Determination of Reference Values for Activated Partial Thromboplastin Time in Healthy Sudanese Adults in Gezira State, Sudan

Mohammed Elnour Yagoub Babiker

(B.Sc. "honor degree" in Medical Laboratories Sciences
University of Shendi 2004)

A Dissertation
Submitted to University of Gezira in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in
Haematology and Immunohaematology

Department of Haematology
Faculty of Medical Laboratories Sciences

University of Gezira

May 2015
Determination of Reference Values for Activated Partial Thromboplastin Time in Healthy Sudanese Adults in Gezira state, Sudan

Mohammed Elnour Yagoub Babiker

Supervision committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Bakri Yousif Mohammed Nour</td>
<td>Main supervisor</td>
<td>.............</td>
</tr>
<tr>
<td>Dr. Albadawi Abdelbagi Talha</td>
<td>Co supervisor</td>
<td>.............</td>
</tr>
<tr>
<td>Mr. Muawia Mohammed Ahmed</td>
<td>Co supervisor</td>
<td>.............</td>
</tr>
</tbody>
</table>

Date: ....../....../ 2015
Determination of Reference Values for Activated Partial Thromboplastin Time in Healthy Sudanese Adults in Gezira state, Sudan

Mohammed Elnour Yagoub Babiker

Examination committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Bakri Yousif Mohammed Nour</td>
<td>Chairman</td>
<td>....::::::</td>
</tr>
<tr>
<td>Dr. Osama Abdalla Elsharif</td>
<td>External Examiner</td>
<td>....::::::</td>
</tr>
<tr>
<td>Dr. Fath Elrahman Mohammed Erabi</td>
<td>Internal Examiner</td>
<td>....::::::</td>
</tr>
</tbody>
</table>

Date: …/…… / 2015
DEDICATION

To those who have always given me their great love, care and kindness and those who have always given me without limits...

To my father and my mother

To those whom I love more... and more....

My brothers...

My sisters....

My friends....

To that who gives me love and hope...

My lovely wife

To those whom do all their efforts to help me...

My teachers...

To all students in University of Gezira...

I dedicate this work.

Mohammed
ACKNOWLEDGMENT

In the beginning my thanks to Allah who gives me the strength and patience to accomplish this work.

Great gratefulness and sincerity should be offered to Dr. Bakri Yousif Mohammed Nour, my supervisor for his advice and guidance.

Highly thankful to Dr. Albadawi Abdelbagi Talha, my co-supervisor, for his care, guidance and support.

I am most grateful to Mr. Muawia Mohammed Ahmed, my co-supervisor, for his kind cooperation.

My sincere gratitude to the Postgraduate Department in University of Gezira, specially, Mr. Badr-Eldeen Alfadil, Mr. Ismail Abd Alrhman and Mrs. Sanaa Elfatih, for their kind cooperation.

My best regards and thanks to my colleagues, specially, the beloved Normal Range Group.

Finally I would like also to express my appreciation and gratitude to all persons who have volunteered to participate in this study, I extend thanks to everyone who helped in this work did not seek the place to mention.
Determination of Reference Values for Activated Partial Thromboplastin Time in Healthy Sudanese Adult in Gezira State, Sudan

Mohammed Elnour Yagoub Babiker

MSc. Haematology and immunohaematology. May, 2015
Faculty of Medical Laboratory Sciences
University of Gezira

Abstract

The haematological reference values are very important for the diagnostic orientation and treatment decision. Thereference values are influenced by many environmental and genetic factors. The use of Western reference value for interpretation of laboratory results in Gezira State may be differ and misdiagnosis may occur. This was prospective analytical cross sectional study carried out in Gezira State from September 2014 to March 2015. The aim of the study was to establish the reference values for activated partial thromboplastin time (APTT) in healthy Sudanese adults living in Gezira State. A total of 657 (281 males and 376 females) healthy volunteers their ages ranged between 18 – 60 years, were randomly selected according to inclusion and exclusion criteria. 2 ml of blood samples were collected into tri-sodium citrate containers, platelets poor plasma were obtained by centrifugation, then after running of commercial controls, all samples were estimated for APTT using Coatron M4 coagulometer device according to manufacturer's instructions. The data was analyzed using Statistical Package for Social Sciences (SPSS) computer program (version 16.0). The results obtained in this study revealed that the reference values for APTT among healthy Sudanese adults living in Gezira State were similar to standard reference values, but the upper limit of APTT in this study was significantly higher (26.3 – 42.7 seconds). Significant difference was enrolled when comparing the values obtained in this study with other populations. In this study there were significant difference in APTT values between study population according to their age, locality, occupation and female’s marital status, while there were no significant differences according to gender, tribe, education and number of birth. The important recommendations in this study were to use the reference values obtained in this study in medical practice in Gezira State instead of Western values quoted from textbooks and to establish reference values for all laboratory tests in Sudan.
تحديد القيم المرجعية لزمن الثروموبلاستين المنشط الجزئي عند السودانيين البالغين الأصحاء

بولاية الجزيرة – السودان

محمد النور يعقوب بابكر

ماجستير في أمراض الدم والمناعية . مايو 2015

كلية علوم المختبرات الطبية
جامعة الجزيرة

ملخص الدراسة

تعتبر الاختبارات العملية مهمة جداً في توجيه التشخيص واتخاذ قرار العلاج. استخدام القيم المرجعية الغريبة في تفسير نتائج الاختبارات العملية في ولاية الجزيرة، قد يكون مختلفاً وقد يؤدي إلى التشخيص الخاطئ. هذه دراسة مستقلة تحليلية مقطعية أجريت في ولاية الجزيرة في الفترة ما بين سبتمبر 2014 إلى مارس 2015. هدفت هذه الدراسة إلى تحديد القيم المرجعية لزمن الثروموبلاستين المنشط الجزئي عند السودانيين البالغين الأصحاء الذين يعيشون في ولاية الجزيرة. تم اختيار عدد 657 شخص تتراوح أعمارهم بين 18 – 60 سنة (281 ذكر و 376 أنثى) عشوائياً وفق معايير الدخول والخروج من الدراسة. تم جمع 2 مل من الدم في حاويات تحتوي ثلاثي سترات الصوديوم كمضاد تخثر. تم تحضير البلازما فورة الصفائح الدموية عن طريق عملية الطرد المركزي. بعد إجراء قياسات بواسطة محلول التحكم التجاري تم إجراء اختبار زمن الثروموبلاستين المنشط الجزئي لكل عينة بواسطة جهاز قياس التجلط حسب إرشادات الجهة المصنعة للجهاز. تم تحليل النتائج بواسطة استخدام برنامج الحزم الإحصائية للعلوم الاجتماعية.

أظهرت النتائج المستخلصة في هذه الدراسة أن القيم المرجعية لزمن الثروموبلاستين المنشط الجزئي في السودانيين البالغين الأصحاء الذين يعيشون في ولاية الجزيرة بسلاج القيم المرجعية المعتمدة. فقط الحد الأعلى لزمن الثروموبلاستين المنشط الجزئي في هذه الدراسة يعتبر أعلى (26.3 – 42.7 ثانية). سجلت فرق معنوية عند مقارنة القيم المتصلا عليها في هذه الدراسات مع المجتمعات الأخرى. في هذه الدراسة وجد أن هناك فرق معنوية في زمن الثروموبلاستين المنشط الجزئي بين مجموع الدراسة حسب العمر، مكان الإقامة، الوضعية والحالة الزوجية للأنثى، بينما لم يوجد فرق معنوية على حسب النوع، القبلة. التعليم وعدد الولادات. أوصت الدراسة باستخدام القيم المرجعية المتصلا عليها في هذه الدراسة كقيم مرجعية في العمل الطبي بولاية الجزيرة بالرغم القيم المرجعية الغريبة المستخلصة من الدراسات. كما أوصت الدراسة أيضاً بضرورة العمل على تحديد القيم المرجعية لكل الاختبارات العملية في السودان.
# Table of contents

<table>
<thead>
<tr>
<th>Number</th>
<th>Name of content</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Covering page</td>
<td>I</td>
</tr>
<tr>
<td>II</td>
<td>Supervision committee</td>
<td>ii</td>
</tr>
<tr>
<td>III</td>
<td>Examination committee</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Dedication</td>
<td>iii</td>
</tr>
<tr>
<td>V</td>
<td>Acknowledgement</td>
<td>iv</td>
</tr>
<tr>
<td>VI</td>
<td>Abstract in English</td>
<td>v</td>
</tr>
<tr>
<td>VII</td>
<td>Abstract in Arabic</td>
<td>vi</td>
</tr>
<tr>
<td>VIII</td>
<td>Table of contents</td>
<td>vii</td>
</tr>
<tr>
<td>IX</td>
<td>List of tables</td>
<td>x</td>
</tr>
<tr>
<td>X</td>
<td>List of figures</td>
<td>xi</td>
</tr>
<tr>
<td>XI</td>
<td>List of abbreviations</td>
<td>xii</td>
</tr>
</tbody>
</table>

## Chapter One

### Introduction

1.1 General introduction 1
1.2 Rationale 2
1.3 Objectives 3
1.3.1 General objective 3
1.3.2 Specific objectives 3

## Chapter Two

### Literature Review

2.1 Reference values 4
2.1.1 The concept of reference values 4
2.1.2 Definition of terms used in reference values study 5
2.1.3 Types of reference values 6
2.1.4 Pre-analytical variables affect coagulation testing 7
2.1.4.1 Appropriate sample collection, processing and storage 7
2.1.4.2 Normal reference range derivation 10
2.1.4.3 Miscellaneous variables 10
2.1.4.4 Physical activity, illness and stress 11
2.1.4.5 Circadian and diurnal rhythms 11
2.1.4.6 Anti-coagulant therapy 11
2.1.5 Strategies for selection of reference individuals 11
2.1.6 Steps involved during establishment of reference values 12
2.1.7 Preparation of individuals and collection of specimens 13
2.1.8 Important of analytical procedures and quality control 13
2.1.9 Determination of reference limits 13
<table>
<thead>
<tr>
<th>Section</th>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.10</td>
<td>Presentation of an observed value in relation to reference values</td>
<td>14</td>
</tr>
<tr>
<td>2.1.11</td>
<td>Reference values for activated thromboplastin time</td>
<td>14</td>
</tr>
<tr>
<td>2.2</td>
<td>Haemostasis</td>
<td>17</td>
</tr>
<tr>
<td>2.2.1</td>
<td>General overview</td>
<td>17</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Components of haemostasis</td>
<td>17</td>
</tr>
<tr>
<td>2.2.2.1</td>
<td>Blood vessels</td>
<td>19</td>
</tr>
<tr>
<td>2.2.2.1.1</td>
<td>The endothelium</td>
<td>20</td>
</tr>
<tr>
<td>2.2.2.2</td>
<td>Platelets</td>
<td>22</td>
</tr>
<tr>
<td>2.2.2.2.1</td>
<td>Platelet structure</td>
<td>22</td>
</tr>
<tr>
<td>2.2.2.2.2</td>
<td>Platelet functions</td>
<td>23</td>
</tr>
<tr>
<td>2.2.2.2.2.1</td>
<td>Platelet pro coagulant activity</td>
<td>24</td>
</tr>
<tr>
<td>2.2.2.2.2.2</td>
<td>Stimulatory agonist of platelet</td>
<td>24</td>
</tr>
<tr>
<td>2.2.2.2.2.3</td>
<td>Inhibitory agonist of platelet</td>
<td>25</td>
</tr>
<tr>
<td>2.2.2.3</td>
<td>Coagulation factors</td>
<td>25</td>
</tr>
<tr>
<td>2.2.2.4</td>
<td>Natural occurring inhibitors of blood coagulation (natural anti-coagulants)</td>
<td>28</td>
</tr>
<tr>
<td>2.2.2.5</td>
<td>Fibrinolysis</td>
<td>29</td>
</tr>
<tr>
<td>2.2.2.5.1</td>
<td>Activation of plasminogen</td>
<td>30</td>
</tr>
<tr>
<td>2.2.2.5.2</td>
<td>Inhibitors of fibrinolysis</td>
<td>31</td>
</tr>
<tr>
<td>2.2.3</td>
<td>Screening tests for haemostasis</td>
<td>31</td>
</tr>
<tr>
<td>2.2.3.1</td>
<td>Prothrombin time (PT)</td>
<td>31</td>
</tr>
<tr>
<td>2.2.3.3</td>
<td>Activated partial thromboplastin time (APTT)</td>
<td>32</td>
</tr>
</tbody>
</table>

**Chapter Three**

**Materials and Methods**

<table>
<thead>
<tr>
<th>Section</th>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Methodology</td>
<td>34</td>
</tr>
<tr>
<td>3.1.1</td>
<td>Study design</td>
<td>34</td>
</tr>
<tr>
<td>3.1.2</td>
<td>Study area and duration</td>
<td>34</td>
</tr>
<tr>
<td>3.1.3</td>
<td>Study population</td>
<td>34</td>
</tr>
<tr>
<td>3.1.4</td>
<td>Sampling and sample size</td>
<td>34</td>
</tr>
<tr>
<td>3.1.5</td>
<td>Study criteria</td>
<td>36</td>
</tr>
<tr>
<td>3.1.5.1</td>
<td>Inclusion criteria</td>
<td>36</td>
</tr>
<tr>
<td>3.1.5.2</td>
<td>Exclusion criteria</td>
<td>37</td>
</tr>
<tr>
<td>3.1.6</td>
<td>Data collection tools</td>
<td>37</td>
</tr>
<tr>
<td>3.1.7</td>
<td>Data analysis</td>
<td>37</td>
</tr>
<tr>
<td>3.1.8</td>
<td>Ethical consideration</td>
<td>37</td>
</tr>
<tr>
<td>3.2</td>
<td>Materials</td>
<td>38</td>
</tr>
<tr>
<td>3.3</td>
<td>Methods</td>
<td>38</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Sample collection and preparation</td>
<td>38</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Laboratory analysis</td>
<td>39</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Activated partial thromboplastin time (APTT) test</td>
<td>39</td>
</tr>
<tr>
<td>3.3.3.1</td>
<td>Principle of the test</td>
<td>39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Section</th>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2.2.2.2.2.2</td>
<td>Stimulatory agonist of platelet</td>
<td>24</td>
</tr>
<tr>
<td>2.2.2.2.2.2.3</td>
<td>Inhibitory agonist of platelet</td>
<td>25</td>
</tr>
</tbody>
</table>
## Chapter Four
### Results and Discussion

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Results</td>
<td>41</td>
</tr>
<tr>
<td>4.1.1</td>
<td>Characterization of the study population</td>
<td>41</td>
</tr>
<tr>
<td>4.1.2</td>
<td>The study of result according to standard reference values</td>
<td>45</td>
</tr>
<tr>
<td>4.1.3</td>
<td>Our study reference values</td>
<td>45</td>
</tr>
<tr>
<td>4.1.4</td>
<td>Effects of the different population factors on activated partial thromboplastin time</td>
<td>46</td>
</tr>
<tr>
<td>4.2</td>
<td>Discussion</td>
<td>50</td>
</tr>
</tbody>
</table>

## Chapter Five
### Conclusion and Recommendations

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Conclusion</td>
<td>53</td>
</tr>
<tr>
<td>5.2</td>
<td>Recommendations</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Appendices</td>
<td>57</td>
</tr>
</tbody>
</table>
# List of tables

<table>
<thead>
<tr>
<th>Number</th>
<th>Table’s name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table (2-1)</td>
<td>Properties of coagulation factors</td>
<td>26</td>
</tr>
<tr>
<td>Table (3-1)</td>
<td>Distribution of sample size in localities</td>
<td>36</td>
</tr>
<tr>
<td>Table (4-1)</td>
<td>Activated partial thromboplastin time results according to standard reference values</td>
<td>45</td>
</tr>
<tr>
<td>Table (4-2)</td>
<td>Reference intervals for activated partial thromboplastin time among the study population</td>
<td>45</td>
</tr>
</tbody>
</table>
# List of Figures

<table>
<thead>
<tr>
<th>Number</th>
<th>Figure’s name</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure (2-1)</td>
<td>The haemostatic mechanism</td>
<td>18</td>
</tr>
<tr>
<td>Figure (2-2)</td>
<td>The anatomy of blood vessel</td>
<td>20</td>
</tr>
<tr>
<td>Figure (2-3)</td>
<td>Platelet ultra-structure</td>
<td>23</td>
</tr>
<tr>
<td>Figure (2-4)</td>
<td>The coagulation cascade reaction</td>
<td>28</td>
</tr>
<tr>
<td>Figure (2-5)</td>
<td>Reactions of PT and APTT</td>
<td>33</td>
</tr>
<tr>
<td>Figure (4-1)</td>
<td>Distribution of the study population according to the gender</td>
<td>41</td>
</tr>
<tr>
<td>Figure (4-2)</td>
<td>Distribution of the study population according to the age groups</td>
<td>41</td>
</tr>
<tr>
<td>Figure (4-3)</td>
<td>Distribution of the study population according to the localities</td>
<td>42</td>
</tr>
<tr>
<td>Figure (4-4)</td>
<td>Distribution of samples according to the occupation</td>
<td>42</td>
</tr>
<tr>
<td>Figure (4-5)</td>
<td>Distribution of the study population according to the tribe</td>
<td>43</td>
</tr>
<tr>
<td>Figure (4-6)</td>
<td>Distribution of the study population according to the educational level</td>
<td>43</td>
</tr>
<tr>
<td>Figure (4-7)</td>
<td>Distribution of the study population according to the female’s marital status</td>
<td>44</td>
</tr>
<tr>
<td>Figure (4-8)</td>
<td>Distribution of the study population according to the number of birth</td>
<td>44</td>
</tr>
<tr>
<td>Figure (4-9)</td>
<td>Comparison means of APTT between males and females</td>
<td>46</td>
</tr>
<tr>
<td>Figure (4-10)</td>
<td>Comparison means of APTT between different localities</td>
<td>46</td>
</tr>
<tr>
<td>Figure (4-11)</td>
<td>Comparison means of APTT between different age groups</td>
<td>47</td>
</tr>
<tr>
<td>Figure (4-12)</td>
<td>Comparison means of APTT between different occupations</td>
<td>47</td>
</tr>
<tr>
<td>Figure (4-13)</td>
<td>Comparison means of APTT between different educational levels</td>
<td>48</td>
</tr>
<tr>
<td>Figure (4-14)</td>
<td>Comparison means of APTT between tribes</td>
<td>48</td>
</tr>
<tr>
<td>Figure (4-15)</td>
<td>Comparison means of APTT between single and married females</td>
<td>49</td>
</tr>
<tr>
<td>Figure (4-16)</td>
<td>Comparison means of APTT between females according to number of birth</td>
<td>49</td>
</tr>
<tr>
<td>Figure (4-17)</td>
<td>Histogram with normal curve for APTT in all population</td>
<td>59</td>
</tr>
<tr>
<td>Figure (4-18)</td>
<td>Histogram with normal curve for APTT in males</td>
<td>59</td>
</tr>
<tr>
<td>Figure (4-19)</td>
<td>Histogram with normal curve for APTT in females.</td>
<td>60</td>
</tr>
</tbody>
</table>
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Complete name</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>Adenosine Di Phosphate</td>
</tr>
<tr>
<td>APC</td>
<td>Activated Protein C</td>
</tr>
<tr>
<td>APTT</td>
<td>Activated Partial Thromboplastin Time</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Tri Phosphate</td>
</tr>
<tr>
<td>ATIII</td>
<td>Anti Thrombin Three</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic Adenosine Mono Phosphate</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic Guanosine Mono Phosphate</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical Laboratory and Standards Institute</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated Intravascular Coagulation</td>
</tr>
<tr>
<td>ECs</td>
<td>Endothelium Cells</td>
</tr>
<tr>
<td>EDRF</td>
<td>Endothelium Derived Relaxing Factor</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetra Acetic acid</td>
</tr>
<tr>
<td>FDPs</td>
<td>Fibrin Degradation Products</td>
</tr>
<tr>
<td>FII</td>
<td>Factor Two</td>
</tr>
<tr>
<td>FV</td>
<td>Factor Five</td>
</tr>
<tr>
<td>FVII</td>
<td>Factor Seven</td>
</tr>
<tr>
<td>FVIIa</td>
<td>Activated Factor Seven</td>
</tr>
<tr>
<td>FVIII</td>
<td>Factor Eight</td>
</tr>
<tr>
<td>FIX</td>
<td>Factor Nine</td>
</tr>
<tr>
<td>FX</td>
<td>Factor Ten</td>
</tr>
<tr>
<td>FXI</td>
<td>Factor Eleven</td>
</tr>
<tr>
<td>FXII</td>
<td>Factor Twelve</td>
</tr>
<tr>
<td>FXIIa</td>
<td>Activated Factor Twelve</td>
</tr>
<tr>
<td>GP</td>
<td>Glyco Protein</td>
</tr>
<tr>
<td>HbAA</td>
<td>Haemoglobin A(Adult Haemoglobin)</td>
</tr>
<tr>
<td>HbSC</td>
<td>Haemoglobin S and C</td>
</tr>
<tr>
<td>HbSS</td>
<td>Haemoglobin S (Sickle Haemoglobin)</td>
</tr>
<tr>
<td>HClII</td>
<td>Heparin Cofactor Two</td>
</tr>
<tr>
<td>HMWK</td>
<td>High Molecular Weight Kininogen</td>
</tr>
<tr>
<td>IFCC</td>
<td>International Federation of Clinical Chemistry</td>
</tr>
<tr>
<td>IL1</td>
<td>Inter Leukin one</td>
</tr>
<tr>
<td>IL2</td>
<td>Inter Leukin Two</td>
</tr>
<tr>
<td>INR</td>
<td>International Normalized Ratio</td>
</tr>
<tr>
<td>ISI</td>
<td>International Sensitivity Index</td>
</tr>
<tr>
<td>LA</td>
<td>Lupus Anti coagulants</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>NRRs</td>
<td>Normal Reference Ranges</td>
</tr>
<tr>
<td>OCS</td>
<td>Open Canalicular System</td>
</tr>
<tr>
<td>PAI - 1</td>
<td>Plasminogen activator Inhibitor – One</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PFA-100</td>
<td>Platelet Function Assay – Hundred</td>
</tr>
<tr>
<td>PG12</td>
<td>ProstaGlandin Twelve</td>
</tr>
<tr>
<td>PS</td>
<td>Protein S</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin Time</td>
</tr>
<tr>
<td>RBCs</td>
<td>Red Blood Cells</td>
</tr>
<tr>
<td>RICK</td>
<td>Radiation and Isotopes Center Khartoum</td>
</tr>
<tr>
<td>RIs</td>
<td>Reference Intervals</td>
</tr>
<tr>
<td>SK</td>
<td>Streptokinase</td>
</tr>
<tr>
<td>TF</td>
<td>Tissue Factor</td>
</tr>
<tr>
<td>TM</td>
<td>ThromboModulin</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>tPA</td>
<td>Tissue Plasminogen activator</td>
</tr>
<tr>
<td>U-PA</td>
<td>Urinary Plasminogen activator</td>
</tr>
<tr>
<td>vWD</td>
<td>Von Willebrand Disease</td>
</tr>
<tr>
<td>vWF</td>
<td>Von Willebrand Factor</td>
</tr>
<tr>
<td>α 2-MG</td>
<td>Alpha two Macro Globulin</td>
</tr>
</tbody>
</table>
Chapter One

1. Introduction

1.1 General introduction:

The use of the word ‘health’ to describe human well-being is relatively recent. The word ‘health’ was derived from the old English word ‘hoelth’, which meant a state of being sound, and was generally used to infer a soundness of the body (Dolfman, 1973).

The definition of health of a reference population, or reference individuals, entails several problems. No definition of health appears to be completely satisfactory, including the definition in the constitution of the World Health Organization: “a state of complete physical, mental and social well-being and not merely the absence of disease and infirmity”. Health is conceptually different in different countries, in the same country at different times and in same individuals at different ages. Thus it is a relative and not an absolute state (Grasbeck, et al 1978).

The condition of individuals must be related to or compared with reference data. On comparing the individual’s data collected during the medical interview, clinical examination, and supplementary investigations with the reference data, the condition of individuals can be interpreted. A patient’s laboratory result simply is not medically useful if appropriate data for comparison are lacking. It is thus the central role of the laboratory scientist to aid the clinician in interpreting observed values by providing relevant reference values and presenting them in a convenient and practical form (Ashavaid, et al 2005).

Reference values are used to describe the dispersion of variables in healthy individuals. They are usually reported as population-based reference intervals (RIs) comprising 95% of the healthy population. The most critical steps in the determination of reference values are the selection of reference individuals based on extensively documented inclusion and exclusion criteria and the use of quality-controlled analytical procedures (Greffe, et al 2009).

A number of factors affect haematological values in apparently healthy individuals, these include the technique and timing of blood collection, transport and storage of specimens, differences in the subject’s posture when the sample is taken, prior physical activity and whether the subject is confined to bed. Variation in the analytic methods used may also affect the measurements. These
can all be standardized. More problematic are the inherent variables as a result of sex, age, occupation, body build, genetic background and adaptation to diet and to environment (especially altitude). These factors must be recognized when establishing physiologically normal values. Furthermore, it is difficult to be certain in any survey of a population for the purposes of obtaining data from which normal ranges may be constructed, that the normal subjects are completely healthy and do not have nutritional deficiencies, mild chronic infections, parasitic infestations or the effects of smoking. Haematological values for the normal and abnormal will overlap and a value within the recognized normal range may be definitely pathological in a particular subject. For these reasons the concept of normal values and normal ranges has been replaced by reference values and the reference range, which is defined by reference limits and obtained from measurements on the reference population for a particular test. The reference range is also termed the reference interval. Ideally, each laboratory should establish a data bank of reference values that take account of the variables mentioned earlier and the test method, so that an individual’s result can be expressed and interpreted relative to a comparable apparently normal population, so far as normal can be defined (Dacie and Lewis, 2011).

Haemostasis is one of a number of protective processes that have evolved in order to maintain a stable physiology (Hoffbrand, et al 2005).

The normal haemostatic response to vascular damage depends on closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors. An efficient and rapid mechanism for stopping bleeding from sites of blood vessel injury is clearly essential for survival. Nevertheless, such a response needs to be tightly controlled to prevent extensive clots developing and to break down such clots once damage is repaired. The haemostatic system thus represents a delicate balance between procoagulant and anticoagulant mechanisms allied to a process for fibrinolysis. The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels (Hoffbrand, et al 2005).

1.2 Rationale:

The major goal of the laboratory investigations is to help the clinicians in the diagnosis orientation and treatment decision, and this goal is very difficult to obtain in the absence of reference values. Because of the lack of reference values for Activated Partial Thromboplastin Time (APTT) in healthy subjects in Gezira State, the investigators interpret their data using reference values obtained from the literature, reagent inserts accompanying the reagent kits or
instrument manuals which derived from populations in Europe and the United States. Factors such as age, gender, ethnicity and environment including altitude and geo-chemicals affect the measurements determined in different populations (Dosoo, et al 2012). Published literature has confirmed that many of the reference values obtained from the developed countries differ significantly from what pertains in most African localities, I have not come across any literature on the Gezira State’s APTT reference values, even an internet search did not yield any finding, thus making it is necessary to establish locally appropriate reference values for Activated Partial Thromboplastin Time (APTT) in our own populations in Gezira State.

1.3 Objectives:

1.3.1 General objective:

- To establish reference values for activated partial thromboplastin time among healthy Sudanese adults living the Gezira State.

1.3.2 Specific objectives:

- To establish reference values for activated partial thromboplastin time (APTT) in healthy Sudanese individuals within the age 18 years and above living in the Gezira State.
- To find out the effect of sex, age, race, education, occupation, female’s marital status, on the activated partial thromboplastin time (APTT).
- To compare these obtained reference values with those obtained in other parts of Sudan or other countries.
Chapter Two

2. Literature Review

2.1 Reference values:

2.1.1 The concept of reference values:

The concept of reference values was introduced in 1969 by Grasbeck and Saris to describe fluctuations of blood analyte concentrations in well-characterized groups of individuals. It was intended to replace the more ambiguous concept of normal values, and to establish a well-defined nomenclature and recommended procedures in the field. There was a clear distinction between healthy reference values measured in healthy populations or individuals and patient reference values measured in patients having various diseases. It is now commonly accepted that reference values describe fluctuations observed in healthy populations or individuals, which makes the definition of health or characterization of health status a critical step (Greffe, et al 2009). Reference values, first introduced as a philosophy, have gained universal acceptance as one of the most powerful tools in laboratory medicine to aid in the clinical decision-making process. However, the recommendations for establishing reference intervals (RIs) described in the original series of articles published by the International Federation of Clinical Chemistry (IFCC) and Laboratory Medicine were sometimes considered too complicated to be applicable in practice; and thus, they have been used erroneously, if used at all. These difficulties have led to a necessary revision of the original recommendations and the publication of common IFCC and Clinical Laboratory and Standards Institute (CLSI) guidelines (C28-A3) in 2008 (Greffe, et al 2009).

Laboratory-based investigations are subject to substantial variability arising from several sources, including differences between subjects (e.g., age, sex, and genetic variation), within-subject variation (e.g., circadian change and pathological change), and variations in sample collection and handling, and laboratory measurement error. In interpreting an individual patient’s laboratory test results, the clinician usually compares the reported values with reference values. Inappropriate reference values may increase the risk of either unnecessary additional investigations or failure to detect underlying disease. In clinical practice, reference values are often printed by the testing laboratory on the same document as the results, although their origins are rarely specified (Tsang, et al 1998).
Reference values for a given patient are usually defined in terms of the spread of results typically encountered from similar subjects who are known to be in good health. Ideally, the ranges are derived by using statistical criteria from a random sample of comparable individuals. To ensure that the values represent those encountered in health, the results from individuals in the sample who are found to have acute or chronic disease are usually excluded. Published reference values have been derived from a variety of samples, including those from healthy volunteers, subjects attending health screening or a routine medical examination, first-time blood donors, pre-employment testing, and subjects in retrospective studies. The extent to which each of these different sampling strategies produces reference values appropriate to any given patient is not always clear (Tsang, et al 1998).

The “reference values” also referred to as “normal reference values” or “normal reference ranges” are defined by Worth as “a set of values of certain type of quantity obtainable from a single individual or a group of individuals corresponding to a stated description (El-Hazmi, 1991).

2.1.2 Definition of terms used in reference values study:

a. Reference individual: is a person selected for testing on the basis of well-defined criteria. Reference individuals are generally assumed to be healthy; however, health is relative and lacks a precise and quantifiable definition. Therefore, reference individuals are selected using well-defined criteria, i.e. inclusion and exclusion criteria, which approximate health. Inclusion and exclusion criteria should be defined precisely, according to the aims of the study, and may differ from one study to another. The RI determined from the individuals selected according to the given criteria will be applicable only to similar individuals, i.e., only to individuals fulfilling the same criteria.

b. Reference population: is a group consisting of all possible reference individuals.

c. Reference sample group: is an adequate number of persons selected to represent the reference population. Although meant to be representative, the characteristics of a reference sample group are not identical to the characteristics of the reference population for the following reasons. First, the reference population is hypothetical because the number of individuals it comprises is unknown. Second, the reference sample group rarely is selected in a completely random manner.
d. **Reference value:** is the value, or test result, obtained by the observation or measurement of a particular type of quantity on a reference individual. A “particular type of quantity” (“measurand” in metrology and “component” or “analyte” in laboratory medicine) implies that most of the theory and application of reference values deals with univariate RIs, i.e., only one analyte at a time, whereas interpretation of results is mostly multivariate. This has led some authors to study multivariate reference regions, which at this time have only limited development. A reference value, which represents one value obtained in one reference individual, is not synonymous with a reference limit, which is a value derived from all results obtained in the reference sample group. The term reference value should not be used to denote a limit of the RI.

e. **Reference distribution:** is the distribution of reference values.

f. **Reference limits:** are the values derived from the reference distribution and are used for descriptive purposes. Reference limits should not be confused with decision limits.

g. **Reference interval:** is the interval between, and including, two reference limits. The RI comprises only a fraction of the values measured in reference individuals, most frequently the central 95% of the distribution located between the 0.025 and 0.975 fractiles as defined by ISO 15189 and IFCC.10,25 as a consequence, 5% of healthy individuals have observed values above or below these reference limits. In other words, it is perfectly normal to observe abnormal results in healthy individuals, it just is not frequent. The term “reference range,” often used as a synonym for RI, is not defined in C28-A3 and therefore should not be used interchangeably.

h. **Observed value:** or patient laboratory test result, is the value obtained in a test subject that is compared with reference values, reference distributions, reference limits, or RIs (Greffe, et al 2009).

2.1.3 **Types of reference values:**

a. **Subject based reference values:** are previous values from the same individual obtained when he / she were in defined state of health.

b. **Population - based reference values:** are those obtained from a group of well-defined reference individuals, and are usually the types of values referred to when the term reference values is used without any qualifying words (Dacie and Lewis, 2011).
2.1.4 Pre-analytical variables affect coagulation testing:

The use of modern laboratory instrumentation with high levels of test reliability and appropriate quality assurance measures will lead to very few analytical errors within haemostasis testing. Nevertheless, incorrect or inappropriate test results are still reported, often due to events outside the control of the laboratories performing the tests. This is due primarily to pre-analytical events associated with sample collection and processing, as well as post-analytical events related to the reporting and interpretation of test results, beside the typing errors.

2.1.4.1 Appropriate sample collection, processing, and storage:

- **Sample collection:**

  All tests have specific collection requirements. Most samples referred for coagulation testing must be drawn into citrate-based anticoagulant tubes (generally 105-109 mM or 129 mM sodium citrate, also referred to as 3.2% or 3.8%, respectively). The current Clinical and Laboratory Standards Institute (CLSI) guidelines favor the use of the lower citrate concentration, except for specific applications. Specimens collected in 129 mM (3.8%) buffered sodium citrate may overestimate the PT and APTT and underestimate fibrinogen if the normal range is based on 3.2% citrated samples. Conversely, samples collected into 129 mM (3.8%) citrate may provide a more stable sample for assessing antiplatelet (e.g., aspirin) therapy response using the PFA-100. The major recommendation therefore is that laboratories standardize to one citrate concentration and develop normal ranges appropriate for that concentration.

  Coagulation samples should preferably be collected before other test samples are drawn, if these contain stronger anticoagulant agents such as ethylene di amine tetra acetic acid (EDTA), for a complete blood count, lithium-heparin, for clinical chemistry testing, as well as clot activators, i.e., thrombin, since these materials may contaminate a subsequent coagulation test sample. A specific sequence of tube collections, so-called “order of draw”, is provided by the CLSI. Tubes should be adequately filled, to the mark noted on the tube if provided, or to no less than 90% of this total volume. Under-filling may cause significant sample dilution and may also provide falsely prolonged clotting times due to the excess calcium-binding citrate present. Sample dilution will also lead to under-estimation of quantitative test results, e.g., clotting factor levels.
Samples should be mixed thoroughly, but gently, by three to six end-over-end tube inversions to ensure adequate mixing of test sample with anticoagulant and to prevent sample clotting. Conversely, too vigorous mixing (e.g., by shaking of tubes) might lead to in vitro haemolysis or spurious factor activation resulting in false shortening of test clotting times and even possible false elevation of clotting factor activity (e.g., FVII) (Favaloro, et al 2012).

**Sample transport:**

Samples should be transported as per current guidelines, non-refrigerated at ambient temperature, 15-22°C, in as short a time as possible. Ideally, testing for routine coagulation tests like the PT and the APTT should be accomplished within 4 hours of collection, although allowable tolerances may be greater than this. However, APTT testing for unfractionated heparin monitoring should preferably be processed within 1 hour due to the potential for heparin neutralization by platelet releasates. Extremes of temperature, i.e., both refrigerated and high, should be avoided. Delays in transport may affect in particular the labile factors (FV, FVIII), leading to prolonged clotting times and in vitro loss of factor activity. In such cases, local centrifugation and separation of plasma followed by freezing and frozen transport of the plasma should be considered (Favaloro, et al 2012).

**Sample processing and storage:**

This should in general proceed as per current CLSI guidelines, noting limitations according to which test is being performed. Most coagulation-based tests, including PT, APTT, and clotting factor assays, are performed on plasma derived from once-centrifuged samples. Some samples, such as those for Lupus Anti – coagulant (LA) testing, should be double centrifuged to ensure platelet-free preparations prior to freezing. Centrifugation should essentially be at an ambient temperature (15°C-22°C). Centrifugation should ideally be at 1500 g for a minimum of 10-15 minutes. Shorter centrifuge times might be acceptable for routine coagulation tests performed immediately post-centrifugation when there are no subsequent test requirements, i.e., plasma not to be frozen or processed for additional assays. Using centrifugal forces greater than 1500 g are not recommended as this may induce platelet activation and lysis of RBCs.

The stability of coagulation samples varies depending on a number of variables such as the blood collection system, whether the samples are stored as whole blood or centrifuged, the temperature at which samples are maintained during storage, the reagent/instrument system used for analysis, and the test parameter to be analyzed. For example, whole blood stored up to 24-48 hours prior
to centrifugation has been reported as acceptable for many haemostasis tests, although not for FV, FVIII, and protein S, but other studies have reported significant changes in some test results over such time periods. Moreover, storage of refrigerated whole blood is now actively discouraged and leads to activation events affecting FVII, FVIII, Von willebrand factor (vWF), and possibly others.

In general, to afford the greatest sample integrity, samples should be processed as quickly as possible, ideally within 1 hour of collection, and testing performed within 4 hours of procurement, or else be processed by centrifugation and plasma frozen. During this short-term storage, whole blood samples should be kept capped and maintained at room temperature. If testing is not to be performed within about 4 hours for the APTT and 24 hours for the Prothrombin time (PT), the plasma should be separated from the cellular fraction of the once or twice-centrifuged sample, without disturbing the cell pellet. For many tests of haemostasis, the separated plasma can be safely frozen for later testing. Separated plasma can generally be maintained at room temperature or refrigerated for a few hours without an adverse effect on coagulation. As a general rule of thumb, testing for samples maintained at around -20°C should be finalized within 2-4 weeks of storage, whereas testing for samples maintained at around -80°C can occur several months and sometimes years later (useful for research studies and prospective trials) (Favaloro, et al 2012).

- **Controlled thawing of frozen plasma samples:**

  Previously frozen samples should be rapidly thawed in a 37°C water bath for 5-10 minutes or until completely thawed. Close monitoring during this time is necessary to avoid inadequate or excessive incubation at 37°C. Once samples are thawed, it is imperative they are thoroughly and adequately mixed prior to testing (Favaloro, et al 2012).

- **Haematocrit:**

  The presence of significant anaemia has not been shown to influence test results. Too high a haematocrit will influence the anticoagulant to plasma ratio and thus test results. An adjustment in the ratio of anticoagulant solution/volume of blood at different packed cell volume when haematocrit values are above 55% may be undertaken using CLSI recommendations, although a simplified method is to remove 0.1 mL of sodium citrate from a 5 mL 3.2% sodium citrate evacuated tube prior to collection (Favaloro, et al 2012).
**Lipemia:**

It is not easy to dichotomize the biological and analytical effect of lipemia on coagulation tests. Acute elevation of the coagulant activity of FVII is observed after consumption of high-fat meals, mostly due to an increase in the concentration of activated FVII (FVIIa). High-fat meals also have a substantial, acute effect on platelet function and may also induce a lowering of some clotting factor activities (e.g., FII, FIX, FX, FVII, FVIIa, and FXIIa). Analytical interferences in some laboratory assays, especially those based on optical clot detection, also occur but are minimized using mechanical or electromechanical-based procedures or using analyzers comparing the absorption of samples at two wavelengths or performing coagulation assays at alternative wavelengths. Nevertheless, regardless of the potential source of interference, biological or analytical, the best approach might be recollection of blood samples at fasting, provided that metabolic problems (i.e., dyslipidaemia) are absent (Favaloro, et al 2012).

### 2.1.4.2 Normal reference range derivations:

Laboratories use normal reference ranges (NRRs) to identify whether a test result is within the normal range or outside this range, and to thus identify an abnormal result. Use of an inappropriate NRR may mean some normal individuals will yield apparently abnormal test results. However, even the use of a typical and potentially appropriate NRR, generated as the mean +/- 2 standard deviations, will identify 5% of the normal population as outside this range, simply based on the statistical model used, i.e., to capture 95% of the normal population. Another way to consider this is to recognize that a standard laboratory NRR will correctly identify only 95 out of every 100 normal test results. Put into clinical context, 5 in every 100, or 1 in every 20, tests a clinician orders, using such NRR estimates, will likely reflect a false abnormal test result, again simply based on the statistical model used to generate the NRR. The relative false positive to true positive rate increases substantially for rare disorders and is a particular problem with congenital disorders such as protein C, protein S, and anti-thrombin, especially when patient cohorts are inappropriately selected for testing (Favaloro, et al 2012).

### 2.1.4.3 Miscellaneous variables:

Age, gender, ethnicity, and blood group might influence reference values for certain parameters of laboratory haemostasis, and/or generate variable test results for some tests. For example, FVIII and VWF and platelet function tests are generally influenced by such factors. Thus,
interpretation of test results should consider these issues to prevent misdiagnosis (Favaloro, et al 2012).

2.1.4.4 Physical activity, illness, and stress:
Excess physical activity in persons immediately prior to collection leads to certain in vivo events (e.g., plasma volume expansion and increased basal metabolism), which may in turn lead to significant effects on haemostasis. However, perhaps the best-known acute effects are related to acute phase reactants, which may rise due to physical activity, illness or stress, and include fibrinogen, VWF, and FVIII. In the worst case scenario, these elevations may result in a misdiagnosis of (mild) haemophilia A or VWD Type 1 patients as a non-haemophilia or non-VWD (false negatives). Blood collection may sometimes be stressful for some individuals (particularly children) leading to acute phase changes in proteins secondary to the phlebotomy itself (Favaloro, et al 2012).

2.1.4.5 Circadian and diurnal rhythms:
Levels of some haemostasis components follow a circadian or diurnal rhythm, with differential levels detectable at different times of the day. For example, fibrinogen and plasminogen activator inhibitor-1 levels tend to be higher in the early morning hours. PFA-100 closure times and possibly VWF may also provide different values throughout a 24-hour period. Although most changes tend to be fairly subtle, in the worst case scenario this might also lead to some clinically significant differences (Favaloro, et al 2012).

2.1.4.6 Anticoagulant therapy:
Testing for thrombophilia is often performed in individuals who have recently suffered a thrombotic event. Individuals are placed on anticoagulant therapy after a thrombosis. Testing while on anticoagulant therapy will affect (both biologically and analytically). Thus, false-positive and false-negative diagnoses can both occur, depending on the extent of the anticoagulant effect, and the test performed (Favaloro, et al 2012).

2.1.5 Strategies for selection of reference individuals:
Of the strategies available for selection of reference individuals, only the direct selection method agrees with the reference values concept as recommended by the International Federation of Clinical Chemistry (IFCC). In this, the individuals are selected from a parent population using defined criteria. However the disadvantages are the problems and cost of obtaining a
representative group of reference individuals. In contrast, in the indirect method the individuals are not considered. The method assumes that the values of an analyte have a distribution with a preponderant central peak, which is composed, mainly of normal values (Ashavaid, et al 2005).

The normal interval can be estimated by extracting the distribution of normal values from this part of the distribution. However, the major disadvantages are the dependence of the lower and upper limits on the particular method used and variation of results not only across hospitals but also for the same hospital at different times depending on the characteristics of the hospital patient group at that particular time. The other strategies are the a priori and the a posteriori sampling method. The a priori (prospective) strategy is best suited for smaller studies and individuals fulfilling defined inclusion criteria are selected for sample collection. On the other hand, a posteriori (retrospective) method consists of database containing both analysis results and information on a large number of individuals. Values of individuals fulfilling defined inclusion criteria are selected (Ashavaid, et al 2005).

2.1.6 Steps involved during establishment of reference values:

While establishing reference values it is necessary that the individuals and methods of production of values be adequately described. Thus it is essential to specify the following factors:

- **Selection criteria:**

For health associated reference values, the International Federation of Clinical Chemistry (IFCC) has laid down certain guidelines. According to this the following individuals should be excluded:

a. **Pathophysiological states** - renal failure, congestive heart disease, chronic respiratory diseases, liver diseases, malabsorption syndromes, and nutritional anaemias.

b. **Systemic diseases** - hypertension, diabetes.

c. **Intake of pharmacologically active agents** - alcohol, and tobacco, oral contraceptives, replacement or supplementation therapy e.g. Insulin.

d. **Modified physiological states** - pregnancy, psychological and mental disorders such as severe stress and depression, exercise or physical training, food intake prior to blood collection.
The selected reference individuals may be further sub classified into more homogenous groups by stratifying them according to age and sex. Age may be categorized by equal intervals (e.g. by decades) or by intervals that are narrower in the periods of life where greater variation is observed (Ashavaid, et al 2005).

2.1.7 Preparation of individuals and collection of specimens:

Ideally, specimens for the production of reference values for clinical use should be collected under conditions as similar as possible to those prevailing in clinical practice. As several factors cause increased variability of analytes, it is necessary to standardize the pre-analytical procedures (Ashavaid, et al 2005).

2.1.8 Importance of analytical procedures and quality control:

It is necessary to specify the essential components while establishing reference values:

a. Analysis method (including information on equipment, reagents, calibration standards, type of raw data, and calculation method).

b. Quality control.

c. Reliability criteria.

The analyzer has to be calibrated with materials provided by the manufacturers. Changes in calibration curve and specificity of the analytical method can be detected by using a number of accuracy control specimens, at both normal and pathological levels of concentration, for the various analytes. During the course of the study there should be no change in the equipment, reagents, calibration standards, and controls. In order to maintain the required precision an adequate number of control specimens should be included at fixed or random positions in each analytical run. Ideally precision controls should be employed at different levels of concentration (Ashavaid, et al 2005).

2.1.9 Determination of reference limits:

In clinical practice, one usually compares an observed patient’s value with the corresponding reference interval, which is bounded by a pair of reference limits. In cases, where the reference distribution of analytes shows Gaussian distribution (symmetrical), parametric methods are used.
According to this, the determination of reference limits (percentile) would be calculated as values two standard deviations below and above the mean. If the reference distribution shows another shape, one may use mathematical functions that transform data to approximately Gaussian shape. In the nonparametric method the percentiles are simply determined by cutting off the required percentage of values in each tail of the subset reference distribution. Using the reference distribution the reference interval can be computed. Three kinds of reference intervals have been suggested: tolerance interval, prediction interval and inter percentile interval. The inter percentile interval is simple to estimate, more commonly used, and recommended by the IFCC. It is defined as an interval bounded by two percentiles of the reference distribution. It is an arbitrary but common convention to define the reference interval as the central 95% interval bounded by the 2.5 and 97.5 percentiles. The inter percentile interval can be determined by both parametric and non-parametric statistical techniques, as mentioned earlier.

2.1.10 Presentation of an observed value in relation to reference values:

An observed value, patient’s value, may be compared with reference values. The set of reference values can be made available to the clinicians in the form of tables, graphs or a few figures, either on result reports, laboratory sheets, or by separate publication. A printed set of reference data on reports is recommended only if one can ensure that it is relevant to the observed value to be reported. When necessary different sets should be given according to age, sex, activity, posture etc.

On the basis of the two reference limits of a reference interval, it is possible to classify an observed value as:
- ‘unusually low’ when situated below the lower reference limit
- ‘usual’ if between or equal to either of the reference limits
- ‘unusually high’ when above the upper reference limit

On reports it is convenient to flag unusual results for e.g. by using ‘L’ for low and ‘H’ for high (Ashavaid, et al 2005).

2.1.11 Reference values for activated partial thromboplastin time:

The international reference interval for activated partial thromboplastin time (APTT) is typically 26 – 40 second. The actual times depend on the reagents used and the duration of the pre incubation period, which varies in manufacturer’s recommendations for different reagents. These variations also greatly alter the sensitivity of the test to minor or moderate deficiencies of the
contact activation system. Each laboratory should calculate its own reference range (Dacie and Lewis, 2011). According to the IFCC it is necessary for every laboratory to have their own set of reference limits. However, in Gezira State most of the laboratories follow reference intervals established in the western population. The reference intervals can be questioned because of differences arising due to variations in diet, lifestyle etc. in Western and Gezira State populations. When I was searching, I found many studies done to evaluate the effects of many diseases, physiological conditions and particular substances on activated partial thromboplastin time. In these studies usually there were control groups of healthy individuals which their activated partial thromboplastin time results may consider as the normal values for healthy individuals.

A study by Ehab et al was undertaken at the Radiation and Isotopes Centre Khartoum (RICK), Sudan, in 2011, to identify the haemostatic abnormalities and vascular damage among the major Sudanese haematological malignancy patients. In this study fifty apparently healthy males and females were considered as control group. The mean of APTT results for control group was 30.5 seconds (Ehab, et al 2014).

A case control study conducted by Abdalla, et al in Khartoum Teaching Hospital in Khartoum State, Sudan, to estimate the PT, APTT, fibrinogen level and platelets count in patients with acute pancreatitis. Fifty normal people that matched the patients group were included as a control group. The mean value of APTT in the control group was 34.0 seconds (Abdalla, et al 2013).

Mahmoud Mohamed Algari had done case control study in Khartoum Teaching Hospital (Khartoum, Sudan), in 2012 to evaluate the effects of liver diseases on PT, APTT and fibrinogen in thirty healthy control subjects, the mean value of APTT was 29.4 seconds. (Algari, 2012).

A study was conducted at the Faculty of Medical Laboratories Sciences, Elneelain University, Khartoum, Sudan in 2013. It is aimed to study the effect of freezing storage (at -20°C) of citrated plasma on PT and APTT. Blood samples were collected from fifty healthy volunteer. PT and APTT were performed immediately after plasma separation using part of the plasma; the rest of the plasma stored at -20°C in three different plastic containers and PT and APTT were performed after one, two, and three weeks on these samples. The result revealed that, the mean value of APTT for immediately performed plasma was 35.3 seconds (Hassan, et al 2013).
A cross sectional study was done by Rania Abd-Alrahman in 2013 to estimate some haemostatic parameters among Sudanese diabetes mellitus type 2 patients in Khartoum state. In this study fifty samples were collected from healthy individuals and considered as control group. The mean of APTT results for control group was 29.4 seconds (Sadaabi, 2013).

A prospective case-control study was done by Erhabor, et al to evaluate the effect of alcohol consumption on platelets, APTT, PT and TT in Kebbi, Kebbi State, Nigeria in 2012. In this study fifty gender and age matched nonalcoholic were monitored as control. The mean value of APTT results for the control group was 34.8 seconds (Ehabor, et al 2014).

A study was done by Erhabor, et al in Family Health Clinic (FHC) of the Department of Community Medicine, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria in 2014, to evaluate the PT and APTT among clients on hormonal contraceptives. Women who have never used any family planning method and or hormonal contraceptives constituted the control participants. The mean value of APTT results for the control group was 38.6 seconds (Osaro, et al 2014).

In Ghana a study made up by Mauryne, et al, in HbSC and HbSS patients in steady state, to evaluate some coagulation parameters in steady state HbSC disease patients. Forty apparently healthy HbAA were considered as control group. The mean value of APTT was 28.1 seconds for the control group (Ajuwon, et al 2014).

A study was conducted by Maureen Andrew et al in Hamilton to determine the postnatal development of the human coagulation system in the healthy full term infants. In this study they collect 29 samples from healthy adults. The mean of APTT results in healthy adult individuals was 33.5 seconds (Andrew et al, 1984).

A study was made up by Appel et al in Netherlands to established reference values for coagulation parameters during childhood and puberty. They obtained blood samples from 52 healthy adults in this study. The mean of APTT for these healthy adults was 34.0 seconds. (Appel et al, 2012).
2.2 Haemostasis:

2.2.1 General overview:

Haemostasis is one of a number of protective process that have evolved in order to maintain a stable physiology. It has many features in common with, and to some extent interacts with, other defence mechanisms in the body, such as the immune system and the inflammatory response. The high blood pressure generated on the arterial side of vertebrate circulation requires a powerful, almost instantaneous but strictly localized pro coagulant response in order to minimize blood loss from site of vascular injury without compromising blood flow generally. Systematic anticoagulant clot-dissolving components have also evolved to prevent extension of the pro coagulant response beyond the vicinity of vascular injury resulting in unwanted thrombus formation in the slow blood flow in the veins. The resultant haemostatic system is thus a complex mosaic of activating or inhibitory feedback or feed-forward pathways, integrating its five major components, blood vessels, blood platelet, coagulation factors, coagulation inhibitors and fibrinolytic elements, (Hoffbrand, et al 2005).

In the most simplistic terms, blood coagulation occurs when the enzyme thrombin is generated and proteolysis soluble plasma fibrinogen, forming the insoluble fibrin polymer, or clot, this provides the physical consolidation of vessel wound repair following injury. Haemostasis refers more widely to the process whereby blood coagulation is initiated and terminated in a tightly regulated fashion, together with the removal, or fibrinolysis, of the clot as part of vascular remodelling, as such, haemostasis describes the global process by which vessel integrity and patency are maintained over the whole organism for its life time (Hoffbrand, et al 2005).

2.2.2 Components of haemostasis:

The haemostatic apparatus consists of blood vessel, platelets, plasma coagulation factors, natural occurring inhibition and fibrinolytic factors. This apparatus is normally quiescent but becomes active within seconds after trauma. When a blood vessels is ruptured, their mechanisms operate locally at the site of injury to curtail the bleeding; vessels wall contraction, platelet’s haemostatic plug formation, platelet adhesion and aggregation, formation and maintenances of fibrin, blood coagulation, Fig. (2-1). All three mechanisms are essential for normal haemostasis, and they
function in concert rather than at random. Other processes also play some role in the control of haemorrhage under special circumstance. (Ratnoff, et al 1996)

The extent of bleeding is influenced by the tension of the extravascular supporting tissue. Haemorrhage into muscles or joints is usually limited, as the resultant increased tissue pressure causes small blood vessels to collapse. Post-partum bleeding from the uterus may be controlled after delivery by the contraction of the myometrium, which applies external pressure on the uterine blood vessel. The relative importance of various haemostatic mechanism, by and large, depend upon the size of the blood vessels injured. Bleeding from small cut in the skin is primarily stopped by formation of a platelet haemostatic plug. In contrast, formation of fibrin clot is required for the control of haemorrhage from large vessels. Abnormal bleeding may result from defect in any of the above mentioned mechanisms. It is customary to divide haemostasis into two continuous separated stage, primary and secondary. (Ratnoff, et al 1996)

Primary haemostasis, the instantaneous plugging of a hole in the vessel wall, is achieved by a combination of vasoconstriction and platelet adhesion and aggregation, the formation of fibrin is not required for haemostasis at this stage. Primary haemostasis is of temporary benefit, haemorrhage may start a fresh unless the friable platelet plug is reinforced by a tough fibrin

Figure (2-1): The haemostatic mechanism (Ratnoff, et al 1996)
mesh. This secondary homeostasis, accomplished by the formation of fibrin, helps maintain a haemostatic plug until healing is completed.

Finally, gradual dissolution of the haemostatic plug by fibrinolysis leads to restoration of normal blood flow. Premature lysis of the fibrin thrombus by accelerated fibrinolysis may result in rebleeding, suggesting a role for fibrinolysis in normal homeostasis (Ratnoff, et al 1996)

2.2.2.1 Blood vessels:

The basic structure of blood vessels can be broken down into three layers, the intima, the media and the adventitia, Fig. (2-2). It is the materials that make up these layers and the size of these layers themselves that differentiate arteries from veins, and indeed one artery or one vein from another artery or veins. The intima is the innermost layer and the surface is covered with a single layer of endothelium cells (ECs), which rest on a basement membrane of sub endothelial microfibrils that are composed of collagen fibres and some elastin. The media or middle layer contains mainly circularly arranged smooth muscle cells and collagenous fibrils and is divided from adventitia by the external elastic lamina. The muscle cells contract and relax, whereas the elastin allows vessels to stretch and recoil. The adventitia outermost layer is composed of collagen fibers and fibroblast that protect the blood vessels and anchor it to surrounding structures. (Hoffbrand, et al 2005)
2.2.2.1.1 The endothelium:

The endothelium function in a multitude of physiological process including the control of cellular trafficking, the regulation of vasomotor tone and maintenances of blood fluidity. Once activated, endothelium cells (ECs) express at their surface, and in some cases release into the plasma, variety of intracellular adhesion molecules e.g., vascular cell adhesion molecules, E-selection P-selection and VWF, which modulate leucocyte and platelets adhesion, inflammation, phagocytosis and vascular permeability. Intact ECs exert a powerful inhibitory influence on haemostasis by virtue of the factors that they synthesize and release or express on their surface. Two of these, prostaglandin I2 (PGI2 or prostacyclin) and nitric oxide (NO) also known as endothelium derived relaxing factor (EDRF). Both substances inhibit aggregation of platelets and leucocytes by raising intra platelet levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) respectively (Hoffbrand, et al 2005).

PGI2 synthesized by ECs its action on platelet involve binding to a specific G.protin coupled receptor (PTGIR) that activate adenylate cyclase, which increase the intra platelet cAMP concentration, this promotes Ca uptake into the dense tubular system and inhibit phosphatidylinositol metabolism, both of which prevent platelet aggregation and the
consequence release of storage granules containing pro coagulant molecules, for example vWF and FV. The effect of PGI2 on the vessel wall is vasodilatation. The precursor of PGI2 is arachidonic acid derived from ECs membrane phospholipids by phospholipase. Arachidonic acid is first converted to prostaglandins PGG2 and PGH2, the so called cyclic end peroxide, by cyclooxygenase, and then to PGI2 by prostacyclin synthetase. Thrombin and other agents that are generated of the site of injury stimulate the synthesis of PGI2 by adjacent ECs, which counteracts the platelet aggregating activity of the protease and there by helps to localized platelet plug formation (Hoffbrand, et al 2005).

Nitric oxide (NO) is synthesized in smooth muscle cells, macrophage and activated platelet as well as by ECs. Stimulate synthesis of NO occurs in ECs exposed to cytokines (IL1, IL2, TNF).

Disruption of the vessel wall following injury leads to exposure of pro coagulant stimuli. Cells of the adventitia or epithelial cells of the surrounding tissue express Tissue factor (TF) constitutively on their surface. Formation of a complex between TF and FVII present in blood flowing from the injured vessel initiates coagulation, resulting in fibrin generation. Exposure of collagen within the sub endothelial layers of the vessel wall leads to immobilization of plasma VWF triggering platelet adhesion, aggregation and activation. Release of contents of platelet α-granules increases the local VWF concentration and activation of ECs results in release of Weibel – Palade bodies, further increasing the local concentration of VWF and expression of the cell adhesion molecule P-selectin, leading to further recruitment of platelets (Hoffbrand, et al 2005).

There are important fibrinolytic factors detectable in the vessel wall; tissue plasminogen activator (tPA), plasminogen activator inhibitor -1 (PAI-1) are synthesized primarily by ECs, urinary plasminogen activator (uPA, urokinase) is mainly derived from fibroblast-like cells in the kidney and gut. Unlike VWF and P-selectin, tPA and PAI.1 are not stored in the Weibel- Palade bodies and in the resting state, synthesis and secretion are slow, resulting in low circulating levels. However stimulated synthesis and release occur in response to a variety of stimuli (Hoffbrand, et al 2005).
2.2.2.2 Platelets:

2.2.2.2.1 Platelets structure:

Platelets are small anucleate cells that play a critical role in haemostasis and thrombosis. Platelets ordinarily circulate in the blood stream in a quiescent state but undergo explosive activation following damage to the vessel wall, leading to rapid formation of a Platelets aggregate or vascular plug and occlusion of the site of damage.

Platelets are extremely small discoid in shape, with dimensions of approximately 3.0μm by 0.5μm and a mean volume of 7 - 11fl. They are present at a high level in the human circulation usually between 150 and 400x10⁹/l. The discoid shape of the Platelet is formed by the Platelet cytoskeleton, which consists of a spectrin based membrane skeleton, circumferential bands of single microtubule that lies beneath the plasma membrane and a rigid actin filament network that fills the cytoplasm of the cell. Much of the rigid structure and platelet strength results from the 2 million copies of action per Platelet, of which approximately 40% are assembled into actin polymers. These polymers connect with each other and with the cytosolic tail of the membrane glycoprotein (GP) 1b α via filamin in a lattice like structure. Platelets contain three main types of storage granules dense, α granules and lysosomes each of which rapidly release their contents upon activation α – granules are the most numerous, with about 80 per platelet, and contain a rich diversity of proteins and membrane receptor that support many processes in haemostasis and in host defence. Dense granules contain high levels of small molecules that support Platelets activation and mediate vasoconstriction. Lysosomes also release their contents on activation. Platelet have a dense network of intracellular membranes known as the dense tubular system, which rapidly liberate their stores of the intracellular messenger Ca+2 in response to the generation of the second messenger inositol triphosphate (IP3). Platelets also have a dense network of invaginations of the surface membrane known as the surface- connected canalicular system, which servers to increase the surface area of the plasma membrane and there by provide more sites for release of intracellular granules and interactions with other surfaces. Platelets contain several mitochondria that generate energy during their short life span of 10 days,

Fig. (2-3) (Hoffbrand, et al 2005).
2.2.2.2 Platelets function:

Haemostatic plug formation divided into three separate yet closely interrelated steps, platelets adhesion, the release reaction and platelets aggregation. Within seconds after injury, platelets accumulate at the site of vascular damage and began to adhere to sub endothelial or perivascular connective tissue that has become exposed to flowing blood. Collagen, fibronectin, laminin and micro fibrils are among the substances present in sub endothelium. They belong to a family of adhesive protein, major component to which platelet adhere are collagen. Platelets bind to collagen through VWF and GP1b-1X. It appears that VWF first adheres to collagen fibers and then change its conformation. This is followed by the binding of platelet to VWF via platelet membrane GP1b-1X. Adhesion appears also to be influenced by other factors including haematocrit and shear rate. After adhesion, platelets undergo a shape change from that of a disk to a more spherical shape with extended pseudopods. At the same time a secretory process occurs by which certain biological active substances stored in platelets granules are actively discharged to the outside of the adherent cells, the release reaction. The release reaction seems to be initiated by contraction of a circumferential band of micro tubules not due to rupture of the
cell membrane. Rather, stored agents are discharged through the open canalicular system (OCS) after the fusion of the granular membranes with OCS membranes. The released substances accelerate platelets plug formation and play a role in the process of tissue repair. ADP induces adhesion of platelet to each other. This phenomenon called platelet aggregation, increases the size of a platelet haemostatic plug at the site of injury. The haemostatic plug at this stage consists of closely packed, degranulated platelets with little fibrin. In addition to ADP a wide variety of agents including epinephrine, collagen, thrombin immune complexes and platelets activating factor, can cause platelet aggregation and secretion.

Thromboxane A2 derived from arachidonic acid metabolism, is a very potent but labile substance that induce platelet aggregation and secretion. Fibrinogen is required for platelet aggregation, which bind to specific receptors on platelet surface GP11b -111a, and there by links platelets to each other (Ratnoff, et al 1996)

2.2.2.2.1 Platelet pro coagulant activity:

A critical function of platelets activation is to provide a negatively charged phospholipids surface for the assembly of two multiprotein complexes that from a vital part of the coagulation cascade, namely the tenase and prothrombinase complexes. The negatively charged platelets surface also support the protein C pathway that serves to limit the coagulation cascade. The formation of the negatively charged lipid surface on activated platelet is commonly described as amino phospholipids exposure or pro coagulant activity. It is formed by the movement of phosphatidyl serine from the inner into outer leaflet of the platelets membrane. The molecular basis of the pro coagulant response including the identity of the enzyme or flipase that promotes the translocation of phosphatidyl serine a cross the membrane (Hoffbrand, et al 2005)

2.2.2.2.2 Stimulatory agonists of platelets:

a. **ADP and ATP:** are stored in dense granules and released rapidly upon platelet activation.

b. **Thromboxane A2:** is generated de novo upon activation of cytosolic phospholipase A2 (PLA2) which liberates arachidonic acid from the 2-position of membrane phospholipids.

c. **Arachidonic acid:** is metabolized by cyclo oxygenase and lipoxygenase enzymes to end peroxide, thromboxane and leukotriene.
d. **Thrombin**: serine protease, the major product of coagulation cascade.
e. **Collagen**: major components of the sub endothelial matrix, mediate platelet adhesion and activation following damage of vasculature.
f. **Fibrinogen**: the major mediator of platelet aggregation and is vital for fibrin formation (Hoffbrand, *et al* 2005).

### 2.2.2.2.3 Inhibitory agonists of platelets:

a. **Nitric oxide (NO)**: mediates powerful inhibition of platelet activation and also promotes vasodilatation, released from endothelial cells that surround the vasculature.
b. **Prostacyclin**: (Prostacyclin I2 or PGI2) synthesized by endothelial cells from arachidonic acid metabolism, exerts powerful inhibitory effect on platelets and also promotes vasodilatation through elevation of cAMP (Hoffbrand, *et al* 2005)

### 2.2.2.3 Coagulation factors:

Coagulation Factors are glycoproteins of greater than 40000 MW. Most of them are produced by the liver and secreted into the circulating blood. There is an evidence that trace amounts of all coagulation factors are also present in the extravascular space. Blood clotting factors or coagulation factors may be classified into several groups according to their functions. Factors XII, XI, pre-kallikrein, X, XI, VII and prothrombin are zymogens of serine proteases and are converted to active enzymes during blood coagulation. In contrast, factors V, VIII, high molecular weight kininogen (HMWK) and tissue factor are not proenzymes but functions as cofactors. Factors and HMWK must be activated or modified in order to function as a cofactor, whereas tissue factor present in extravascular spaces, must make contact with blood to function. Factors X, IX, VII and prothrombin are called vitamin K-dependent factors because they require vitamin K for their complete synthesis. These proteins contain 10-12 unique amino acids, \( \gamma \)-carboxyglutamic acid (GLa) at the amino terminal end of the molecule, which are incorporated into a polypeptide chain of the vitamin K-dependent clotting factors by the action of a carboxylase in hepatocytes, vitamin K is required for this reaction as essential cofactors (Ratnoff, *et al* 1996)

Blood coagulation results from the conversion of a soluble plasma protein, fibrinogen, to insoluble fibrin. The conversion is catalyzed by an enzyme thrombin. Thrombin is not normally present in the circulating blood but exists as an inert precursor, prothrombin. The generation of thrombin from prothrombin is mediated in vitro by either or both of two sequential chains of
reactions, described as the extrinsic and intrinsic pathways. Exposure of blood to injured tissue initiates blood clotting by the extrinsic pathway, this is called extrinsic because tissue thromboplastin (tissue factor) comes from outside the blood. The prothrombin time assay screens for the integrity of this pathway. When blood is withdrawn carefully so that it is not contaminated with tissue juice, it still clots in a glass tube. This pathway is called intrinsic, because all the substances required for clotting appear to be present within the blood. The intrinsic pathway is triggered by contact of factor XII (Hageman factor) with surfaces foreign to the normal vascular wall. The partial thromboplastin time (PTT) are good monitor for this pathway. The difference in the two pathways lies in the way factor X is activated. The two pathways share a common pathway after activation of factor X (Ratnoff, et al 1996).

Table (2-1): Properties of coagulation factors (Ratnoff, et al 1996)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Synonyms</th>
<th>Molecular weight</th>
<th>Plasma concentration (mg/dl)</th>
<th>In vivo half life (hr)</th>
<th>Chromosomal location of gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
<td>340.000</td>
<td>200-400</td>
<td>100-150</td>
<td>4q26</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
<td>70.000</td>
<td>10</td>
<td>80-100</td>
<td>11p11-12q</td>
</tr>
<tr>
<td>III</td>
<td>Tissue thromboplastin factor</td>
<td>44.000</td>
<td>0</td>
<td></td>
<td>1pter-p12</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium ion</td>
<td>40</td>
<td>9-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Labile factor, proaccelerin</td>
<td>330.000</td>
<td>1</td>
<td>24</td>
<td>1q21-q21</td>
</tr>
<tr>
<td>VII</td>
<td>Stable factor</td>
<td>48.000</td>
<td>0.05</td>
<td>6</td>
<td>13q34</td>
</tr>
<tr>
<td>VIII</td>
<td>Anti hemophilic factor (AHF)</td>
<td>330.000</td>
<td>0.01</td>
<td>12</td>
<td>Xq28</td>
</tr>
<tr>
<td>VWF</td>
<td>Von wille brand factor</td>
<td>(250.000)</td>
<td>1</td>
<td>24</td>
<td>12pter-p12</td>
</tr>
<tr>
<td></td>
<td>Christmas factor</td>
<td>55.000</td>
<td>0.3</td>
<td>24</td>
<td>Xq26.3-q27.2</td>
</tr>
<tr>
<td>-X</td>
<td>Stuart- power factor</td>
<td>59.000</td>
<td>1</td>
<td>25-60</td>
<td>13q34</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecedent (PTA)</td>
<td>160.000</td>
<td>0.5</td>
<td>40-80</td>
<td>4q35</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor</td>
<td>80.000</td>
<td>3</td>
<td>50-70</td>
<td>5q33-qter</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilizing factor (FSF)</td>
<td>320.000</td>
<td>1-2</td>
<td>150</td>
<td>6p24-p25</td>
</tr>
<tr>
<td>Pre-kallikrein</td>
<td>Fletcher factor</td>
<td>85.000</td>
<td>5</td>
<td>35</td>
<td>4q35</td>
</tr>
<tr>
<td>High molecular Weight kininogen (HMWK)</td>
<td>Fitzgerald, flaujeas, Williams factor</td>
<td>120.000</td>
<td>6</td>
<td>150</td>
<td>3q26-qter</td>
</tr>
</tbody>
</table>

The blood coagulation cascade has been suggested to function as an amplifier in which an initially small stimulus leads to the explosive formation of thrombin through sequential enzymatic steps. Many of the blood coagulation reactions proceed efficiently only on surface membrane of stimulated cells, such as activated platelets, leukocyte and smooth muscle cells exposed by injury. This helps restrict the coagulation process to the site of vascular injury (Ratnoff, et al 1996)
2.2.2.4 Natural occurring inhibitors of blood coagulation: (Natural anticoagulants)

Human plasma contains many agents that inhibit the activity of activated clotting factors and fibrinolytic enzymes.

1. Anti-thrombin III (ATIII):
Plasma protein of 65000 MW produced by liver. It inhibits thrombin by forming a stable 1:1 complex between an arginine residue of AT III and the serine active site of thrombin. ATIII is a major inhibitor of thrombin, it also inhibits Factors XIIa, XIa, Xa, IXa, VIIa, kallikrein and plasmin. Its action is greatly accelerated by heparin.

2. Heparin cofactor II (HCII):
Plasma protein selectively inhibits thrombin and its activity. Stimulated markedly in presence of heparin. Differs from AT III in that HCII does not inhibit the activity of other activated clotting factors and is stimulated by dermatan sulfate.

3. Alpha-1 antitrypsin:
It is responsible for about 70% and 35% respectively of the FXIa and FXa neutralizing activity in plasma and little effect on thrombin inhibition.
4. **α 2 – macroglobulin (α 2-MG):**
It is responsible for approximately 50%, 20% and 10% of the inhibition of kallikrein, thrombin and FXa respectively.

5. **Thrombomodulin (TM):**
TM is an integral transmembrane receptor found on endothelial cells in virtually all body tissue. TM forms a1:1 complex with thrombin preventing binding to its various procoagulant substrates.

6. **Activated protein C (APC):**
This is generated from its vitamin K-dependent precursor by the action of thrombin, thrombin activation of protein C is enhanced when thrombin is bound to thrombomodulin. Activated protein C destroys factor Va and factor VIIIa reducing further thrombin generation. Once generated APC interact with protein S (PS) bound to the phospholipids surface of activated platelets, enhancing APC anti-coagulant activity against FVa and FVIIIa.

7. **Protein S (PS):**
This is a cofactor for protein C which acts by enhancing binding of APC to the phospholipids surface. It circulates bound to C4b binding protein but some, 30-40%, remains unbound and active, free protein S (Hoffbrand, et al 2005).

2.2.2.5 **Fibrinolysis:**
The coagulation and fibrinolytic systems are two separated but inter linked enzyme cascades that regulate the production and breakdown of fibrin. Fibrinolysis the dissolution of fibrin clots may be considered as an essential host defense mechanism against fibrin deposition in the vasculature. Premature lysis of fibrin clots may lead to re bleeding from the site of injury. Fibrinolysis also seems to be associated with many other physiologic and pathologic processes such as tissue repair, ovulation and malignant transformation (Ratnoff, et al 1996).

Fibrinolysis is achieved by a potent plasma proteolytic enzyme plasmin. Plasmin not present in normal circulating blood but rather is in the form of an inert precursor Plasminogen is a plasma glycoprotein of 90.000 MW composed of a single polypeptide chain and present in normal plasma at a concentration of 20 - 40 mg/dl also is produced by the liver, Plasminogen is converted to plasmin by the action of specific enzymes collectively known as plasminogen activator. The activation of plasminogen involves a proteolytic cleavage of the single chain
zymogen and leads to the formation of plasmin. Plasmin is a serine protease and hydrolyzes susceptible arginine and lysine bonds in many proteins including fibrin, fibrinogen, factor V and factor VII. When fibrin or fibrinogen is digested by plasmin, a series of fragments known as fibrin (or fibrinogen) degradation products (FDPs) or fibrin (or fibrinogen) split products (FSP) are produced. FDP inhibit both platelet aggregation and the action of thrombin on fibrinogen, and they may contribute to a bleeding tendency during fibrinolysis (Ratnoff, et al 1996).

2.2.2.5.1 Activation of plasminogen:

Two major types of plasminogen activators are known intrinsic and extrinsic.

1. **Intrinsic activators:** are agents endogenous to blood and may convert plasminogen to plasmin when blood comes into contact with a foreign surface include factor XII and other contact factors such as pre-

2. **Extrinsic activators:** are agents extrinsic to blood and were widely distributed in almost all body tissue including vascular endothelium. The extrinsic activators include the following:

   I. **Tissue plasminogen activators (t-PA):** produced and secreted from endothelium, has high affinity for fibrin and close proximity to blood so play an important role in physiologic fibrinolysis. The t-PA released on demand as a result of local or systemic stimuli such as local thrombotic venous occlusion, physical exercise, epinephrine or thrombin increases the release of t-PA.

   II. **Urinary plasminogen activator (Urokinase) (U-PA):** a serine protease produced by epithelial cells in the urogenital tract and excreted in to urine. Three types of urokinase have been isolated from human urine: single chain prourokinase, HMW urokinase and LMW urokinase.

   III. **Striptokinase (SK):** derived from beta-haemolytic streptococci, it is not an enzyme and forming a complex with plasminogen (Ratnoff, et al 1996)
2.2.2.5.2 Inhibitors of fibrinolysis:

1. **Plasminogen activators inhibitor type 1 (PAI-1):** It is an important fast acting inhibitor of t-PA, u-PA and to a small extent plasmin which is secreted by endothelial cells and also found in platelet α-granules. In plasma it occurs in two forms a functionally active (free) form and as an inactive complex with t-PA. There is growing evidence that elevated levels of PAI-1 are associated with an increased incidence of venous and arterial thrombosis.

2. **Plasminogen activator inhibitor type 2 (PAI-2):** This is an inhibitor of t-PA produced mainly by placenta and may thus contribute to the inhibition of fibrinolysis which occurs during pregnancy, it also synthesized in monocyte and epidermal cells. The potency is at least 10-fold less than that of PAI-1.

3. **α2-antiplasmin (α2-AP):** This is the predominant plasmin inhibitor, it forms a stable 1:1 complex with plasmin. It is a single chain glycoprotein synthesized by the liver, has half time about 60 hours. The physiological importance is supported by the fact that a congenital deficiency (known as Miyasato disease) is associated with a clinical significant bleeding disorder due to uncontrolled fibrinolytic activity, and that levels are reduced in disseminated intravascular coagulation (DIC) and during thrombotic therapy.

4. **Lipoprotein A (LpA):** The protein portion of lipoprotein A is termed Apo (a), it is synthesized in the liver and circulate in plasma, has considerable structural homology with PLG. It inhibits fibrinolysis by increase expression of PAI-1 lipoprotein A, which has clinical importance indicated by that the raised levels are associated with an increased incidence of thrombosis (Hoffbrand, et al 2005).

2.2.3 Screening tests for haemostasis:

2.2.3.1 Prothrombin Time (PT):

Although originally intended to measure Prothrombin (factor II) concentration, it was later realized that the PT measure much more. It is performed by adding tissue factor (in the form of phospholipids complex or thromboplastin, originally derived from brain tissue but more recently prepared as a recombinant protein) and calcium to hypo calcaemic (i.e. anti-coagulated with citrate) plasma. Tissue factor reacts with factor VII to activate the extrinsic pathway of coagulation cascade. In addition to factor VII the PT reflect the activities of factor V, factor X and factor II (prothrombin) assembled with phospholipids and calcium to form the
prothrombinase complex which yields thrombin that then produces polymerizing fibrin to form a clot. Therefore the PT is sensitive to the activities of factor II, V, VII, X and fibrinogen, but to different degrees and with different clinical implications. It is also important to realize that the relationship between PT and the degree of factor deficiency is not linear.

A derivative of the PT is the International Normalized Ratio (INR), which was develop to monitor anticoagulation with warfarin. The difficulty with using the PT for this purpose is that the sensitivity at the PT to the degree of anticoagulation created by warfarin is dependent upon the particular reagents and instrument used to measure the PT, and these vary among different laboratories. To compensate for this variability each laboratory must adjust its PT according to the following formula:

\[ \text{INR} = \frac{\text{patient PT}}{\text{control PT}} \]

In which the control is the mid-point of the laboratory normal range and the ISI (International Sensitivity Index) is a value determined for each bath of each commercial thromboplastin. Thromboplastin that are less sensitive to the effect of warfarin have greater ISI. The INR should not be used to assess liver function since the test is calibrated specifically for plasma from patient taking warfarin. It is sensitivity to factor deficiency related to liver disease cannot be predicted. Although the PT is not used to monitor other anticoagulation, it is prolonged by relatively high concentration of heparin and by therapeutic concentrations of the newer anticoagulants lepirudin and argatroban (Kitchens, et al 2002)

2.2.3.2 Activated partial thromboplastin time (APTT):

This standard assay is performed by adding phospholipids (lacking tissue factor, hence a partial thromboplastin) and particulate matter (to activate the contact pathway of coagulation hence the activated PTT) such as kaolin to citrated plasma along with calcium. The APTT is affected by all of the coagulation factors in the intrinsic and common pathways (factors XII, XI, IX, X, VIII, V and II). While the APTT is an extremely useful screening test, it does not simulate physiologic coagulant mechanisms (Kitchens, et al 2002).

The APTT can be prolonged by a deficiency of any of the factors in the intrinsic or common pathway, by the presence of a circulating inhibitors, or because of a fibrinogen abnormality. Factor deficiencies must be relatively severe that is level below 30-40% of normal, multiple
deficiencies or both to significantly prolong the APTT. Although the APTT is somewhat less sensitive than the PT to low levels of vitamin K-dependent factors, the APTT is more sensitive to the presence of circulating anticoagulants, including heparin and the lupus anticoagulant. To identify a circulating anticoagulant, the laboratory can perform a repeated APTT with a 1:1 mix of patient and normal plasma. Although the admixture of normal plasma will nearly completely correct a prolonged APTT secondary to a factor deficiency it will have little, or no impact on the APTT that is prolonged because of a high titer circulating anticoagulant. A prolonged APTT resulting from heparin in the sample, either because of therapeutic anticoagulant or inadvertent contamination by drawing the sample through a line, can be determined by mixing the patient plasma with polybrene, this will neutralize the heparin and correct the APTT (Hillman, et al 2005).

Fig. (2-5): Reactions of PT and APTT
Chapter Three

3. Materials and Methods

3.1 Methodology:

3.1.1 Study design:

This was prospective analytical cross sectional study aimed to establish reference values for activated partial thromboplastin time (APTT) among healthy Sudanese adults living in Gezira State.

3.1.2 Study area and duration:

This study was done in Gezira state from September 2014 to March 2015. The Gezira State lies between latitudes (13-32 and 15-30) North and longitudes (22-32 and 20-34) East. It is bordered by Khartoum State to the North, Sinnar State to the South, Gadarif State to the East and White Nile State to the West. It has an area of 27,549 km². Total population is 2,796,330 in the census performed in 2008. Gezira state was inhabited by a mixture of races and tribes from inside and outside Sudan. The name comes from the Arabic word for Island. Wad Madani is the capital of the state. The Gezira is a well-populated area suitable for agriculture. The region has benefited from the Gezira Scheme, a program to foster cotton farming begun in 1925. At that time the Sinnar Dam and numerous irrigation canals were built. The Gezira became the Sudan's major agricultural region with more than 2.5 million acres (10,000 km²) under cultivation (Sudan.gov.sd, 2012).

3.1.3 Study population:

Adult people in Gezira State according to inclusion and exclusion criteria.

3.1.4 Sampling and sample size:

The research was used stratified random sampling with proportional to size technique. A sample of normal persons was carried out in seven localities in Gezira State. The sample was included possible normal persons in these localities at any time. The sample size was determined by using
stratified random sampling formula. The sample size (n) was determined by using the following formula:

\[ n = \frac{N \sum_{j=1}^{I} N_j p_j q_j}{N^2 A + \sum_{j=1}^{I} N_j p_j q_j} \]

\[ A = \frac{B^2}{4} = \frac{(0.04)^2}{4} = 0.0004 \]

\[ P = 50\% \text{ thus } P \text{ is } 0.5 \text{ and } Q \text{ is } 0.5. \]

Where B is the expected error that the researcher accepted which is (0.04) and N is the total population. Nj denotes the stratum j Pj (0.5) is estimated proportion in the stratum j (this assumed equally for strata). Since we have N equals 1694653, the total number of possible normal persons in the seven localities, Nj is illustrated in the table below:

\[ \begin{align*}
\text{N} &= \frac{1694653 \left( 190515 \times 0.5 \times 0.5 + 287428 \times 0.5 \times 0.5 + 219535 \times 0.5 \times 0.5 + 103559 \times 0.5 \times 0.5 \right)}{1694653^2 \times (0.0004) + \left( 190515 \times 0.5 \times 0.5 + 287428 \times 0.5 \times 0.5 + 219535 \times 0.5 \times 0.5 + 103559 \times 0.5 \times 0.5 \right)} \\
\text{N} &= 624.7696 \\
\text{N} &= 625
\end{align*} \]

This was the minimum sample size.

Thus the minimal total sample size was 625 individual, divided into seven localities according to the locality population as following in table (3-1):
Table (3-1): Distribution of sample size in localities.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Population</th>
<th>Pop %</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Elkamleen</td>
<td>190515</td>
<td>0.112</td>
<td>70</td>
</tr>
<tr>
<td>2. Elhassahesa</td>
<td>287428</td>
<td>0.170</td>
<td>106</td>
</tr>
<tr>
<td>3. Sharg Elgezira</td>
<td>219535</td>
<td>0.130</td>
<td>81</td>
</tr>
<tr>
<td>4. Om Elgora</td>
<td>103559</td>
<td>0.061</td>
<td>38</td>
</tr>
<tr>
<td>5. Ganoub Elgezira</td>
<td>263159</td>
<td>0.155</td>
<td>97</td>
</tr>
<tr>
<td>6. Madani Elkobra</td>
<td>200911</td>
<td>0.119</td>
<td>74</td>
</tr>
<tr>
<td>7. Elmanagil</td>
<td>429546</td>
<td>0.253</td>
<td>158</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1694653</strong></td>
<td><strong>1.000</strong></td>
<td><strong>625</strong></td>
</tr>
</tbody>
</table>

Notice:

Note that this was the minimum sample size calculated for Gezira State the researchers might have to increase this size as possible as they wish according to cost, time and efforts for more statistical precision and tests’ conditions (Population Studies Center U o G, 2014).

3.1.5 Study criteria:

3.1.5.1 Inclusion criteria:

- Sudanese nationality.
- Both gender.
- Age group (18 years and above).
- Living in Gezira state for at least 1 year before the time of sampling.
- Apparently free from illnesses and in normal health state.
3.1.5.2 Exclusion criteria:

- Any acute illness within the last two weeks before the day of sampling.
- History of any chronic illness from which the individual is still suffering, e.g. diabetes, hypertension.
- Individuals used anticoagulant drugs.
- History of significant blood loss, blood donation or surgical operation within the last three months before the day of sampling.
- Pre symptomatic carriers of the common diseases that affecting parameters under study, e.g. malaria, silent UTI.
- Modified physiological states that cause changes in parameters under study, e.g. pregnancy, fasting individuals, psychological and mental disorders such as severe stress and depression.
- Common extraneous influences affecting parameters under study e.g. cigarette smoking, alcohol intake.

3.1.6 Data collection tools:

The data was collected by using a questionnaire. A questionnaire was designed to include all needed information (appendix 1).

3.1.7 Data analysis:

Data was analyzed by using Statistical Package for Social Sciences (SPSS) computer program (version 16.0).

3.1.8 Ethical Consideration:

Ethical approval of this study was obtained from the ministry of health in Gezira State. Permission was obtained from health services units in localities. Informed consent was obtained from participants before collection of samples (appendix 2). The specimens and information were collected from the individuals under privacy and confidentiality and were not used for any purposes rather than this study. Each individual included in this study was given his/her tests results.
3.2 Materials:

The following materials were utilized in this study:

- Lab coat.
- Gloves.
- Cotton.
- 70% isopropanol alcohol pad.
- Tourniquet.
- Syringes (5 ml).
- Tri sodium citrate anticoagulant vac container tubes.
- Centrifuge (Hettich, serial number 0114728).
- Automatic micropipettes.
- Tips.
- Eppendorf tubes.
- Coatron M4 coagulometer device (manufactured by TECO medical instruments, production GmbH, Germany. SN M4-2171).
- Coagulometer cuvettes.
- Coagulation controls (From Biosystems, code: 61007 and 61008).
- APTT reagents (Biomed).
- Racks.

3.3 Methods:

3.3.1 Sample collection and preparation:

Blood sample collection was done by clean venepuncture technique using needle and syringe. 2 ml of venous blood was withdrawn in vac container tube containing aqueous tri-sodium citrate anticoagulant (32 g/l), the blood was immediately mixed with anticoagulant by inverting the tube several times, and the tube was labelled. A non-allergenic adhesive spot was placed over the venepuncture site. Then blood samples were centrifuged at 1200 - 2000 rpm for 15 minutes, this provided platelet poor plasma (PPP). Immediately after centrifugation the plasma was removed into an Eppendorf tube and stopper.
3.3.2 Laboratory analysis:

Laboratory analysis was done as soon as possible and within not more than four hours from the time of collection in faculty of medical laboratories sciences, haematology lab. Analysis was done by using Coatron M4 coagulometer for coagulation profile, after commercial controls from BioSystems had been run, according to the manufacturer’s instructions.

- **Coatron M4:**

  Is a highly sensitive 4 channel photo-optical instrument that offers clotting, chromogenic and immune turbid metric testing capabilities. A very bright LED optic ensures accurate and precise results, even when icteric or lipemic samples are used. The receiver signal is detected and converted to an electric current. During the actual test the system is searching for the best signal amplification, therefore it will support a wide range of different reagents i.e. very turbid thromboplastin or very clear reagents. Additionally the software is based on optical density (extinction), which absorbs outside light effects (operation manual).

3.3.3 Activated partial thromboplastin time (APTT) test:

The APTT is a screening test for the intrinsic clotting system, i.e. factors XII, XI, IX, VIII, It will also detect deficiencies of factors, prothrombin, V, X, and fibrinogen. It is mainly used to monitor patients receiving unfractionated heparin anticoagulation.

3.3.3.1 Principle of the test:

Plasma is added to a kaolin-phospholipid suspension and calcium chloride reagent at 37°C and the time taken for a clot to form is measured.

3.3.3.2 Procedure:

Activated partial thromboplastin time was measured by using Coatron M4 coagulometer applied clotting method as the following:

- The (PPP) was prepared for all samples.
- 25 μL of plasma was pipetted to the cuvette.
- Plasma was pre-warmed for 2 min at 37°C in warming wells.
- 25 μL of kaolin-phospholipid reagent was added to plasma, incubate for three min.
- Cuvette was transferred to measuring position.
- Then 25 μL of pre-warmed calcium reagent was added.
- The test was automatically started and the result was displayed in seconds.
Chapter Four
4. Results and Discussion

4.1 Results:

4.1.1 Characterization of the study population:

- **Gender:** From the 657 individuals included in this study, 281 (42.8%) were males and 376 (57.2%) were females (Figure 4-1).

![Gender Distribution](image1.png)

*Figure (4-1): Distribution of the study population according to the gender.*

- **Age groups:** The age groups of the study population were, 193 (29.4%) aged less than 21 years, 283 (43.1%) aged between 21-30 years, 99 (15.1%) aged between 31-40 years and 82 (12.5%) aged more than 40 years (Figure 4-2).

![Age Distribution](image2.png)

*Figure (4-2): Distribution of the study population according to the age groups.*
- **Locality**: All individuals live in Gezira state at the time of study for at least a year, their localities were, 90 (13.7%) from Madani Elkobra, 39 (5.9%) from Om Elgara, 107 (16.3%) from Elhassahesa, 84 (12.8%) from Sharg Elgezira, 98 (14.9%) from Ganoub Elgezira, 71 (10.8%) from Elkamleen and 168 (25.6%) from Elmanagil (Figure 4-3).

![Figure (4-3): Distribution of the study population according to the localities.](image)

- **Occupation**: In the study population, 364 (55.4%) were students, 122 (18.6%) were workers, 155 (23.6%) were employees and 16 (2.4) were other jobs such as private sector and housewives (Figure 4-4).

![Figure (4-4): Distribution of samples according to the occupation.](image)
- **Tribe**: Concerning tribe, 244 (37.1%) were Middle tribes, 234 (35.6%) were Northern tribes, 21 (3.2%) were Western tribes, 7 (1.1%) were Eastern tribes and 151 (23%) were other tribes such as Arabic tribes and West Africa tribes (Figure 4-5).

![Figure (4-5): Distribution of the study population according to the tribe.](image)

- **Education**: The educational level of the study population was, 13 (2%) none educated, 44 (6.7%) primary education, 93 (14.2%) secondary education and 507 (77.1%) university education (Figure 4-6).

![Figure (4-6): Distribution of the study population according to the educational level.](image)
**Female’s marital status:** 274 (72.9%) of females were single and 102 (27.1%) were married (Figure 4-7).

**Figure (4-7):** Distribution of the study population according to the female’s marital status.

**Number of birth:** According to the number of birth in females, 53 (14.1%) were found to birth between 1-3 times, 28 (7.4%) between 4-6 times, 5 (1.3%) more than 6 times and 290 (77.2%) were either non birthed or non-married (Figure 4-8).

**Figure (4-8):** Distribution of the study population according to the number of birth.
4.1.2 The study of results according to standard reference values:

The study results were compared with the international reference values for activated partial thromboplastin time. We found that 585 (89%) of results were within the standard reference values, 7 (1.1%) were below the standard reference values and 65 (9.9%) were above the standard reference values (Table 4-1).

Table (4-1) Activated partial thromboplastin time results according to standard reference values.

<table>
<thead>
<tr>
<th>Results</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>585</td>
<td>89.0</td>
</tr>
<tr>
<td>High</td>
<td>65</td>
<td>9.9</td>
</tr>
<tr>
<td>Low</td>
<td>7</td>
<td>1.1</td>
</tr>
<tr>
<td>Total</td>
<td>657</td>
<td>100.0</td>
</tr>
</tbody>
</table>

4.1.3. Our study reference values:

The reference values for activated partial thromboplastin time (mean ± 2 SD) were found to be 34.54 ± 8.24 seconds for all population, 34.21 ± 7.84 seconds for males and 34.78 ± 8.50 seconds for females (Table 4–2) (appendix 3).

Table (4-2): Reference intervals for activated partial thromboplastin time among the study population.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± 2 SD</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>All population</td>
<td>34.54 ± 8.24</td>
<td>26.30 – 42.78 seconds</td>
</tr>
<tr>
<td>Males</td>
<td>34.21 ± 7.84</td>
<td>26.37 – 42.05 seconds</td>
</tr>
<tr>
<td>Females</td>
<td>34.78± 8.50</td>
<td>26.28 – 43.28 seconds</td>
</tr>
</tbody>
</table>
4.1.4 Effects of the different population factors on activated partial thromboplastin time:

- **Gender:** There was no significant difference in APTT means between males and females in this study (P. value = 0.074) (figure 4-9).

![Figure (4 - 9): Comparison means of APTT between males and females.](image)

- **Locality:** In this study there was highly significant difference in APTT means between population in different localities (P value = 0.000) (figure 4-10).

![Figure (4 – 10): Comparison means of APTT between different localities.](image)
- **Age:** There was highly significant difference in APTT means between different age groups in this study (P value = 0.000) (Figure 4-11).

![Figure 4-11](image)

**Figure (4-11):** Comparison means of APTT between different age groups.

- **Occupation:** In this study there was significant difference in APTT means between different types of occupations (P value = 0.012) (Figure 4-12).

![Figure 4-12](image)

**Figure (4-12):** Comparison means of APTT between different occupations.
• **Education:** There was no significant difference in APTT means between population with different educational levels in this study (P value = 0.649) (Figure 4-13).

![Figure (4-13): Comparison means of APTT between different educational groups.](image)

• **Tribe:** In this study there was no significant difference in APTT means between different tribes (P value = 0.280) (Figure 4-14).

![Figure (4-14): Comparison means of APTT between tribes.](image)
- **Female’s marital status:** There was significant difference in APTT means between single and married females in this study (P value = 0.006) (Figure 4-15).

![Figure 4-15](image)

**Figure (4-15):** Comparison means of APTT between single and married females.

- **Number of birth:** In this study there was no significant difference in APTT between females according to number of birth (P value = 0.248) (Figure 4-16).

![Figure 4-16](image)

**Figure (4-16):** Comparison means of APTT between females according to the number of birth.
4.2 Discussion:

The haematological reference values are very important for diagnostic orientation and treatment decision. The haematological reference values were determined many years ago for the Caucasian’s populations. Recently, several authors tried to establish reference values in haematology for African countries. However there are some discrepancies from one study to another which may be related to different factors such as age, sex, geographic origin, altitude, ethnic origin, and pathologic conditions, influence the haematological values (Kueviakoe, et al 2011).

The activated partial thromboplastin time (APTT) is a central component of any screen for haemostatic patency. The test is used to detect the deficiency of intrinsic clotting factors VIII, IX, XI, and XII, the presence of lupus anticoagulant and the monitoring of unfractionated heparin therapy (Lawrie, et al 2013).

This is the first documented study for activated partial thromboplastin time (APTT) reference values done in the Gezira State from September 2014 to March 2015. A total of 657 healthy adults individuals were participated in this study from different localities in the Gezira State according to inclusion criteria, 281 (42.8%) were males and 376 (57.2%) were females (Figure 4-1) and age varies from 18 years to 60 years with a mean of 27.5 years.

A number of factors were studied for their effect on APTT, Such as sex, age, residence, education, occupation and female’s marital status.

The results obtained in this study can help the doctors to improve their patient’s health condition. According to this study the activated partial thromboplastin time (APTT) for Sudanese healthy adult individuals living in Gezira state should be between 26.30 – 42.78 seconds (Table 4 – 2). These values are similar to those found on textbooks especially practical haematology (Dacie and Lewis, 2011), but the upper limit of APTT in this study is consider higher.

The mean values of APTT among Sudanese healthy adults living in Gezira state is similar to those means obtained in Khartoum, Sudan by Nasma Ali Hassan et al (Hassan, et al 2013) and Sanaa Eltahir Abdalla (Abdalla, et al 2013 ), also is similar to study done in Kebbi State, Nigeria by Erhabor et al (Ehabor, et al 2014), also is similar to study in the Hamilton, Ontario by Maureen Andrew et al (Andrew et al, 1984 ), and study done by Appel et al in the Netherlands (Appel, et al 2012), while higher than means those obtained in Khartoum, Sudan by Mahmoud

In this study when we compared the mean of APTT between males and females, found that, there was no significant different between them, the p. value was (0.074) (figure 4-9), and this agreed with Rania Abd Alrahman that found there was no significant different on APTT between males and females, p. value was > 0.05 (Sadaabi, 2013).

This study showed a highly significant difference on the APTT means between individuals in different localities p. value (0.000) (Figure 4-10). Elkamleen had the highest means while Managil had the lowest means. This difference might be due to the highly difference in number of participated individuals in each locality, environment and life style.

According to this study there was a highly significant difference on the APTT means among the different age groups, p. value (0.000) (Figure 4-11). This study showed a strong correlation between the means of APTT and age, it became shortened with increase in age.

In this study the occupation showed significant difference on the APTT among the study population, p. value was (0.012) (Figure 4-12). The students had the highest mean, followed by the employees, then the workers and the lowest were whom with other occupations. This difference might be due to the difference in sample size of participants in each group or due to the type of occupation and its special environment.

In this study education had no significant difference on APPT mean with p. value (0.649) (Figure 4-13).

Tribes had no effects on this study parameter (APTT), there was no significant difference on the APTT mean among different tribes with p. value of 0.280 (Figure 4-14).
Results from this study showed a significant difference on APTT mean according to female marital status, p. value was (0.006) (Figure 4-15). Single females had higher mean than married females.

This significant difference might be a result of physiological changes in married females let them more susceptible for hypercoagulability state and so had shortened APTT.

In this study number of birth showed no significant difference on the APTT means, with P. value (0.248) (Figure 4-16).
Chapter Five
5. Conclusion and Recommendations

5.1 Conclusion:

- The reference values for APTT among healthy Sudanese adults living in Gezira state were similar to standard reference values, but the upper limit of APTT in this study is significantly higher (26.3 – 42.7 seconds).

- When the observed values of this study were compared with those drawn from different populations in Sudan, Europe and Africa, significant differences were emerged. These differences are not fixed; our results were found to be either lower or higher. Never the less, a number of values were closely similar to values of other countries.

- APTT values in this study had affected by age, localities, occupation and female marital status.

- In this study there were no significant difference in APTT values according to gender, education, tribes and number of birth.

5.2 Recommendations:

- The reference values obtained in this study are recommended to be locally used in the medical practice in Gezira State instead of the Western values quoted from textbooks.

- Reference values for all laboratory tests are very important to be determined locally in Sudan. These reference values should be determined on a larger number of subjects and should cover the different parts of Sudan to reveal significant differences due to geographical and ethnic factors.

- Furthermore studies to establish reference values for coagulation parameters not included in this study, such as thrombin time and fibrinogen level are recommended.
REFERENCES


Appel, IM; Grimminck, B; Geerts, J; Stigter, R; Cnossen, MH and Beishuizen, A (2012). Age dependency of coagulation parameters during childhood and puberty. Journal of thrombosis and haemostasis. 10 (22): 54–63.


Hillman, RS; Ault, KA; and Rinder, HM (2005). Haematology in Clinical Practice. (Fourth edition), McGraw Hill,USA.


Kitchens, CS; Alving, BM; and Kessler, CM; (2002). Consultative Haemostasis and Thrombosis,WB Saunders , USA.


Sadaabi, RA; Mohamed, BA (2013). Estimation of some Haemostatic Parameters among Sudanese Diabetes Mellitus Type 2 Patients in Khartoum state. *Sudan University of Science and Technology*, college of Medical Laboratory Science 56p.
Appendix (1)

Questionnaire

Determination of reference values for Activated Partial Thromboplastin Time in Healthy Sudanese Adults in Gezira State, Sudan

Date: ........

Time: ............... (am / pm) NO.........................

Name: ........................................................... Age: ..............................

Locality: ................................. Phone number: ........................

Tribe: ............................... Occupation: ........................

Sex: male ( ) female ( )

Social status: single ( ) married ( )

Number of birth: 1-3 ( ) 4-6 ( ) more than 6 ( )

Education: Non ( ) Primary ( ) Secondary ( ) University ( )

RESULTS:

Urine culture: positive ( ) Negative ( )

Blood film for malaria: positive ( ) Negative ( )

APTT: .............................................. Seconds.
Appendix (2)

Informed consent

بسم الله الرحمن الرحيم

جامعة الجزيرة

كلية علوم المختبرات الطبية

برنامج ماجستير علوم المختبرات الطبية

تخصص أمراض الدم

الاسم: ...........................................

سوف يتم اخذ عينة دم من الوري دم يعتبر 2.5 مل بواسطة حقنة طعن. وذلك بعد مسح
منطقة اخذ العينة بواسطة المطهر. كل الأدوات المستخدمة لأخذ العينة محكمة ومسقعة فيها
كل وسائل السلامة العملية، وليس هناك آثار جانبية للعملية.

ربما يحصل لتورم بسيط في منطقة اخذ العينة وسوف يزول بعد فترة قصيرة.

أوافق أنا المذكور أعلاه على التبرع بعينة الدم لإجراء هذه الدراسة.

الإمضاء: ...........................................

التاريخ: ...........................................
Appendix (3)

Normal curves histograms for APTT

Figure (4-17): showed histogram with normal curve for APTT in all population.

Figure (4-18): showed histogram with normal curve for APTT in males.
Figure (4-19): showed histogram with normal curve for APTT in females.