserted correctly into the vein, and 2.5 ml of blood were taken from the antecubital vein of the forearm, tourniquet was released, needle removed, and 2.5ml blood was drained into K_3EDTA container, and mixed with anticoagulant gently several times.

2.4 Analytic motheds

Hematological analyzer (The Sysmex KX-21)

2.4.1 Procedure

(1) The sample mixed sufficiently.

(2) The plug while removed taking care not to allow blood scatter.

(3) The sample probe set to the tube, and in that condition, pressed the start switch.

(4) The buzzer sounds two times - "beep, beep" - and when the LCD screen had displayed "Analyzing," the tube was removed. After that, the unit executed automatic analysis and displayed the result on the LCD screen. (Stiene, *etal*, 1998).

2.5 Ethical consideration

The study was conducted after permission from the institution ethical committee. Written consent of cases and controls were taken.

2.6 Statistical analysis

Data were processed and analyzed using Statistical Package for Social Sciences (SPSS) version 16.0. Independent Samples t-test was used to calculate P value. Differences were considered statistical significant when P value ≤ 0.05.

Chapter Three

CHAPTER THREE
RESULTS

A total of 150 subjects were included in this study, of which 100 were diagnosed with congestive heart failure; 65 of them are male and 35 are female and 50 healthy people as control; 27 of them are male and 23 are female showed in table (3-1). In table (3-2) the patiets were divided into four age groups, their frequencies were as follows, patients with age less than 25 years were 6 and control were 5, those between 26- 45 years were 9 and control were 14, 46-65 years were 22 in patients and control were 15 and 63 patients between 66-85 years of age and control were 16.

The RDW-SD values were found to be significantly higher ($P < 0.05$) in the congestive heart failure cases group (57.30±.729 fL) compared to control group (42.30±.378 fL). Also the RDW-CV values were found to be significantly higher ($P < 0.05$) in the congestive heart failure cases group (18.28±.267 %) compared to control group (13.85±.613 %) showed in table (3-3).

In table (3-4) the effect of the red cell distribution width (RDW-SD and RDW-CV) related to gender in congestive heart failure patients were found to be insignificant.

In table (3-5) the effect of the red cell distribution width (RDW-SD) related to age group in congestive heart failure patients were found to be insignificant in all age group except the group of 66-85 years was found to be significantly higher ($P < 0.05$) (58.75±1.039 fL). While the effect of the red cell distribution width (RDW-CV) related to age group in congestive heart failure patients were found to be insignificant in all age group.

Table (3-1): Distribution of study subjects according to gender

Subject	Gender		Total
	Male	Female	
Case	65 (65.0%)	35 (35.0%)	100 (100.0%)
Control	27 (54.0%)	23 (46.0%)	50 (100.0%)

Table (3-2): Distribution of study subjects according to age group

Subject	Age group				Total
	Less than 25 years	26-45 years	46-65 years	66-85 years	
Case	6 (6.0%)	9 (9.0%)	22(22.0%)	63(63.0%)	100(100.0%)
Control	5 (10.0%)	14(28.0%)	15(30.0%)	16(32.0%)	50(100.0%)

Table (3-3): Results of Red cell distribution width (RDW-SD and RDW-CV) related to Subject

	Subject	**N**	**Mean ± Std**	**P. value**
RDW-SD	Case	100	57.30 ± .729	0.000
	Control	50	42.30 ± .378	0.000
RDW-CV	Case	100	18.28 ± .267	0.000
	Control	50	13.85 ± .613	0.000

Table (3-4): Effect of gender on the red cell distribution width (RDW-SD and RDW-CV) in congestive heart failure patients

	Gender	N	Mean ± Std	P. value
RDW-SD	Male	65	57.50 ± .880	0.718
	Female	35	56.94 ± 1.308	0.725
RDW-CV	Male	65	18.34 ± .324	0.758
	Female	35	18.16 ± .477	0.764

Table (3-5): Effect of age group on the red cell distribution width (RDW-SD and RDW-CV) in congestive heart failure patients

	Age group	N	Mean ± Std	P. value
RDW-SD	Less than 25	6	55.27 ± 1.660	.431
	26 - 45	9	53.49 ± 1.403	.431
	46 – 65	22	55.27 ± .984	.064
	66 - 85	63	58.75 ± 1.039	.018
RDW-CV	Less than 25	6	17.93 ± .541	.828
	26 - 45	9	17.62 ± 1.076	.801
	46 – 65	22	17.82 ± .467	.272
	66 - 85	63	18.56 ± .358	.216

Discussion

4.1 Discussion

RDW is a parameter, which demonstrates variations in the dimensions of circulating erythrocytes (i.e. anisocytosis). Routinely, the value of this marker can be learnt during a simple whole blood count. Up to now RDW values have been assessed in hematological indications as malnutritional anemias and diseases leading to the destruction of erythrocytes (Tonelli**,** *et al.* 2008). Recent studies have indicated RDW as an applicable parameter in the prediction of risk and determination of prognosis in cardiovascular diseases including heart failure, diseases of peripheral arteries and lungs, myocardial infarction, and angina pectoris (Tonelli**,** *et al.* 2008). The first outcome derived from this study is that increased of the RDW values in the patients with congestive heart failure in comparison with the control group. These results agree with Felker *et al* who founded that increased RDW was a strong independent predictor of greater morbidity and mortality in patients with chronic heart failure and concluded that RDW is an important marker for prediction of mortality and morbidity of chronic heart failure (Felker, *et al.*2007). Also the results agree with Mustafa who reported that there was significant increased RDW in Patients with congestive heart failure and concluded that the RDW can be used as prognostic value for Patients with congestive heart failure (Mustafa, 2014).

The second outcome derived from this study is the significantly increase RDW values in older more than young people particularly over 65years; this agrees with Mustafa who concluded that there was significant increased RDW value with older (Mustafa, 2014). The third outcome derived from this study is there no change in RDW related to gender.

4.2 Conclusion

This study concluded that:

- The red cell distribution width can be used as a biomarker for congestive heart failure.
- There was significant increase of the red cell distribution width in older subjects particularly over 65years.
- There was no association between the red cell distribution width and gender.

4.3 Recommendations

- More studies with a large sample size and long following duration should be conducted in the future to verify these results.
- Epidemiological studies should be done in all country to determine the prevalence of congestive heart failure in Sudanese people.
- This study should prompt further evaluation of the association between RDW and outcome in heart failure to improve understanding of pathophysiology and to better risk-stratify patients with chronic heart failure.

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Appendix

Appendix (1): Questionnaire

بسم الله الرحمن الرحيم

جامعة السودان للعلوم و التكنولوجيا

كلية الدراسات العليا

استبيان لمشروع بحث بعنوان:

Determination of Red Cell Distribution Width (RDW) in Patients with Congestive Heart Failure

- Age :……years
- Sex: Male…… Female……
- RDW-SD…….
- RDW-CV…….
- Diagnosis ……....

Appendix (2): اقرار الموافقة

بسم الله الرحمن الرحيم

جامعة السودان للعلوم و التكنولوجيا
كلية الدراسات العليا

اقرار الموافقة

الاسم:.....

سوف يتم اخذ عينه دم من الوريد بواسطة حقنة طعن، وذلك بعد مسح مكان أخذ العينة بواسطة مطهر،كل الأدوات المستخدمة معقمه و فيها وسائل السلامة المعملية . وأنا أقر بأن العينات سوف البحث مع مراعاة السرية يتم تحليلها فقط لغرض.

اوافق انا المذكور اعلاه بأخذ عينة لاجراء الدراسة.

الاسم : .....

الامضاء : .....