INTRODUCTION

von Willebrand factor (vWF) is essential for normal hemostasis. It carries and protects coagulation factor VIII in the circulation, and it recruits platelets to the site of clot formation during primary hemostasis. vWF cross-links platelets with exposed collagen at a site of vessel damage, and together with fibrinogen, it cross-links platelets during subsequent platelet aggregation. The efficacy of vWF in primary hemostasis depends on its level and function *(Davies et al., 2007)*.

Low vWF levels are associated with bleeding and lead to the hemorrhagic disorder von Willebrand disease (vWD) *(Sadler et al., 2006)*.

In contrast, increased vWF levels are associated with risk for ischemic heart disease and myocardial infarction *(Lowe, 2005)*.

Three subtypes of vWD are defined according to a quantitative deficiency (types 1 and 3) or qualitative deficiency (type 2). Type 1 vWD is the commonest form and is characterized by a partial quantitative deficiency of vWF *(Sadler et al., 2006)*.

The mechanisms that control plasma vWF level are not understood. Approximately 70% of variability is explained by genetic factors, *(De Lang et al., 2006)* of which the ABO blood group is a major influence *(Orstavik et al., 1985)*.

In Egypt, being always center for multicultural population, the normal range for vWF has to be estimated to proper diagnoses of vWD and response to acute phase reactant.

AIM OF THE WORK

1- To determine the normal range in our population in order to use it as a reference in our laboratory.

2- To assess the correlation between vWF and blood groups A1, A2 and O.

ESTABLISHING REFERENCE RANGE FOR CLINICAL LABORATORY TEST RESULTS

Reference range is essential for clinical laboratory test interpretation and patient care *(Katayev et al., 2010)*. Reference range is the most common decision support tool used for interpretation of numerical pathology reports. As laboratory results may be interpreted by comparison with these ranges, the quality of the reference ranges can play large role in result interpretation as the quality of the result itself. The recommended protocol for setting a reference range is to perform a reference range study according to standard published procedures *(Jones and Barker, 2008)*.

Reference ranges have several advantages in routine clinical applications including their simplicity, ease of storage and retrieval from laboratory computer systems and pocket notebooks, and their high degree of acceptance by the medical community through long use *(Boyd, 2010)*.

Definition of reference range:

Reference range is defined as an interval that when applied to the population serviced by the laboratory correctly includes most of the subjects with characteristics similar to the reference group and excludes the others *(Ceriotti et al., 2007)*.

The reference range for many laboratory tests is defined by threshold values between which the test results of a specified percentage (usually 95%) of apparently healthy individuals would fall. This definition results in exclusion of the 2.5% of individuals with the lowest results and the 2.5% of individuals with the highest results from the reference interval [5% of all cases will fall outside of the reported reference range] (Figure 1) *(Boyd, 2010)*.

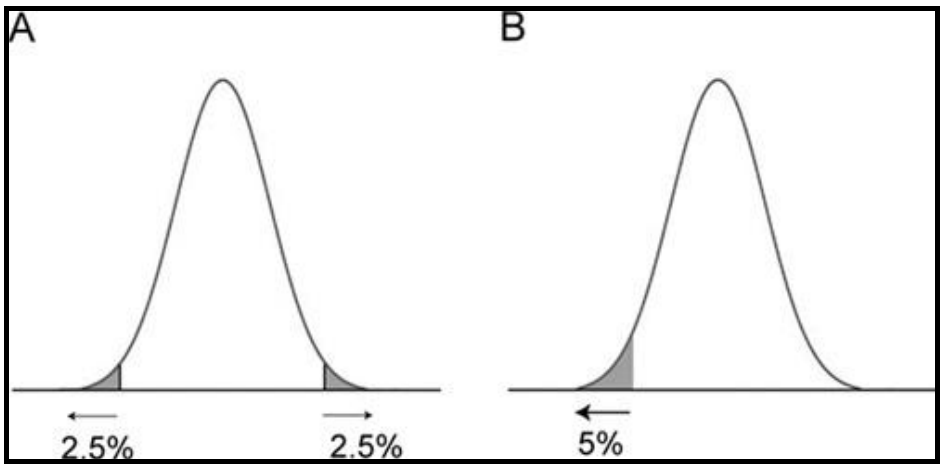

Fig. (1): The 95% reference ranges are derived by identifying the most outlying 5% of observed values in a reference population *(Boyed, 2010)*.

Reference ranges are specific to the laboratory that produces the test results. For many analytes, different laboratories use different kinds of equipment and different kinds of testing methods. This means that each laboratory must establish its own reference ranges using data from its own equipment and methods. The laboratory must supply the test result with accompanying reference ranges on the laboratory report *(Sacher et al., 2000)*.

The sources of biological reference ranges and medical decision points must be documented and should include references to the information used in deciding the ranges any statistical processes used, literature studies considered and the personnel

involved in deciding the ranges *(Jones and Barker, 2008)*.

The reliability of a reference ranges study should be a function of its accuracy and reproducibility and have a direct relationship with the number of observations used and method of standardization *(Katayev et al., 2010)*.

Establishment of reference ranges for coagulation molecules for each laboratory is important *(Jang et al., 2010)* as prothrombin time, partial thromboplastin time, factors VIII, IX, and XI, Ristocetin cofactor activity and von Willebrand factor antigen (vWF:Ag) *(Flanders et al., 2005)*.

Factors affecting reference range:

Many factors must be considered in the determination of normal values or reference range. One extremely important factor is the choice of which population to study. Population should be selected who reflect the overall healthy persons. Possible approaches that can be used include studying a random sample from a normal population such as volunteer blood donors, medical students or medical technologists *(Boyd, 2010)*.

When performing a reference range studies the subjects being tested (the reference population) should be as similar as possible to that for which the test will be applied *(Ichihara et al., 2008)*.

For many tests, there is no single reference range that applies to everyone because the tests performed may be affected by the age, sex, diet, circadian rhythm, race, posture, medications, physical activity, socioeconomic status, medical history and fasting status. Few select reference ranges listed are specific for adults only and there are no ranges included for children or adolescents. The reason is that from infancy through adolescence, a child's body goes through many changes and growing cycles *(Flanders et al., 2005)*.

The laboratory where child's sample is tested has established reference ranges for the different stages of child development. Many other factors can affect reference range such as obesity, diabetes, coronary artery disease, thyroid gland problem and hereditary disorder as family history of bleeding disorders and race also affect the reference range *(Jones and Barker, 2008)*.

It has been recommended that a reference range can be established by selecting a statistically sufficient group (a minimum of 120) of healthy reference subjects. This put limitations on the ability of laboratories to set reference ranges as many laboratories are unable to collect the minimum number of samples. A new protocol allows a laboratory to validate a reference range with a smaller number of samples as only 20 normal individuals can be used to establish reference range *(Horn et al., 2009)*.

When evaluating 20 samples, if no more than two results are outside the proposed reference range, it is statistically valid for the laboratory to adopt the proposed reference range as its own. If three or four results lie outside the reference range, another 20 samples must be collected *(Horowitz et al., 2008)*.

If a laboratory was able to obtain a selected statistically sufficient number of healthy subjects and perform all the necessary testing, the next step would require statistical analysis of data *(Katayev et al., 2010)*.

Calculation of the reference range:

Two methods have commonly been used for calculation of the reference range from study data:

A. With the parametric approach where the central 95% boundaries are specified by the mean ± 2SD, the data follow a Gaussian (normal) distribution or can be transformed to a normal distribution by one of several two-step transformation methods.

B. With the non-parametric approach, where the central 95% boundaries is determined by trimming off the lowest and highest 2.5% of observations. The latter method is used for skewed and other non-normal data distributions.

(Boyd, 2010)

The test result may fall outside of the established reference range despite of the fact that individuals are in a good health due to few reasons such as statistical variability as when performing the same test on the same sample multiple times, 1 out of 20 (or 5%) determinations will fall outside an established range, based on the laws of probability. Sometimes, if the test is repeated on this same

sample, the result will then be within range. Also biological variability affects the reference ranges so that one result may fall outside a reference range even though the person is in good health *(Sacher et al., 2000)*.

ENDOTHELIAL MARKERS

Endothelial cell Physiology:

The inner lining (intima) of all blood vessels consists of a monolayer of flattened, orthogonal cells referred to as the endothelium, positioned on the internal elastic lamina. The vascular endothelium is not just a cell lining, but plays an active role via various mediators in the equilibrium of haemostasis and fibrinolysis *(Pusztaszeri et al., 2005)*.

The endothelium has many vital and diverse physiological roles, such as regulation of blood vessel tone, permeability and metabolism. Impairment of endothelial function manifests clinically as oedema, hypertension, abnormal vasoconstriction and hypercoagulability. Impaired endothelial function is also the initial step in atherogenesis, which is largely responsible for ischaemic heart disease and thrombotic strokes decades later, hypertension and diabetes mellitus *(Chong et al., 2003)*.

Increased numbers of circulating endothelial cells (CECs) in the peripheral blood are present in various pathological condition including inflammatory

disease, acute myocardial infarction, chronic stable heart failure, unstable angina and critical limb ischaemia *(Chong et al., 2006)*.

Since CECs are rare in the blood of healthy persons their levels imply the most severe form of blood vessel injury. Desquamated and detached CECs imply that areas of the endothelium are denuded, thus exposing the underlying prothrombotic sub-endothelial layer to the circulating blood. As a consequence, this may activate platelets and the coagulation cascade and be partly responsible for the prothrombotic or hypercoagulable state in these conditions and may contribute to the high morbidity and mortality *(Gibbes et al., 2001)*.

Plasma markers associated with endothelial damage:

1. von Willebrand Factor (vWF):

vWF is a glycoprotein that mediates platelet adhesion to subendothelium at sites of vascular injury and binds and stabilizes factor VIII (FVIII) in the circulation. It appears to be expressed exclusively on endothelial cell where it shows a granular pattern of reactivity and also expressed in the cytoplasm of

megakaryocytes. It is stored in Weibel Palade bodies *(Bowen, 2010)*.

The release of vWF from storage pools in the vascular endothelium is stimulated by the administration of adrenaline, vasopressin and nicotinic acid as well as interleukin-1. vWF is also released from storage granules by thrombin, fibrin and histamine. vWF levels are also influenced by non pathological conditions, for example, vWF increases with exercise, pregnancy and with drugs such as cyclosporin *(Chong et al., 2003)*.

The measurement of plasma vWF is an index of endothelial damage in vascular disease. Raised levels of plasma soluble vWF:Ag is an index of endothelial cell activation or dysfunction. vWF is also known to be an acute phase reactant affected by inflammatory cytokines and may be elevated even in the absence of endothelial damage *(Horvath et al., 2004)*.

High levels of vWF are a prognostic indicator for myocardial infarction and re-infarction. vWF is also a prognostic indicator of other cardiovascular events such as stroke and the requirement for arterial surgery in patients with hypertension, intermittent claudication, angina and ischaemic heart disease. In

addition, high vWF predicts the development of thromboembolic events, acute respiratory failure, acute and chronic renal insufficiency, diabetic nephropathy, vasculitis and prognosis in patients with rheumatoid arthritis and systemic sclerosis *(Chong et al., 2003)*.

2. Soluble thrombomodulin (sTM):

Thrombomodulin is a transmembrane proteo-glycan with a molecular mass of 75kDa, located on the vascular and lymphatic endothelium surfaces and function as an anticoagulant. It has a high affinity for thrombin forming a 1:1 thrombin-thrombomodulin complex that inhibits fibrin formation, platelet activation and protein S inactivation by thrombin. The complex also activates protein C which will inactivate factors Va and VIIIa of the intrinsic pathway *(Chong et al., 2003)*.

sTM is released from endothelial cells following cell membrane injury. Levels of sTM are elevated in some condition as diabetes mellitus and atheromatous arterial disease. High levels of sTM lead to increase vascular complications and hypertension *(Gedikli et al., 2010)*.