Title:

Determination of Haematological and Hormonal changes in Females with Thyroid Disorders at Reproductive Age at Al Mak Nimir University Hospital - Shendi – River Nile State - Sudan

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

2017
قال تعالى:

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Thesis title: Determination of Haematological & Hormonal changes in Females with Thyroid Disorders at Reproductive Age at Almak Nimir University Hospital- Shendi, River Nile State - Sudan

Degree program: PhD in Haematology.

Field of Study: Haematology

Supervisor: Prof. Gamal Mahmoud Alimairi.

Academic years: 2011-2017

Key wards: Hypothyroidism, Hyperthyroidism, \textit{CBC, PT, PTT, TSH, FSH, Prolactin}. 
Dedication

To my Father Soul
Mother,
Husband,
Daughters & Son
Brothers & Sister
All
Families

Teachers & Students

To all my colleagues in Shendi University

I dedicate this simple effort with my love and best wishes
Acknowledgements

All thanks to Allah from the start to the end…..

I would like to express my thanks and gratefulness to my supervisor Prof Gamal Mahmoud Alimairi for his guidance and support during conducting this study.

I am deeply indebted to Mr. Elfatih Mohammed Abdalla for his help and continuous encouragement to make this research possible, and to all the staff of the Modern Medical Analysis Centre for their help.

My special thanks to the members and staff of the laboratory of Elmek Nimir University Hospital for their help, also to the members and staff of Shendi University for their effort and support. I thank Dr. Osama Khedir for his help in collecting the sample from patients.

Finally my appreciations are extended to all those who helped me in the research.
English Abstract:

**Background:**
Thyroid hormones have a crucial role in metabolism and proliferation of blood cells. Thyroid dysfunction induces different effects on blood cells such as anaemia, erythrocytosis, leukopaenia, thrombocytopaenia, and in rare cases causes’ pancytopenia. It alters RBC indices including (MCV, MCH, MCHC and RDW), affects coagulation system and alters the level of hormones of reproduction.

**Objectives:**
This study aimed to evaluate the effect of noncancerous thyroid disorders on blood cells count and RBC indices, WBCs t & differential, platelet and MPV and some coagulation profile (PT and APTT) to correlate thyroid disorders & complications of pregnancy (abortions and deep venous thrombosis) and to determine the effect of thyroid disorders on Prolactin and FSH hormones in females at reproductive age.

**Materials and methods:**
The study includes (150) females (60) with hypothyroidism, (40) with hyperthyroidism and (50) healthy females as control group. The range of ages was (18 - 48) years. The study was conducted in Elmek Nimir University Hospital in the referred clinic of medicine after confirmation of diagnosis by estimation of TSH, T₃ and T₄ levels and before receiving treatment, then (6 ml) of venous blood was collected from each female after accepting the consent form and filling the questionnaire, during the period from 2014 to 2017. The blood dispensed into three blood containers, (2.25 ml) in trisodium citrate then centrifuged to obtain plasma for coagulation profile study, (2.5 ml) in lithium heparin and centrifuged to obtain plasma for hormonal estimation and (1.25) in EDTA anticoagulant for complete blood count. Then, complete blood count was measured by auto-haematology analyzer; coagulation profile was performed by coagulyzer and hormones by automatic immunoassay system. Finally obtained results were analyzed by computer program SPSS software version (20).
Results
Analysis of the obtained data revealed that Hb showed statistically significant difference between female with thyroid disorders compared with control group (P-value <0.05). Red blood cells count showed no difference between female with thyroid disorders compared with control group (P-value >0.05), most red blood cell indices and parameters including PCV were statistically significant difference between the two groups of females and control (P-value <0.05). MCV and MCH showed no significant difference between hypothyroidism and control (P-value >0.05), but had significant difference between hyperthyroidism and control (P-value <0.05). RDW in these two groups showed statistically significant difference with control group (P-value <0.05).

Comparison of the two groups of females under study with the control group did not show statistically significant difference in WBC (P-value >0.05).

In the differential count of WBCs only the neutrophil in hyperthyroidism and monocyte in hypothyroidism & basophil in the two groups showed statistically significant differences.

The study did not show statistically significant difference in PLT count in the two groups of females and control (P-value >0.05), but MPV had significant difference in hypothyroidism (P-value <0.05).

The result showed statistically significant difference in PT & INR (P-Value <0.05), and not in PTT, although it was decreased when compared to the control group.

Regarding to this study (22) females with hypothyroidism had a history of abortion (36.7%). In hyperthyroidism (13) females had abortion (32.5%), one female had deep venous thrombosis (DVT, 2.5%). But another female had menorrhagia (2.5%).

The results showed that the difference in prolactin level between
hyperthyroidism and control is statistically significant \((P.Value < 0.05)\), but did not show any significant difference between hypothyroidism and control group \((P-Value > 0.05)\).

There was no significant difference between the two groups of females in the follicular phase of \(FSH\) compared with the control \((P-value > 0.05)\) but the \(FSH\) in females with hypothyroidism was within normal range, whereas in females with hyperthyroidism was higher than the normal range, \((10.5, 31.3\) and \(NR: 4.5-11\) respectively. In midcycle the level of \(FSH\) in hypothyroidism and hyperthyroidism within normal range \((5.9, 15.9\) and \(NR: 3.6-20.6\) respectively. \(FSH\) level in luteal phase compared with the normal range was high \((hypothyroidism 30.8, hyperthyroidism 37.6 and NR: 1.5-10.8)\) and showed significant difference when compared with control group \((P-value > 0.05)\).

**Conclusion & recommendations:**

The study concluded that haemoglobin was low, Platelet count was slightly decreased, minor coagulation abnormalities were observed in thyroid disorders noncancerous compared with control. There were disturbances in hormones of reproduction that lead to defect in menstrual cycle and then lead to problems in reproduction (infertility). So the study recommended to screening the female patients with hypothyroidism and hyperthyroidism for haematological changes to avoid the anaemia, coagulation defect, to decrease the risk of such complications (bleeding tendency, thrombosis) to avoid the problems of reproduction.

**Key Words:** Hypothyroidism, Hyperthyroidism, \(CBC, PT, PTT, TSH, FSH,\) Prolactin
الخلاصة

خلفية:

تلعب هورمونات الغدة الدرقية دورًها مهماً في استقلاب وتائر خلايا الدم. بسبب الخلل في وظيفة الغدة تتأثر مختلفة في خلايا الدم مثل الأنيميا، نقص الخلايا البيضاء، نقص الصفائح الدموية ونحو.

 حالات نادرة نقص في كل الخلايا. وايضاً يثير في معاملات كريات الدم الحمراء مثل متوسط حجم الكره ونهر ملتوسط هيموغلوبين الكره وتوزيع هيموغلوبين الكريه وتوزيع الخلايا أيضاً يؤثر في تخثر الدم ومستوى هورمونات الإنجاب.

الأهداف:

صممت هذه الدراسة بهدف تقييم تأثيرات ضرر اعراض الغدة الدرقية الفعال سرطانية على خلايا الدم ومعاملات كريات الدم الحمراء و كريات الدم البيضاء والعد التفريقي لها والصفائح الدموية ومتوسط حجم الصفائح وايضاً بعض اختبارات تخثر (زمن البروتامين والترموبلاستين) لربط العلاقة بين مشاكل النزف وحودة الجلطة وايضاً قياس هورمونات الإنجاب (هرمون البرولاكتين والهرمون المنبه للجريب) عند الإناث في فترة الإنجاب.

المواضو والطريقة:

شملت الدراسة 150 إمرأة (60) منهم مصابات بنقص نشاط الغدة الدرقية و(40) بزيادة نشاط الغدة و (50) صححيات كعينة ضابطة. تتراوح أعمارهن من (18 إلى 48) سنة واجريت الدراسة في مستشفى المركز الجامعي. أولاً تم قياس هورمونات الغدة ونهر الدم وريدي من كل أنثى بعد مواجهتها على الإقراض ومنع الأسباب في الفترة من 2014 إلى 2017. تم توزيع الدم على ثلاثة حاويات (2.25) مل في مضاد تجلط سترات الصوديوم الثلاثي وتم تدوير العينة للحصول على البلازما لإجراء اختبارات التجلط و(2.5) مل في هيبارين وايضاً تدويرها للحصول على البلازما لقياس هورمونات (2.5) مل فيදین لقياس هورمونات الدم الكامل ومن ثم قياس الفحص الكامل للدم بجهات التحليل الذاتي لدم وفحصات التحليل بواسطة جهاز التحليل و أيضاً تم قياس هورمونات الإنجاب وتم تحليل النتائج المحصلة عليها بواسطة برنامج الحزم الإحصائي (20).

النتائج:

أوضحت النتائج المحصلة عليها أنه يوجد فرق ذو دلالة إحصائية في مستوى متوسط تركيز الهيموغلوبين عند الإناث المصابات بنقص أو زيادة نشاط الغدة عند مقارنتها بالعينة الضابطة لأن قيمة الثقة أقل من (0.05).

تعداد كريات الدم الحمراء لم يوضح فرق ذو دلالة إحصائية عند الإناث المصابات والعينة الضابطة. ومعظم معاملات كريات الدم الحمراء مثل حجم الكره الحشوي يوجد فرق ذو دلالة إحصائية بين...
المجموعتين والعينتين الضابطتين، متوسط حجم الكرية ومتوسط هيموغلوبين الكرية لا يوجد فرق عند الإناث المصابات بنقص نشاط الغدة لكن يوجد فرق مع المصابات بزيادة نشاط الغدة والعينات الضابطتين، وأظهرت الدراسة أنه يوجد فرق في حجم توزيع خلايا الدم الحمراء لأن قيمة الثقة أقل من 0.05.

عند مقارنة مجموعة الدراسة مع العينة الضابطية لا يوجد فرق في تعداد كريات الدم البيضاء قيمة الثقة أكبر من 0.05.أما في العد التفريقي أوضحت الخلايا العدلة فرق في حالة زيادة نشاط الغدة وحيدة النواة في حالة نقص نشاط الغدة، وكذلك الخلايا القاعدية في المجموعتين أوضحت فرق ذو دلالة إحصائية.

لم توضح الدراسة فرق ذو دلالة إحصائية في تعداد الصفحات الدموية في المجموعتين بالمقارنة مع العينة الضابطية لأن قيمة الثقة أكبر من 0.05، لكن يوجد فرق في متوسط حجم الصفحات مع نقص نشاط الغدة قيمة الثقة أقل من 0.05.

أوضحت الدراسة فرق ذو دلالة إحصائية في زمن الثرومين في حالة نقص أو زيادة نشاط الغدة عند مقارنتها مع العينة الضابطية، ولا يوجد فرق في زمن الثرومين بلاستين بالرغم نشاط الغدة مقارنة بالعينه الضابطية قيمة الثقة أقل من 0.05.

أيضًا بالرجوع للدراسة نجد أن (22) أنثى مصابات بنقص نشاط الغدة (36.7%) لديهن مشاكل نزف كالإجهاض و(13) أنثى مصابات بزيادة نشاط الغدة (32.5%) لديهن إجهاض وأثنتي واحدة مصابة بجلطة (2.5%) وأثنتي مصابة بنزف الشعر (2.5%).

أوضح الدراسة وجود فرق ذو دلالة إحصائية في هرمون البرولاكتين بين الإناث المصابات بزيادة نشاط الغدة والعينات الضابطتين قيمة الثقة أقل من 0.05، ولا يوجد فرق في حالة نقص نشاط الغدة قيمة الثقة أكبر من 0.05.

لا يوجد فرق ذو دلالة إحصائية بين مجموعتي الدراسة في مستوي الهرمون المحفز للجريبات في الطور البيضاري للدوة مقارنة مع العينة الضابطية قيمة الثقة أقل من 0.05. لكن مستوي الهرمون في حالة نقص نشاط الغدة في المدي الطبيعي بينما في حالة زيادة نشاط الغدة أعلى من المدي الطبيعي (4.5-11) والمدي الطبيعي (10.5و31.5) والدبي الطبيعي (5.9و15.9) والمدي الطبيعي (6.3-20) على التوالي. وفي الطر مشاكل في حالة نقص نشاط الغدة (30.8) وزيادة نشاط الغدة (37.6) والمدي الطبيعي أعلى في حالة نقص نشاط الغدة (10.8) وأوضح فرق ذو دلالة إحصائية عند مقارنته بالعينة الضابطية قيمة الثقة أقل من 0.05.
الخاتمة والتصويت:
توصلت الدراسة إلى أن مستوي الهيموغلوبين أقل، نقص في الصفحات الدموية وتلك الاضطرابات بسيطة في جهاز التجلط في حالة اضطرابات الغدة الدرقية غير السرطانية مقارنة مع العينة الضابطة.
كذلك يوجد خلل في مستوى هورمونات الإنجاب والتي تؤدي إلى خلل في الدورة الشهرية ثم تؤدي إلى مشاكل في الإنجاب (العقم). أيضاً أوصت الدراسة بالكشف للإناث المصابات بنقص أو زيادة نشاط الغدة للتهيجات الدموية لتجنب حدوث الإنيميا ومشاكل التجلط لتقليل المضاعفات (مشاكل النزف وحدوث الجلطات) وكذلك لتجنب مشاكل الإنجاب.
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**Chapter three**

**Materials And Methodology**

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<tr>
<td>Hb-A</td>
<td>Adult Haemoglobin</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen-Presenting Cells</td>
</tr>
<tr>
<td>ART</td>
<td>Assisted Reproductive Technology</td>
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<tr>
<td>AITD</td>
<td>Autoimmune Thyroid Disease</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>Carbon Dioxide</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster Differentiation</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
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<tr>
<td>CFU</td>
<td>Colony –Forming Unit</td>
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<td>CBC</td>
<td>Complete Blood Count</td>
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<td>COH</td>
<td>Controlled Ovarian Hyperstimulation</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>FL</td>
<td>Femtoliter</td>
</tr>
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<td>Fe^{2+}</td>
<td>Ferrous</td>
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<td>luteinizing hormone</td>
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<td>Major Histocompatibility II</td>
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<td>MCH</td>
<td>Mean Corpuscular Haemoglobin</td>
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<td>MCHC</td>
<td>Mean Corpuscular Haemoglobin Concentration</td>
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<td>MCV</td>
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<td>MPV</td>
<td>Mean Platelet Volume</td>
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<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic acid</td>
</tr>
<tr>
<td>MUP</td>
<td>Methyl lumbelliferyl Phosphate</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<td>µg,</td>
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<tr>
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<td>MicroLiter</td>
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<td>µU/ml</td>
<td>Microunit per milliliter</td>
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<td>min</td>
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<tr>
<td>Mol/L</td>
<td>Mole/Liter</td>
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<tr>
<td>M-CSF</td>
<td>Monocyte–Coloncy Stimulating Factor</td>
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<tr>
<td>ng/mL</td>
<td>Nanogram/milliliter</td>
</tr>
<tr>
<td>ng/dl</td>
<td>nanograms per deciliter</td>
</tr>
<tr>
<td>NR</td>
<td>Normal Range</td>
</tr>
<tr>
<td>O\textsubscript{2}</td>
<td>Oxygen</td>
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<tr>
<td>PCV</td>
<td>Packed Cell Volume</td>
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<td>Parathyroid Hormone</td>
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<td>PTT</td>
<td>Partial Thromboplastin Time</td>
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<td>Pg/d</td>
<td>Picogram/day</td>
</tr>
<tr>
<td>pg/d</td>
<td>picograms per day</td>
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<tr>
<td>PCOS</td>
<td>Polycystic Ovarian Syndrome</td>
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<tr>
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<td>Polymorphonuclear</td>
</tr>
<tr>
<td>PRL</td>
<td>Prolactin</td>
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<tr>
<td>PTU</td>
<td>Propylthiouracil</td>
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<td>PR</td>
<td>Prothrombin ratio</td>
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<td>RAIU</td>
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<td>RBCs</td>
<td>Red Blood Cells</td>
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<td>RDW</td>
<td>Red Cell Distribution Width</td>
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<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>rpm</td>
<td>Round per minute</td>
</tr>
<tr>
<td>Sec</td>
<td>Second</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SHBG</td>
<td>Sex Hormone-Binding Globulin</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
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<tr>
<td>SOCS</td>
<td>Suppressors of cytokine signaling</td>
</tr>
<tr>
<td>Tg</td>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>TgAb</td>
<td>Thyroglobulin Antibody titer</td>
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<td>THBR</td>
<td>Thyroid hormone binding ratio</td>
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<td>TMAb</td>
<td>Thyroid microsomal antibody titer</td>
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<td>TSH</td>
<td>Thyroid stimulating Hormone</td>
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<td>TRH</td>
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<td>T₄</td>
<td>Thyroxine</td>
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<tr>
<td>TBG</td>
<td>Thyroxine binding globulin</td>
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<td>TGF-β</td>
<td>Transforming growth factor-β</td>
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<td>Tuberoinfundibulum</td>
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<td>TNF</td>
<td>Tumor necrosis factor</td>
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<td>US</td>
<td>United state</td>
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<td>VWF</td>
<td>Von Willebrand factor</td>
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<td>WBC</td>
<td>White Blood Cell</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>IFN-γ</td>
<td>γ-interferon</td>
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<tr>
<td>δ-ALA</td>
<td>δ-aminolaevulinic acid</td>
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Chapter One

Introduction
Justification
Objectives
1-1: Introduction

The thyroid is a small gland located below the skin and muscles at the front of the neck. *Thyroid Stimulating Hormone (TSH)* controls the thyroid gland by inducing the transport of iodine into the gland, and then the subsequent secretion of thyroxine *(T4)* and *Triiodothyronine (T3)* into circulation. *(T3)* is the most active metabolite, followed by *(T4)* and then the inactive reverse *(rT3)*. The thyroid affects nearly every organ system in the body and appears to be a major regulator of metabolism. Low *(T3)* is seen with malnutrition, anorexia, severe burns, and febrile illnesses. The thyroid produces hormones that play key roles in growth and development, changes in thyroid function can have a major effect on reproductive function before, during and after conception. Thyroid disease is a common endocrinopathy found in (1%) of women of reproductive age. The prevalence of hypothyroidism in women in the reproductive age (20-40 years) varies between (2 and 4 %.). *(1, 2)* In this age group, *autoimmune thyroid disease (AITD)* is the most common cause of hypothyroidism. *(3, 4)* *Hypothyroidism* is associated with a broad spectrum of reproductive disorders ranging from abnormal sexual development through menstrual irregularities to infertility. *(5, 6)* *Hypothyroidism* is associated with increased production of *thyroid releasing hormone (TRH)*, which stimulates pituitary to secrete *TSH* and *prolactin (PRL)*. *Hypothyroidism* (underactive thyroid) affects about (0.5%) of women of reproductive age. Also the thyroid disease is associated with an increased risk of problems during pregnancy, including miscarriage, preeclampsia, poor fetal growth, premature birth and stillbirth.

Both overactive and underactive thyroid can have significant effects on reproductive function. *(7)* The prevalence of hyperthyroidism is about (1%) and it is
about (6 – times) more common in women, (3) and can cause a woman to have difficulties in not only getting pregnant, but also had complications.

**Thyroid Disorders in Women:**
Thyroid problems can affect female patients at any age. The functions of the thyroid gland have much to do with a woman's reproductive system, particularly if the thyroid is overactive or underactive. Effects of this imbalance in hormone levels may have the following effects on a woman's body:

**Puberty and menstruation:**
Thyroid disorders can cause abnormally early or late onset of puberty and menstruation. In addition, abnormally high or low levels of thyroid hormone can cause very light, very heavy menstrual periods, or very irregular menstrual periods, or amenorrhea).

**Reproduction:**
An overactive or underactive thyroid may also affect ovulation (the release of an egg for fertilization). Thyroid disorders may prevent ovulation from occurring at all. In addition, the ovaries are at an increased risk for cyst development if the woman has an underactive thyroid (hypothyroid). Severe hypothyroidism can actually cause milk production in the breast, while preventing ovulation.

**Pregnancy and postpartum:**
Thyroid disorders during pregnancy can harm the fetus and may lead to thyroid problems in the mother after birth, such as postpartum thyroiditis.

**Menopause:**
Thyroid disorders may cause the early onset of menopause (before age 40 or in the early 40s). In addition, some symptoms of hyperthyroidism (overactive thyroid), such as lack of menstruation, hot flashes; insomnia, and mood swings may be mistaken for early menopause. Treating hyperthyroidism sometimes can alleviate symptoms of, or the actual onset of, early menopause. Millions of people in United
State (US) have thyroid disease, most of them are women. About (200) million people in the world have some form of thyroid disease.
1-2: Justification:
Thyroid diseases become a worldwide problem, at the same time insufficiency of interrelation informations about the topic pushed me to decide going on trying to fill up the gaps if possible. On the other hand, in Sudan no studies were handled or carried out in details before concerning and detailing this topic, so this encouraged me to perform and to conduct more further and detailed studies, to try to find out the alterations, interrelations and variations profiles. therefore, a hospital based study is to be conducted to determine the haematological parameters, coagulation and hormonal abnormalities by performing complete blood count, coagulation studies (PT&PTT) and to estimate the hormone prolactin and FSH in women with thyroid disorders at reproductive age (18-48 years), at Almek Nimir University Hospital. Abnormalities in thyroid function can have an adverse effect on reproductive health and results in reduced rates of conception, increased miscarriage risk, adverse pregnancy and neonatal outcomes. The thyroid produces hormones that play key roles in growth and development, changes in thyroid function can have a major effect on reproductive function before, during and after conception. Thyroid disorders are associated with haematological abnormalities and with various abnormalities in coagulation system.
1-3: Objectives:

1.3.1: General objective:
To determine haematological and hormonal changes in females with thyroid disorders (non-cancerous) at reproductive age (18-48 years) at Almek Nimir University Hospital.

1.3.2: Specific objectives:
1. To evaluate effects of thyroid disorders on blood cells count and red blood cells indices (complete blood count).
2. To estimate the frequency of anaemia in thyroid disorders.
3. To estimate the prothrombin and partial thromboplastin time in females with thyroid disorders.
4. To correlate between bleeding tendency and deep venous thrombosis in thyroid disorders.
5. To evaluate the primary and secondary haemostasis in females with thyroid disorders.
6. To estimate the prolactin hormone in females with thyroid disorders.
7. To estimate the FSH hormone in females with thyroid disorders.
Chapter Two

Literature Review
2-1: History

thyroid was first identified by the anatomist Thomas Wharton in 1656.\(^8\) Thyroxine was identified only in the 19\(^{th}\) century.

2-2: Anatomy:

The thyroid gland is a butterfly-shaped organ and is composed of two cone-like lobes or wings, lobus dexter (right lobe) and lobus sinister (left lobe), connected via the isthmus. The organ is situated on the anterior side of the neck, lying against and around the larynx and trachea, reaching posteriorly the oesophagus and carotid sheath. It starts cranially at the oblique line on the thyroid cartilage (just below the laryngeal prominence, or 'Adam's Apple'), and extends inferiorly to approximately the 5\(^{th}\) or 6\(^{th}\) tracheal ring.\(^9\) It is difficult to demarcate the gland's upper and lower border with vertebral levels because it moves position in relation to these during swallowing. The thyroid gland is covered by a fibrous sheath, the capsula glandulæ thyroidea, composed of an internal and external layer. The external layer is anteriorly continuous with the lamina pretrachealis fasciae cervicalis and posterior laterally continuous with the carotid sheath. The gland is covered anteriorly with infrahyoid muscles and laterally with the sternocleidomastoid muscle also known as sternomastoid muscle. On the posterior side, the gland is fixed to the cricoid and tracheal cartilage and cricopharyngeus muscle by a thickening of the fascia to form the posterior suspensory ligament of Berry.\(^{10}\)\(^{11}\) the thyroid glands firm attachment to the underlying trachea is the reason behind its movement with swallowing.\(^{12}\) In variable extent, Lalouette's Pyramid, a pyramidal extension of the thyroid lobe, is present at the most anterior side of the lobe. In this region, the recurrent laryngeal nerve and the inferior thyroid artery pass next to or in the ligament and tubercle. Between the two layers of the capsule and on the posterior side of the lobes, there are on each side two parathyroid glands.
Examination Committee Members

Thesis

Determination of Haematological and Hormonal changes in Females with Thyroid Disorders at Reproductive Age at Al Mak Nimir University Hospital - Shendi – River Nile State - Sudan

Supervisor:

Prof. Gamal Mahmoud Alimairi

External Examiner:

Dr. Sufian Khalid

Internal Examiner:

Prof. Rashid Eltabey Abdalla
The thyroid isthmus is variable in presence and size, and can encompass a cranially extending pyramid lobe (lobus pyramidalis or process suspyramidalis), remnant of the thyroglossal duct. The thyroid is one of the larger endocrine glands, weighing (2-3 grams) in neonates and (18-60 grams) in adults, and is increased in pregnancy. The thyroid is supplied with arterial blood from the superior thyroid artery, a branch of the external carotid artery, and the inferior thyroid artery, a branch of the thyrocervical trunk, and sometimes by the thyroidima artery, branching directly from the brachiocephalic trunk. The venous blood is drained via superior thyroid veins, draining in the internal jugular vein, and via inferior thyroid veins, draining via the plexus thyroideus impar in the left brachiocephalic vein. Lymphatic drainage passes frequently the lateral deep cervical lymph nodes and the pre- and paratracheal lymph nodes. The gland is supplied by parasympathetic nerve input from the superior laryngeal nerve and the recurrent laryngeal nerve.\(^{12}\)

**Figure (2-1): Anatomy of thyroid gland\(^{12}\)**
2-3: Evolution:

Thyroid cells phylogenetically derived from primitive iodide-concentrating gastroenteric cells (endostyle) which, during evolution, migrated and specialized in uptake and storage of iodine in follicular cellular structures, also in order to adapt the organisms from iodine-rich sea to iodine-deficient land. Venturi et al suggested that iodide has an ancestral antioxidant function in all iodide-concentrating cells from primitive algae to more recent vertebrates. \(^{(13)}\) In 2008, this ancestral antioxidant action of iodides has been experimentally confirmed by Küpper et al. \(^{(14)}\) Since (700) million years ago thyroxine is present in fibrous exoskeletal scleroproteins of the lowest invertebrates (Porifera and Anthozoa), without showing any hormonal action. When some primitive marine chordates started to emerge from the iodine-rich sea and transferred to iodine-deficient fresh water and finally land, their diet became iodine deficient. Therefore, during progressive slow adaptation to terrestrial life, the primitive vertebrates learned to use the primitive thyroxine in order to transport antioxidant iodide into the cells. Therefore, the remaining triiodothyronine \((T_3)\), the real active hormone, became active in the metamorphosis and thermogenesis for a better adaptation of the organisms to terrestrial environment (fresh water, atmosphere, gravity, temperature and diet). In fact, the U.S. Food and Nutrition Board and Institute of Medicine recommended daily allowance of iodine ranges from (150 micrograms /day) for adult humans to (290 micrograms /day) for lactating mothers. However, the thyroid gland needs no more than (70 micrograms /day) to synthesize the requisite daily amounts of \((T_4)\) and \((T_3)\). These higher recommended daily allowance levels of iodine seem necessary for optimal function of a number of body systems, including lactating breast, gastric mucosa, salivary glands, oral mucosa, thymus, epidermis, choroid plexus and brain, \(^{(15)}\) etc. \(^{(16)(17)(18)}\)
2-4: Embryological development:

In the fetus, at (3–4 weeks) of gestation, the thyroid gland appears as an epithelial proliferation in the floor of the pharynx at the base of the tongue between the tuberculum impar and the copula linguæ at a point later indicated by the foramen cecum. The thyroid then descends in front of the pharyngeal gut as a bilobed diverticulum through the thyroglossal duct. Over the next few weeks, it migrates to the base of the neck. During migration, the thyroid remains connected to the tongue by a narrow canal, the thyroglossal duct. Thyrotropin-releasing hormone TRH and thyroid-stimulating hormone TSH start being secreted from the fetal hypothalamus and pituitary at (18-20 weeks) of gestation, and fetal production of thyroxine $T_4$ reach a clinically significant level at (18–20 weeks).  

Fetal triiodothyronine $T_3$ remains low (less than 15 ng/dL) until (30 weeks) of gestation, and increases to (50 ng/dL) at term.  

Fetal self-sufficiency of thyroid hormones protects the fetus against e.g. brain development abnormalities caused by maternal hypothyroidism.  

However, preterm births can suffer neuro-developmental disorders due to lack of maternal thyroid hormones due their own thyroid being insufficiently developed to meet their postnatal needs. The portion of the thyroid containing the parafollicular C cells, those responsible for the production of calcitonin, are derived from the neural crest. This is first seen as the ultimobranchial body, which joins the primordial thyroid gland during its descent to its final location in the anterior neck. Aberrations in embryological development can cause various forms of thyroid dysgenesis.
2-5: Histology:

At the microscopic level, there are three primary features of the thyroid: (22)

Table (2-1): histology of the thyroid:

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicles</td>
<td>The thyroid is composed of spherical follicles that selectively absorb iodine (as iodide ions, I) from the blood for production of thyroid hormones, but also for storage of iodine in thyroglobulin, in fact iodine is necessary for other important iodine-concentrating organs as breast, stomach, salivary glands, thymus etc. (see iodine in biology). Twenty-five percent of all the body's iodide ions are in the thyroid gland. Inside the follicles, colloid serves as a reservoir of materials for thyroid hormone production and, to a lesser extent, acts as a reservoir for the hormones themselves. Colloid is rich in a protein called thyroglobulin.</td>
</tr>
<tr>
<td>Thyroid epithelial cells (or &quot;follicular cells&quot;)</td>
<td>The follicles are surrounded by a single layer of thyroid epithelial cells, which secrete $T_3$ and $T_4$. When the gland is not secreting $T_3/T_4$ (inactive), the epithelial cells range from low columnar to cuboidal cells. When active, the epithelial cells become tall columnar cells.</td>
</tr>
<tr>
<td>Parafollicular cells (or &quot;C cells&quot;)</td>
<td>Scattered among follicular cells and in spaces between the spherical follicles is another type of thyroid cell, parafollicular cells, which secrete calcitonin.</td>
</tr>
</tbody>
</table>
2-6: Physiology:

The primary function of the thyroid is production of the hormones triiodothyronine $T_3$, thyroxine $T_4$, and calcitonin. Upto (80%) of the $T_4$ is converted to $T_3$ by peripheral organs such as the liver, kidney and spleen. $T_3$ is several times more powerful than $T_4$, which is largely a prohormone, perhaps four $^{(23)}$ or even ten times more active. $^{(24)}$

![Thyroid system diagram](image)

**Figure (2-2): Thyroid system**
The system of the thyroid hormones $T_3$ and $T_4$.\(^{(25)}\)

**Figure (2-3): The system of the thyroid hormones $T_3$ and $T_4$.\(^{(25)}\)**

Synthesis of the thyroid hormones, as seen on an individual thyroid follicular cell.\(^{(26)}\)
- Thyroglobulin is synthesized in the rough endoplasmic reticulum and follows the secretory pathway to enter the colloid in the lumen of the thyroid follicle by exocytosis.

**$T_3$ and $T_4$ regulation:**

The production of thyroxine and triiodothyronine is regulated by thyroid-stimulating hormone $TSH$, released by the anterior pituitary. The thyroid and thyrotropes form a negative feedback loop: $TSH$ production is suppressed when the $T_4$ levels are high. The $TSH$ production itself is modulated by thyrotropin-releasing hormone $TRH$, which is produced by the hypothalamus and secreted at an increased rate in situations such as cold exposure (to stimulate thermogenesis). $TSH$ production is blunted by somatostatin $SRIH$, rising levels of glucocorticoids and sex hormones (oestrogen and testosterone), and excessively high blood iodide concentration. An additional hormone produced by the thyroid contributes to the
regulation of blood calcium levels. Parafollicular cells produce calcitonin in response to hypercalcaemia. Calcitonin stimulates movement of calcium into bone, in opposition to the effects of parathyroid hormone *PTH*. However, calcitonin seems far less essential than *PTH*, as calcium metabolism remains clinically normal after removal of the thyroid (thyroidectomy), but not the parathyroids.

**2-7: Thyroid function test:**

**Table (2-2): Thyroid function tests:**

<table>
<thead>
<tr>
<th>Test</th>
<th>Abbreviation</th>
<th>Normal ranges&lt;sup&gt;(27)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum thyrotropin/thyroid-stimulating hormone</td>
<td>TSH</td>
<td>0.3–3.0 μU/ml</td>
</tr>
<tr>
<td>Free thyroxine</td>
<td>FT&lt;sub&gt;4&lt;/sub&gt;</td>
<td>7–18 ng/l = 0.7–1.8 ng/dl</td>
</tr>
<tr>
<td>Serum triiodothyronine</td>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.8–1.8 μg/l = 80–180 ng/dl</td>
</tr>
<tr>
<td>Radioactive iodine-123 uptake</td>
<td>RAIU</td>
<td>10–30%</td>
</tr>
<tr>
<td>Radiiodine scan (gamma camera)</td>
<td>N/A</td>
<td>N/A - thyroid contrasted images</td>
</tr>
<tr>
<td>Free thyroxine fraction</td>
<td>FT&lt;sub&gt;4&lt;/sub&gt;F</td>
<td>0.03–0.005%</td>
</tr>
<tr>
<td>Serum thyroxine</td>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>46–120 μg/l = 4.6–12.0 μg/dl</td>
</tr>
<tr>
<td>Thyroid hormone binding ratio</td>
<td>THBR</td>
<td>0.9–1.1</td>
</tr>
<tr>
<td>Free thyroxine index</td>
<td>FT&lt;sub&gt;4&lt;/sub&gt;I</td>
<td>4–11</td>
</tr>
<tr>
<td>Free triiodothyronine I</td>
<td>FT&lt;sub&gt;3&lt;/sub&gt;</td>
<td>230–619 pg/d</td>
</tr>
<tr>
<td>Free T3 Index</td>
<td>FT&lt;sub&gt;3&lt;/sub&gt;I</td>
<td>80–180</td>
</tr>
<tr>
<td>Thyroxine-binding globulin</td>
<td>TBG</td>
<td>12–20 ug/dl T4 +1.8 μg</td>
</tr>
<tr>
<td>TRH stimulation test</td>
<td>Peak TSH</td>
<td>9–30 μIU/ml at 20–30 min.</td>
</tr>
<tr>
<td>Serum thyroglobulin I</td>
<td>Tg</td>
<td>0-30 ng/m</td>
</tr>
<tr>
<td>Thyroid microsomal antibody titer</td>
<td>TMAb</td>
<td>Varies with method</td>
</tr>
<tr>
<td>Thyroglobulin antibody titer</td>
<td>TgAb</td>
<td>Varies with method</td>
</tr>
</tbody>
</table>

- μU/ml = mU/l, microunit per milliliter - ng/dl, nanograms per deciliter
• μg, micrograms - pg/d, picograms per day,
• μIU/ml = mIU/l, micro-international unit per milliliter

2-8: Significance of iodine:

In areas of the world where iodine is lacking in the diet the thyroid gland can become considerably enlarged, a condition called endemic goiter. Pregnant women on a diet that is severely deficient of iodine can give birth to infants who can present with thyroid hormone deficiency (congenital hypothyroidism), manifesting in problems of physical growth and development as well as brain development (a condition referred to as endemic cretinism). In many developed countries, newborns are routinely tested for congenital hypothyroidism as part of newborn screening. Children with congenital hypothyroidism are treated supplementally with levothyroxine, which facilitates normal growth and development. Thyroxine is critical to the regulation of metabolism and growth throughout the animal kingdom. Among amphibians, for example, administering a thyroid-blocking agent such as propylthiouracil (PTU) can prevent tadpoles from metamorphosing into frogs; in contrast, administering thyroxine will trigger metamorphosis. Because the thyroid concentrates this element, it also concentrates the various radioactive isotopes of iodine produced by nuclear fission. In the event of large accidental releases of such material into the environment, the uptake of radioactive iodine isotopes by the thyroid can, in theory, be blocked by saturating the uptake mechanism with a large surplus of non-radioactive iodine, taken in the form of potassium iodide tablets. One consequence of the Chernobyl disaster was an increase in thyroid cancers in children in the years following the accident. (28)

The use of iodised salt is an efficient way to add iodine to the diet. It has eliminated endemic cretinism in most developed countries, and some governments
have made the iodination of flour, cooking oil, and salt mandatory. Potassium iodide and sodium iodide are typically used forms of supplemental iodine. As with most substances, either too much or too little can cause problems. Recent studies on some populations are showing that excess iodine intake could cause an increased prevalence of autoimmune thyroid disease, resulting in permanent hypothyroidism. (29)

2-9: Thyroid-Disorders:
Thyroid disorders include hyperthyroidism (abnormally increased activity), hypothyroidism (abnormally decreased activity) and thyroid nodules, which are generally benign thyroid neoplasms, but may be thyroid cancers. All these disorders may give rise to goiter, that is, an enlarged thyroid.

2-9-1: Hyperthyroidism:
Hyperthyroidism, or overactive thyroid, is the overproduction of the thyroid hormones $T_3$ and $T_4$, and is most commonly caused by the development of Graves' disease, an autoimmune disease in which antibodies are produced which stimulate the thyroid to secrete excessive quantities of thyroid hormones. The disease can result in the formation of a toxic goiter as a result of thyroid growth in response to a lack of negative feedback mechanisms. It presents with symptoms such as a thyroid goiter, protruding eyes (exophthalmos), palpitations, excess sweating, diarrhoea, weight loss, muscle weakness and unusual sensitivity to heat. Beta blockers are used to decrease symptoms of hyperthyroidism such as increased heart rate, tremors, anxiety and heart palpitations, and anti-thyroid drugs are used to decrease the production of thyroid hormones, in particular, in the case of Graves' disease. These medications take several months to take full effect and have side-effects such as skin rash or a drop in white blood cell count, which decreases the ability of the body to fight off infections. These drugs involve frequent dosing and
often require frequent doctor visits and blood tests to monitor the treatment, and may sometimes lose effectiveness over time. Due to the side-effects and inconvenience of such drug regimens, some patients choose to undergo radioactive iodine - (131) treatment. Radioactive iodine is administered in order to destroy a proportion of or the entire thyroid gland, since the radioactive iodine is selectively taken up by the gland and gradually destroys the cells of the gland. Alternatively, the gland may be partially or entirely removed surgically, though iodine treatment is usually preferred since the surgery is invasive and carries a risk of damage to the parathyroid glands or the nerves controlling the vocal cords. If the entire thyroid gland is removed, hypothyroidism results.\(^\text{30}\)

2-9-2:Hypothyroidism:

Hypothyroidism is the underproduction of the thyroid hormones \(T_3\) and \(T_4\). Hypothyroid disorders may occur as a result of congenital thyroid abnormalities (see congenital hypothyroidism), autoimmune disorders such as Hashimoto's thyroiditis, iodine deficiency (more likely in poorer countries) or the removal of the thyroid following surgery to treat severe hyperthyroidism and/or thyroid cancer. Typical symptoms are abnormal weight gain, tiredness, baldness, cold intolerance, and bradycardia. Hypothyroidism is treated with hormone replacement therapy, such as levothyroxine, which is typically required for the rest of the patient's life. Thyroid hormone treatment is given under the care of a physician and may take a few weeks to become effective.\(^\text{31}\)

Negative feedback mechanisms result in growth of the thyroid gland when thyroid hormones are being produced in sufficiently low quantities as a means of increasing the thyroid output; however, where the hypothyroidism is caused by iodine insufficiency, the thyroid is unable to produce \(T_3\) and \(T_4\) and as a result; the
thyroid may continue to grow to form a non-toxic goiter. It is termed non-toxic as it does not produce toxic quantities of thyroid hormones, despite its size.

2-9-3: **Initial hyperthyroidism followed by hypothyroidism:**

This is the overproduction of $T_3$ and $T_4$ followed by the underproduction of $T_3$ and $T_4$. There are two types: Hashimoto's thyroiditis and postpartum thyroiditis. Hashimoto's thyroiditis or Hashimoto's Disease is an autoimmune disorder whereby the body’s own immune system reacts with the thyroid tissues in an attempt to destroy it. At the beginning, the gland may be overactive, and then becomes underactive as the gland is damaged resulting in too little thyroid hormone production or hypothyroidism. Some patients may experience "swings" in hormone levels that can progress rapidly from hyper-to-hypothyroid (sometimes mistaken as severe mood swings, or even being bipolar, before the proper clinical diagnosis is made). Some patients may experience these "swings" over a longer period of time, over days or weeks or even months.

Hashimoto's is more common in females than males, usually appearing after the age of (30), and tends to run in families meaning it can be seen as a genetic disease. Also more common in individuals with Hashimoto's Thyroiditis are type (1) diabetes and celiac disease.\(^{(32)}\)

Postpartum thyroiditis occurs in some females following the birth of a child. After delivery, the gland becomes inflamed and the condition initially presents with over activity of the gland followed by under activity. In some cases, the gland may recover with time and resume its functions. In others it may not. The etiology is not always known, but can sometimes be attributed to autoimmunity, such as Hashimoto's Thyroiditis or Graves ‘disease.
2-9-4: Thyroid-malignancy:
Cancers do occur in the thyroid gland and are more common in females. In most cases, the thyroid cancer presents as a painless mass in the neck. It is very unusual for the thyroid cancers to present with symptoms, unless it has been neglected. One may be able to feel a hard nodule in the neck. Diagnosis is made using a needle biopsy and various radiological studies. (33)

2-9-5: Thyroid-diseases:(non-cancerous):
Many individuals may find the presence of thyroid nodules in the neck. The majority of these thyroid nodules are benign (non-cancerous). The presence of a thyroid nodule does not mean that one has thyroid disease. Most thyroid nodules do not cause any symptoms, and most are discovered on an incidental examination. Doctors usually perform a needle aspiration biopsy of the thyroid to determine the status of the nodules. If the nodule is found to be non-cancerous, no other treatment is required. If the nodule is suspicious then surgery is recommended.

2-9-6: Other-disorders:
Limited research shows that seasonal allergies may trigger episodes of hypo- or hyperthyroidism. (34)(35) A ectopic thyroid is an entire or parts of the thyroid located in another part of the body than what is the usual case.

2-10: Thyroid disease and female reproduction:
The menstrual pattern is influenced by thyroid hormones directly through impact on the ovaries and indirectly through impact on SHBG, PRL and GnRH secretion and coagulation factors. Treating thyroid dysfunction can reverse menstrual abnormalities and thus improve fertility. In infertile women, the prevalence of autoimmune thyroid disease (AITD) is significantly higher compared to parous age-matched women. This is especially the case in women with endometriosis and
polycystic ovarian syndrome PCOS. AITD does not interfere with normal foetal implantation and comparable pregnancy rates have been observed after assisted reproductive technology (ART) in women with and without AITD. During the first trimester, however, pregnant women with AITD carry a significantly increased risk for miscarriage compared to women without AITD, even when euthyroidism was present before pregnancy. It has also been demonstrated that controlled ovarian hyperstimulation (COH) in preparation for ART has a significant impact on thyroid function, particularly in women with AITD. It is therefore advisable to measure thyroid function and detect AITD in infertile women before ART, and to follow-up these parameters after COH and during pregnancy when AITD was initially present. Women with thyroid dysfunction at early gestation stages should be treated with L-thyroxine to avoid pregnancy complications. Whether thyroid hormones should be given prior to or during pregnancy in euthyroid women with AITD remains controversial. To date, there is a lack of well-designed randomized clinical trials to elucidate this controversy. Procreation is a fundamental evolutionary process necessary to sustain life and involves spatio-temporally regulated endocrine, cellular and molecular events. Before ovarian follicles are expelled, oocyte maturation demands a favourable endocrine environment, including normal levels of thyroid hormones. The major factors that establish uterine receptivity for implantation and further embryo development are progesterone, oestrogens and the immunological system. \(^{36}\) Infertility and reproductive impairment can be compromised by abnormalities in both the endocrine and the immune system. A close interplay between thyroid hormones and normal steroid action and secretion exists, necessary for normal ovarian function and thus fertility. Women with thyroid dysfunction often have menstrual irregularities, infertility and increased morbidity during pregnancy. \(^{37,38}\)
2-11: Haemopoiesis:
(Or haematopoiesis ) is the formation of blood cells The processes that regulate haemopoiesis and the early stages of formation of red cells (erythropoiesis), granulocytes and monocytes (myelopoiesis) and platelets (thrombopoiesis). The site of haemopoiesis in the first few weeks of gestation the yolk sac is the main site of haemopoiesis. And from (6 weeks) until (6-7 months) of fetal life the liver and spleen are the major haemopoiesis organs and continue to produce blood cells until about that (2 weeks) after birth. The bone marrow is the most important site from (6 to 7 months) of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells.

In infancy all the bone marrow is haemopoietic but during childhood there is progressive fatty replacement of marrow throughout the long bones so that in adult life haemopoiesis marrow is confined to the central skeleton and proximal ends of the femurs and humeri. Even in these haemopoietic areas, approximately (50%) of the marrow consists of fat. The remaining fatty marrow is capable of reversion to haemopoiesis and in many diseases there is also expansion of haemopoiesis down the long bones. Moreover, the liver and spleen can resume their fetal haemopoietic role ('extramedullary haemopoiesis').

2-11-1: Haemopoietic stem cell and progenitor cells:
Haemopoiesis starts with a pluripotential stem cell that can give rise to the separate cell lineages. Cell differentiation occurs from the stem cell via the committed haemopoietic progenitors which are restricted in their developmental potential. The existence of the separate progenitor cells can be demonstrated by in-vitro culture techniques. The earliest detectable mixed myeloid precursor which gives rise to granulocytes, erythrocytes, monocytes and megakaryocytic and is termed CFU (colony-forming unit)-GEMM. The stem cell has the capability for self-renew also
that marrow cellularity remains constant in a normal healthy steady state. One stem cell is capable of producing about \((10)^6\) mature blood cells after (20) cell divisions. (39)

2-11-2: Haemopoietic growth factors:
The haemopoietic growth factors are glycoprotein hormones that regulate the proliferation and differentiation of haemopoietic progenitor cells and the function of mature blood cells. They may act locally at the site where they are produced by cell-cell contact or circulate in plasma. The growth factors may cause cell proliferation but can also stimulate differentiation, maturation, prevent apoptosis and affect the function of mature cells.

They share a number of common properties and act at different stages of haemopoiesis. Stromal cells are the major source of growth factors except for erythropoietin, (90%) of which is synthesized in the kidney, and thrombopoietin, made largely in the liver. The action of one growth factor on a cell may stimulate production of another growth factor or growth factor receptor. SCF and Flt ligand (Flt-L) act locally on the pluripotential stem cells and on early myeloid and lymphoid progenitors. Interleukin 3 (IL-3) and GM-CSF are multi-potential growth factors with overlapping activities. G-CSF and thrombopoietin enhance the effects of Flt-L, IL-3 and GM-CSF on survival and differentiation of the early haemopoietic cells.

These factors maintain a pool of haemopoietic stem and progenitor cells on which later acting factors erythropoietin, G-CSF, M-CSF, IL-5 and thrombopoietin act to increase production of one or other cell lineage in response to the body's need. Granulocyte and monocyte formation can be stimulated by infection or inflammation through release of IL-1 and tumor necrosis factor (TNF) which then stimulate stoma cells to produce growth factors in an interacting network. Cytokines such as transforming growth factor-\(\beta\) (TGF-\(\beta\)) and \(\gamma\)-interferon (IFN-\(\gamma\))
can exert a negative effect on haemopoiesis and may have a role in the
development of a plastic anaemia.\textsuperscript{(39)}

**2-11-3: Haemoglobin synthesis:**

The main function of red cells is to carry $O_2$ to the tissues and to return
carbon dioxide ($CO_2$) from the tissues to the lungs. In order to achieve this
gaseous exchange they contain the specialized protein haemoglobin. Each red
cell contains approximately (640) million haemoglobin molecules. Each
molecule of normal adult haemoglobin ($Hb$) $A$ (the dominant haemoglobin in blood
after the age of (3-6 months) consists of four \textit{polypeptide chains}$\alpha_2\beta_2$, each with its
own haem group. The molecular weight of $Hb$ $A$ is (68 000). Normal adult blood
also contains small quantities of two other haemoglobins: $HbF$ and $HbA2$. These
also contain $a$-chains, but with $\gamma$ and $\delta$ chains, respectively, instead of $\beta$.
The major switch from fetal to adult haemoglobin occurs (3-6 months) after birth.

Haem synthesis occurs largely in the mitochondria by a series of biochemical
reactions commencing with the condensation of glycine and succinyl coenzyme A
under the action of the key rate limiting enzyme $\delta$ –amino-laevulinic acid ($ALA$)
synthase. Pyridoxal phosphate (\textit{vitamin B6}) is a coenzyme for this reaction which
is stimulated by erythropoietin. Ultimately, protoporphyrin combines with iron in
the ferrous ($Fe^{2+}$) state to form haem, each molecule of which combines with a
globin chain made on the polyribosomes. A tetramer of (4) globin chains each with
its own haem group in a 'pocket' is then formed to make up a haemoglobin
molecule.\textsuperscript{(39)}
2-11-4: Erythropoiesis:

The process by which red blood cells (erythrocytes) are produced. It is stimulated by decreased $O_2$ in circulation, which is detected by the kidneys, which then secrete the hormone erythropoietin. This hormone stimulates proliferation and differentiation of red cell precursors, which activates increased erythropoiesis in the haemopoietic tissues, ultimately producing red blood cells. In postnatal birds and mammals (including humans), this usually occurs within the red bone marrow.\(^{(40)}\) In the early fetus, erythropoiesis takes place in the mesodermal cells of the yolk sac. By the 3\textsuperscript{rd} or 4\textsuperscript{th} month, erythropoiesis moves to the spleen and liver.\(^{(41)}\) After (7) months, erythropoiesis occurs in the bone marrow. Increased
level of physical activity can cause an increase in erythropoiesis. However, in humans with certain diseases and in some animals, erythropoiesis also occurs outside the bone marrow, within the spleen or liver. This is termed extramedullary erythropoiesis. The bone marrow of essentially all the bones produces RBCs until a person is around (5 years) old. The tibia and femur cease to be important sites of haematopoiesis by about age (25); the vertebrae, sternum, pelvis and ribs, and cranial bones continue to produce red blood cells throughout life.

Erythrocyte differentiation:
In the process of red blood cell maturation, a cell undergoes a series of differentiations. The following stages of development all occur within the bone marrow:

- Haemocytoblast, a multipotent haematopoietic stem cell.
- Common myeloid progenitor, a multipotent stem cell.
- Unipotent Stem Cell.
- Pronormoblast, also commonly called proerythroblast or rubriblast.
- Basophilic normoblast/early normoblast, also commonly called erythroblast.
- Polychromatophilic normoblast/intermediate normoblast.
- Orthochromatic normoblast/late normoblast. Nucleus is expelled before becoming a reticulocyte.
- Reticulocyte.

The cell is released from the bone marrow after stage (7), and so of circulating red blood cells there are (~1%) reticulocytes. After (1 to 2 days), these ultimately become "erythrocytes" or mature red blood cells. These stages correspond to specific appearances of the cell when stained with
Wright's stain and examined by light microscopy, but correspond to other biochemical changes. In the process of maturation, a basophilic pronormoblast is converted from a cell with a large nucleus and a volume of (900 fL) to an enucleated disc with a volume of (95 fL). By the reticulocyte stage, the cell has extruded its nucleus, but is still capable of producing haemoglobin. Essential for the maturation of RBC'S are Vitamin B₁₂ (cobalamin) and Vitamin B₉ (Folic acid). Lack of either of these causes maturation failure in the process of erythropoiesis, which manifests clinically as reticulocytopaenia, an abnormally low amount of reticulocytes.

Characteristics seen in erythrocytes during erythropoiesis:
The following characteristics can be seen changing in the erythrocytes when they are maturing:

- They show a reduction in the cell size.
- The cytoplasmic matrix increases in amount.
- Staining reaction of the cytoplasm changes from blue to pinkish red (this is because of the decrease in the amount of RNA and DNA). Initially the nucleus was large in size and contained open chromatin. But with the maturation of RBC's the size of the nucleus decreases and finally disappears with the condensation of the chromatin material.³⁴³

Regulation of erythropoiesis:
A feedback loop involving erythropoietin helps regulate the process of erythropoiesis so that, in non-disease states, the production of red blood cells is equal to the destruction of red blood cells and the red blood cell number is sufficient to sustain adequate tissue oxygen levels but not so high as to cause sludging, thrombosis, or stroke. Erythropoietin is produced in the kidney and liver
in response to low oxygen levels. In addition, erythropoietin is bound by circulating red blood cells; low circulating numbers lead to a relatively high level of unbound erythropoietin, which stimulates production in the bone marrow. Recent studies have also shown that the peptide hormone hepcidin may play a role in the regulation of haemoglobin production, and thus affect erythropoiesis. The liver produces hepcidin. Hepcidin controls iron absorption in the gastrointestinal tract and iron release from reticuloendothelial tissue. Iron must be released from macrophages in the bone marrow to be incorporated into the haem group of haemoglobin in erythrocytes. There are colonies forming units that the cells follow during their formation. These cells are referred to as the committed cells including the granulocyte monocyte colony forming units. Also, loss of function of the erythropoietin receptor or JAK2 in mice cells causes failure in erythropoiesis, so production of red blood cells in embryos and growth is disrupted.

Also, if there is no feedback inhibition, such as SOCS (Suppressors of Cytokine Signaling) proteins in the system, that would cause gigantism in mice.\(^{(44)}\)\(^{(45)}\)

**2-11-5: White blood cells or leukocytes:**

(also spelled "leucocytes"; from the Greek word leuko- meaning "white"), are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five\(^{(46)}\) different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a haematopoietic stem cell. They live for about (3 to 4 days) in the average human body. Leukocytes are found throughout the body, including the blood and lymphatic system.\(^{(47)}\) The number of leukocytes in the blood is often an indicator of disease. There are normally between \((4\times10^9 \text{ and } 1.1\times10^{10})\) white blood cells in a litre of blood, and ranging from (7 and 21 micrometers) in diameter, they make up approximately (1\%) of blood in a healthy adult.\(^{(48)}\) An increase in the
number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopaenia. The physical properties of leukocytes, such as volume, conductivity, and granularity, may change due to activation, the presence of immature cells, or the presence of malignant leukocytes in leukaemia.

**Types of white blood cells:**

There are several different types of white blood cells. They all have many things in common, but are all distinct in form and function. A major distinguishing feature of some leukocytes is the presence of granules; white blood cells are often characterized as granulocytes or a granulocytes: granulocytes (polymorphonuclear leukocytes): leukocytes characterized by the presence of differently staining granules in their cytoplasm when viewed under light microscopy. These granules are membrane-bound enzymes that act primarily in the digestion of endocytosed particles. There are three types of granulocytes: neutrophils, basophils, and eosinophils, which are named according to their staining properties.

**Agranulocytes:**

(mononuclear leukocytes): leukocytes characterized by the apparent absence of granules in their cytoplasm. Although the name implies a lack of granules these cells do contain non-specific azurophilic granules, which are lysosomes.\(^{(49)}\) The cells include lymphocytes, monocytes, and macrophages.\(^{(50)}\)

**Neutrophil:**

Neutrophils defend against bacterial or fungal infection and other very small inflammatory processes that are usually first responders to microbial infection; their activity and death in large numbers forms pus. They are commonly referred to as **polymorphonuclear (PMN) leukocytes**, although, in the technical sense, **PMN** refers to all granulocytes. They have a multi-lobed nucleus that may appear like multiple nuclei, hence the name **polymorphonuclear leukocyte**. The cytoplasm may
look transparent because of fine granules that are pale lilac. Neutrophils are very active in phagocytosing bacteria and are present in large amount in the pus of wounds. These cells are not able to renew their lysosomes (used in digesting microbes) and die after having phagocytosed a few pathogens. Neutrophils are the most common cell type seen in the early stages of acute inflammation, and make up (60-70%) of total leukocyte count in human blood.\(^{48}\) The life span of a circulating human neutrophil is about (5-4 days).\(^{52}\)

**Eosinophils:**
These cells are similar to neutrophils, except that the cytoplasmic granules are coarser and more deeply red staining and there are rarely more than three nuclear lobes. Eosinophils myelocytes can be recognized but earlier stages are indistinguishable from neutrophil precursors. The blood transit time for eosinophils is longer than for neutrophils. They enter inflammatory exudates and have a special role in allergic responses, defense against parasites and removal of fibrin formed during inflammation.\(^{39}\)

**Basophils:**
These are only occasionally seen in normal peripheral blood. They have many dark cytoplasmic granules which overlie the nucleus and contain heparin and histamine in the tissues they become mast cells. They have *immunoglobulin* E (*IgE*) attachment sites and their degranulation is associated with histamine release.\(^{39}\)

**Lymphocyte:**
Lymphocytes are much more common in the lymphatic system. Lymphocytes are distinguished by having a deeply staining nucleus that may be eccentric in location, and a relatively small amount of cytoplasm. The blood has three types of lymphocytes:
• **B cells** make antibodies that bind to pathogens to enable their destruction.

• **T cells** divided into:
  
  • **CD4**-helper T cells: T cells having co-receptor CD4 are known as CD4+ T cells. These cells bind antigen presented by antigen-presenting cells via T-cell receptor interacting with MHC II complex on APC. Helper T cells coordinate the immune response. In acute HIV infection, these T cells are the main index to identify the individual's immune system activity.

  o **CD8**-cytotoxic T cells: T cells having co-receptor CD8+ are known as CD8+ T cells. These cells bind antigens presented on MHC I complex of virus-infected or tumour cells and kill them. All nucleated cells possess MHC I on its surface.

  o γδ T cells possess an alternative T cell receptor as opposed to CD4+ and CD8+ αβ T cells and share characteristics of helper T cells, cytotoxic T cells and natural killer cells.

  o Natural killer cells are able to kill cells of the body that have lost MHC I molecule, as they have been infected by a virus or have become cancerous.

**Monocyte:**

Monocytes share the "vacuum cleaner" (phagocytosis) function of neutrophils, but are much longer lived as they have an additional role: they present pieces of pathogens to T cells so that the pathogens may be recognized again and killed, or so that an antibody response may be mounted. Monocytes eventually leave the bloodstream to become tissue macrophages, which remove dead cell debris as well
as attacking microorganisms. Neither of these can be dealt with effectively by the neutrophils. Unlike neutrophils, monocytes are able to replace their lysosomal contents and are thought to have a much longer active life. They have the kidney shaped nucleus and are typically a granulated. They also possess abundant cytoplasm.

Once monocytes move from the bloodstream out into the body tissues, they undergo changes (differentiate) allowing phagocytosis and are then known as macrophages.

**2-11-6: Platelets:**

**2-11-6-1: Platelets production:**

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte-the megakaryoblast-arises by a process of differentiation from the haemopoietic stem cell. The megakaryocyte matures by endomitotic synchronous replication (i.e. DNA replication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two. Very early on invaginations of plasma membrane are seen, called the demarcation membrane, which evolves through the development of the megakaryocyte into a highly branched network. At a variable stage in development, most commonly at the eight nucleus stage, the cytoplasm becomes granular. Mahler megakaryocytes are extremely large, with an eccentric placed single lobulated nucleus and a low nuclear to cytoplasmic ratio. Platelets form by fragmentation of megakaryocyte cytoplasm, approximately each megakaryocyte giving rise to (1000-5000 platelets). The time interval from differentiation of the human stem cell to the production of platelets averages approximately (10 days). Thrombopoietin is the major regulator of platelet production and is constitutively
produced by the liver and kidneys. Thrombopoietin increases the number and rate of maturation of megakaryocytes via c-Mpl receptor. Platelet levels start to rise (6 days) after the start of therapy and remain high for (7-10 days). Unfortunately, thrombopoietin is not available for routine clinical practice. Platelets also have c-Mpl receptors for thrombopoietin and remove it from the circulation. Therefore, levels are high in thrombocytopenia as a result of marrow an a plasia and vice versa. The normal platelet count is approximately (250 x 10^9/L) (range (150-400 x 10^9/L) and the normal platelet lifespan is (7-10 days) up to one-third of the marrow output of platelets may be trapped at any one time in the normal spleen but this rises to (90%) in cases of massive splenomegaly .

2-11-6-2:Platelet structure:
Platelets are extremely small and discoid, (3.0 x 0.511 m) in diameter, with a mean volume (7-11 fL). The glycoproteins of the surface coat are particularly important in the platelet reactions of adhesion and aggregation which are the initial events leading to platelet plug formation during haemostasis. Adhesion to collagen is facilitated by glycoprotein la (GPla). Glycoproteins Ib (defective in Bernard-Soulier syndrome) and IIb/IIIa (defective in thrombasthaenia) are important in the attachment of platelets to Von Willebrand factor (VWF) and hence to vascular subendothelium where metabolic interactions occur. The binding site for lib /IIa is also the receptor for fibrinogen which is important in platelet-platelet aggregation. The plasma membrane invaginates into the platelet interior to form an open membrane (canalicular) system which provides a large reactive surface to which the plasma coagulation proteins may be selectively absorbed. The membrane phospholipids (previously known as platelet factor 3) are of particular importance in the conversion of coagulation factor (X to Xa) and prothrombin (factor II) to thrombin (factor IIa).
2-11-6-3: Platelet function

The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur. The immobilization of platelets at the sites of vascular injury requires specific platelet-vessel wall (adhesion) and platelet-platelet (aggregation) interactions. The blood flow conditions determine the specific receptor ligand interactions. (39)

2-12: Pathology:

General medical disorders: Disorders of volume:

- Injury can cause blood loss through bleeding. (53) A healthy adult can lose almost (20%) of blood volume (1 L) before the first symptom, restlessness, begins, and (40%) of volume (2 L) before shock sets in. Thrombocytes are important for blood coagulation and the formation of blood clots, which can stop bleeding. Trauma to the internal organs or bones can cause internal bleeding, which can sometimes be severe.

- Dehydration can reduce the blood volume by reducing the water content of the blood. This would rarely result in shock (apart from the very severe cases) but may result in orthostatic hypotension and fainting.

Disorders of circulation:

- Shock is the ineffective perfusion of tissues, and can be caused by a variety of conditions including blood loss, infection, and poor cardiac output.

- Atherosclerosis reduces the flow of blood through arteries, because atheroma lines arteries and narrows them. Atheroma tends to increase
with age, and its progression can be compounded by many causes including smoking, high blood pressure, excess circulating lipids (hyperlipidaemia), and Diabetes mellitus.

- Coagulation can form a thrombosis, which can obstruct vessels.
- Problems with blood composition, the pumping action of the heart, or narrowing of blood vessels can have many consequences including hypoxia (lack of oxygen) of the tissues supplied. The term *ischaemia* refers to tissue that is inadequately perfused with blood, and *infarction* refers to tissue death (necrosis), which can occur when the blood supply has been blocked (or is very inadequate)

**Haematological disorders:**

**Anaemia:**

- Insufficient red cell mass (anaemia) can be the result of bleeding, blood disorders like Thalassaemia, or nutritional deficiencies; and may require blood transfusion. Several countries have blood banks to fill the demand for transfusible blood. A person receiving a blood transfusion must have a blood type compatible with that of the donor.
- Sickle-cell anaemia

- **Disorders of cell proliferation:**
  - Leukaemia is a group of cancers of the blood-forming tissues.
  - Non-cancerous overproduction of red cells (polycythaemia Vera) or platelets (essential thrombocytosis) may be premalignant.
  - Myelodysplastic syndromes involve ineffective production of one or more cell lines.
• **Disorders of coagulation:**
  o Haemophilia is a genetic illness that causes dysfunction in one of the blood's clotting mechanisms. This can allow otherwise inconsequential wounds to be life-threatening, but more commonly results in haemarthrosis, or bleeding into joint spaces, which can be crippling.
  o Ineffective or insufficient platelets can also result in coagulopathy (bleeding disorders).
  o Hypercoagulable state (thrombophilia) results from defects in regulation of platelet or clotting factor function, and can cause thrombosis.

• **Infectious disorders of blood:**
  o Blood is an important vector of infection. *HIV*, the virus that causes *AIDS*, is transmitted through contact with blood, semen or other body secretions of an infected person. *Hepatitis B and C* are transmitted primarily through blood contact. Owing to blood-borne infections, blood stained objects are treated as a biohazard.
  o Bacterial infection of the blood is bacteraemia or sepsis. Viral Infection is viraemia. Malaria and trypanosomiasis are blood-borne parasitic infections.

**2-13: Complete Blood Count (CBC):**

(CBC) gives important information about the kinds and numbers of cells in the blood, especially red blood cells, white blood cells, and platelets. A *CBC* helps doctors to check patient’s symptoms, such as weakness, fatigue, or bruising. A
CBC also helps doctors to diagnose conditions, such as anaemia, infection, and many other disorders. The CBC test usually includes:

**2-13-1: Red blood cell (RBC) count:**
Red blood cells carry oxygen from the lungs to the rest of the body. They also carry carbon dioxide back to the lungs so it can be exhaled. If the RBC count is low (anaemia), the body may not be getting the oxygen it needs. If the count is too high (a condition called polycythaemia), there is a chance that the red blood cells will clump together and block tiny blood vessels (capillaries). This also makes it hard for your red blood cells to carry oxygen.\(^{(54)}\)

**2-13-2: Haematocrit (HCT, packed cell volume, PCV):**
This test measures the amount of space (volume) red blood cells take up in the blood. The value is given as a percentage of red blood cells in a volume of blood. For example, a haematocrit of (38) means that (38%) of the blood's volume is made of red blood cells. Haematocrit and haemoglobin values are the two major tests that show if anaemia or polycythaemia is present.\(^{(54)}\)

**2-13-3: Haemoglobin (Hgb):**
The haemoglobin molecule fills up the red blood cells. It carries oxygen and gives the blood cell its red color. The haemoglobin test measures the amount of haemoglobin in blood and is a good measure of the blood's ability to carry oxygen throughout the body.\(^{(54)}\)

**2-13-4: Red blood cell indices:**
There are three red blood cell indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). They are measured by a machine, and their values come
from other measurements in a *CBC*. The *MCV* shows the size of the red blood cells. The *MCH* value is the amount of haemoglobin in an average red blood cell. The *MCHC* measures the concentration of haemoglobin in an average red blood cell. These numbers help in the diagnosis of different types of anaemia. *Red cell distribution width (RDW)* can also be measured which shows if the cells are all the same or in different sizes or shapes.\(^{(54)}\)

**2-13-5: White blood cell (WBC, leukocyte) count:**

White blood cells protect the body against infection. If an infection develops, white blood cells attack and destroy the bacteria, virus, or other organism causing it. White blood cells are bigger than red blood cells but fewer in number. When a person has a bacterial infection, the number of white cells rises very quickly. The number of white blood cells is sometimes used to find an infection or to see how the body is dealing with cancer treatment.\(^{(54)}\)

**White blood cell types (WBC differential):**

The major types of white blood cells are neutrophils, lymphocytes, monocytes, eosinophils and basophils. Immature neutrophils, called band neutrophils, are also part of this test. Each type of cell plays a different role in protecting the body. The numbers of each one of these types of white blood cells give important information about the immune system. Too many or too few of the different types of white blood cells can help find an infection, an allergic or toxic reaction to medicines or chemicals, and many conditions, such as leukaemia.\(^{(54)}\)

**2-13-6: Platelet (thrombocyte) count:**

Platelets (*thrombocytes*) are the smallest type of blood cell. They are important in blood clotting. When bleeding occurs, the platelets swell, clump together, and form a sticky plug that helps stop the bleeding. If there are too few platelets,
uncontrolled bleeding may be a problem. If there are too many platelets, there is a chance of a blood clot forming in a blood vessel. Also, platelets may be involved in hardening of the arteries (atherosclerosis).\(^{(54)}\)

**2-13-6-1: Mean platelet volume (MPV):**

Mean platelet volume measures the average amount (volume) of platelets. Mean platelet volume is used along with platelet count to diagnose some diseases. If the platelet count is normal, the mean platelet volume can still be too high or too low.\(^{(54)}\)

**2-14: Coagulation Profile:**

**2-14-1: Prothrombin-time-(PT):**

History: The prothrombin time was discovered by Dr Armand Quick and colleagues in 1935,\(^{(55)}\) and a second method was published by Dr Paul Owren,\(^{(56)}\) also called the "p and p" or "prothrombin and pro-convert in" method. It aided in the identification of the anticoagulants dicumarol and warfarin,\(^{(57)}\) and was used subsequently as a measure of activity for warfarin when used therapeutically. The INR was introduced in the early 1980s when it turned out that there was a large degree of variation between the various prothrombin time assays, a discrepancy mainly due to problems with the purity of the thromboplastin (tissue factor) concentration.\(^{(58)}\) The INR became widely accepted worldwide, especially after endorsement by the World Health Organization (WHO).\(^{(59)}\)

The *prothrombin time (PT)* and its derived measures of *prothrombin ratio (PR)* and *international normalized ratio (INR)* are measures of the extrinsic pathway of coagulation. This test is also called "ProTime INR" and "INR PT". They are used to determine the clotting tendency of blood, in the measure of warfarin dosage, liver damage, and vitamin K status. *PT* measures *factors I, II, V, VII, and X*. It is used in
conjunction with the *activated partial thromboplastin time (a-PTT)* which measures the intrinsic pathway.

**Laboratory measurement:**
Normal range is the reference range for prothrombin time is usually around (10-13 seconds); the normal range for the *INR* is (0.8–1.2). Clinicians desiring therapeutic anticoagulation may aim for a higher *INR* - in many cases ranging from (2-3) - using anticoagulants such as warfarin. \(^{(60)}\)

\[
\text{INR} = \text{(patient’s PT/Normal control PT)}^{\text{ISI}}
\]

**Interpretation:**
The prothrombin time is the time it takes plasma to clot after addition of tissue factor (obtained from animals such as rabbits, or recombinant tissue factor, or from brains of autopsy patients). This measures the quality of the *extrinsic pathway* (as well as the *common pathway*) of coagulation. The speed of the *extrinsic pathway* is greatly affected by levels of functional *factor VII* in the body. *Factor VII* has a short half-life and the carboxylation of its glutamate residues requires *vitamin K*. The prothrombin time can be prolonged as a result of deficiencies in *vitamin K*, warfarin therapy, malabsorption, or lack of intestinal colonization by bacteria (such as in newborns). In addition, poor *factor VII synthesis* (due to liver disease) or increased consumption (in disseminated intravascular coagulation) may prolong the *PT*. A high *INR* level such as *INR=5* indicates that there is a high chance of bleeding, whereas if the *INR=0.5* then there is a high chance of having a clot. Normal range for a healthy person is (0.9–1.3), and for people on warfarin therapy, (2.0–3.0), although the target *INR* may be higher in particular situations, such as for those
with a mechanical heart valve, or bridging warfarin with a low-molecular weight heparin (such as enoxaparin) perioperatively.

2-14-2: Partial thromboplastin time (PTT):

History:
The a-PTT was first described in 1953 by researchers at the University of North Carolina at Chapel Hill. The partial thromboplastin time (PTT) or activated partial thromboplastin time (a PTT or APTT) is a performance indicator measuring the efficacy of both the "intrinsic" (now referred to as the contact activation pathway) and the common coagulation pathways. Apart from detecting abnormalities in blood clotting, it is also used to monitor the treatment effects with heparin, a major anticoagulant. It is used in conjunction with PT which measures the extrinsic pathway. Kaolin cephalin clotting time (KccT) is a historic name for the activated partial thromboplastin time. The typical reference range is between (3 and 50 seconds) (depending on laboratory). Shortening of the PTT is considered to have little clinical relevance, but some research indicates that it might increase risk of thromboembolism. Normal PTT times require the presence of the following coagulation factors: I, II, V, VIII, IX, X, XI, & XII. Notably, deficiencies in factors VII or XIII will not be detected with the PTT test. Prolonged APTT may indicate:

- Use of heparin (or contamination of the sample).
- Antiphospholipid antibody (especially lupus anticoagulant, which paradoxically increases propensity to thrombosis).
- Coagulation factor deficiency (e.g. haemophilia).
- Sepsis - coagulation factor consumption.
- Presence of antibodies against coagulation factors (factor inhibitors).
To distinguish the above causes, mixing tests are performed, in which the patient's plasma is mixed (initially at a (50:50) dilution with normal plasma. If the abnormality does not disappear, the sample is said to contain an "inhibitor" (heparin, anti-phospholipids antibodies or coagulation factor specific inhibitors), while if it does correct a factor deficiency is more likely. Deficiencies of factors VIII, IX, XI and XII and rarely Von Willebrand factor (if causing a low factor VIII level) may lead to a prolonged aPTT correcting on mixing studies.

2-15:Hormones:

2-15-1:Prolactin:

Prolactin (PRL), also known as luteotropic hormone or luteotropin, is a protein that in humans is best known for its role in enabling mammals, usually females, to produce milk; however, it is influential over a large number of functions with over (300) separate actions of PRL having been reported in various vertebrates. Prolactin is secreted from the pituitary gland in response to eating, mating, estrogen treatment, ovulation, and nursing. Prolactin is secreted in a pulsatile fashion in between these events.

Prolactin also plays an essential role in metabolism, regulation of the immune system, and pancreatic development. Although often associated with human milk production, prolactin plays a wide range of other roles in both humans and other vertebrates.

Prolactin also acts in a cytokine-like manner and as an important regulator of the immune system. It has important cell cycle related functions as a growth-, differentiating- and anti-apoptotic factor. As a growth factor, binding to cytokine like receptors, it also has profound influence on haematopoiesis, angiogenesis and is involved in the regulation of blood clotting through several pathways. The hormone acts in endocrine, autocrine, and paracrine manner through the prolactin receptor and a large number of cytokine receptors.
Pituitary prolactin secretion is regulated by endocrine neurons in the hypothalamus, the most important ones being the neuro-secretory tuberoinfundibulum (TIDA) neurons of the arcuate nucleus, which secrete dopamine (aka Prolactin Inhibitory Hormone) to act on the $D_2$ receptors of lactotrophs, causing inhibition of prolactin secretion. Thyrotropin-releasing factor (thyrotropin-releasing hormone) has a stimulatory effect on prolactin release, however $PRL$ is the only adenohypophyseal hormone whose principal control is inhibitory.

**Effects:**

$PRL$ has a wide range of effects. It stimulates the mammary glands to produce milk (lactation): increased serum concentrations of $PRL$ during pregnancy cause enlargement of the mammary glands of the breasts and prepare for the production of milk. Milk production normally starts when the levels of progesterone fall by the end of pregnancy and a suckling stimulus is present. Sometimes, newborn babies (males as well as females) secrete a milky substance from their nipples known as witch's milk. This is in part caused by maternal $PRL$ and other hormones. $PRL$ also has been found to play an important role in maternal behavior.\(^{(66)}\) It provides the body with sexual gratification after sexual acts: The hormone counteracts the effect of dopamine, which is responsible for sexual arousal. This is thought to cause the sexual refractory period. The amount of $PRL$ can be an indicator for the amount of sexual satisfaction and relaxation. Unusually high amounts are suspected to be responsible for impotence and loss of-libido.

Highly elevated levels of prolactin decrease the levels of sex hormones - oestrogen in women and testosterone in men.\(^{(67)}\) The effects of mildly elevated levels of $PRL$ are much more variable, in women either substantial increase or decrease of oestrogen levels may result.
PRL is sometimes classified as a gonadotropin \(^{(68)}\) although in humans it has only a weak luteotropic effect while the effect of suppressing classical gonadotropin hormones is more important. \(^{(69)}\) PRL within the normal reference ranges can act as a weak gonadotropin but at the same time suppresses GnRH secretion. The exact mechanism by which it inhibits GnRH is poorly understood although expression of prolactin receptors (PRL-R) have been demonstrated in rat's hypothalamus, the same has not been observed in GnRH neurons. \(^{(70)}\) Physiologic levels of prolactin in males enhance LH-receptors in Leydig cells, resulting in testosterone secretion, which leads to spermatogenesis. \(^{(71)}\) PRL also stimulates proliferation of oligodendrocyte precursor cells. These cells differentiate into oligodendrocytes, the cells responsible for the formation of myelin coatings on axons in the central nervous system. \(^{(72)}\) PRL also has a number of other effects including contributing to pulmonary surfactant synthesis of the fetal lungs at the end of the pregnancy and immune tolerance of the fetus by the maternal organism during pregnancy. Prolactin delays hair re-growth in mice.\(^{(73)}\) Prolactin promotes neurogenesis in maternal and fetal brains.\(^{(74)}\)(75)

2-15-2: Follicle-stimulating hormone:

Follicle-stimulating hormone (FSH) is a gonadotropin, a glycoprotein polypeptide hormone. FSH is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland, \(^{(76)}\) and regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and luteinizing hormone (LH) work together in the reproductive system.

Activity:

FSH regulates the development, growth, pubertal maturation and reproductive processes of the human body.

- In both males and females, FSH stimulates the maturation of germ cells.
In males, *FSH induces* Sertoli cells to secrete *androgen-binding proteins* (ABPs), regulated by inhibin's negative feedback mechanism on the anterior pituitary.

In females, *FSH initiates* follicular growth, specifically affecting granulosa cells. With the concomitant rise in *inhibin B, FSH levels* then decline in the late follicular phase. This seems to be critical in selecting only the most advanced follicle to proceed to ovulation. At the end of the luteal phase, there is a slight rise in *FSH* that seems to be of importance to start the next ovulatory cycle.

Control of *FSH release* from the pituitary gland is unknown. Low frequency gonadotropin-releasing hormone (*GnRH* *) pulses* increase *FSH mRNA levels* in the rat, \(^{(77)}\) but is not directly correlated with an increase in *circulating FSH*. \(^{(78)}\) *GnRH* has been shown to play an important role in the secretion of *FSH*, with hypothalamic-pituitary disconnection leading to a cessation of *FSH*. *GnRH administration* leads to a return of *FSH secretion*. *FSH* is subject to oestrogen feed-back from the gonads via the hypothalamic pituitary gonadal axis.

**Effect of** (*FSH*) **in female:**

*FSH stimulates* the growth and recruitment of immature ovarian follicles in the ovary. In early (small) antral follicles, *FSH* is the major survival factor that rescues the small antral follicles (2–5 mm in diameter for humans) from apoptosis (programmed death of the somatic cells of the follicle and oocyte). In the luteal-follicle phase transition period the serum levels of progesterone and oestrogen (primarily oestradiol) decrease and no longer suppress the release of *FSH*, consequently *FSH peaks* at about day three (day one is the first day of menstrual flow). The cohort of small antral follicles is normally sufficiently in number to produce enough *inhibin B* to lower *FSH serum* levels.
In addition, there is evidence that gonadotropin surge-attenuating factor produced by small follicles during the first half of the follicle phase also exerts a negative feedback on pulsatile (LH) secretion amplitude, thus allowing a more favorable environment for follicle growth and preventing premature luteinization. (79) As a woman nears perimenopause, the number of small antral follicles recruited in each cycle diminishes and consequently insufficient inhibin B is produced to fully lower FSH and the serum level of FSH begins to rise. Eventually the FSH level becomes so high that down regulation of FSH receptors occurs and by post menopause any remaining small secondary follicles no longer have neither FSH nor LH receptors. (80)

When the follicle matures and reaches (8–10 mm) in diameter it starts to secrete significant amounts of estradiol. Normally in humans only one follicle becomes dominant and survives to grow to (18–30 mm) in size and ovulate, the remaining follicles in the cohort undergo atresia. The sharp increase in estradiol production by the dominant follicle (possibly along with a decrease in gonadotrophin surge-attenuating factor) cause a positive effect on the hypothalamus and pituitary and rapid GnRH pulses occur and an LH surge results.

The decrease in FSH production by inhibiting GnRH production in the hypothalamus. (81) The decrease in serum FSH level causes the smaller follicles in the current cohort to undergo atresia as they lack sufficient sensitivity to FSH to survive. Occasionally two follicles reach the (10 mm) stage at the same time by chance and as both are equally sensitive to FSH both survive and grow in the low FSH environment and thus two ovulations can occur in one cycle possibly leading to non identical (dizygotic) twins.
High FSH levels:
The most common reason for high serum FSH concentration is in a female who
was undergoing or has recently undergone menopause. High levels of Follicle-
Stimulating Hormone indicated that the normal restricting feedback from the
gonad is absent, leading to an unrestricted pituitary FSH production. If high FSH
levels occurred during the reproductive years, it is abnormal conditions with high
FSH levels include: Premature menopause also known as premature ovarian failure
(POF).

1. Poor ovarian reserve also known as premature ovarian aging.
2. Gonadal dysgenesis, Turner syndrome.
3. Castration.
4. Klinefelter syndrome.
5. Systemic Lupus Erythematosus also known as Lupus. (82)

Most of these conditions are associated with sub fertility and/or infertility.
Therefore, high FSH levels are an indication of subfertility and/or infertility.

Low FSH levels:
Diminished secretion of FSH can result in failure of gonadal function
(hypogonadism). This condition is typically manifested in males as failure in
production of normal numbers of sperms. In females, cessation of reproductive
cycles is commonly observed. Conditions with very low FSH secretions are:

1. Polycystic Ovarian Syndrome (PCOS).
2. Polycystic Ovarian Syndrome + obesity + infertility.
3. Hypothalamic suppression.
4. Hypopituitarism.
5. Hyper prolactinaemia.
7. Gonadal suppression therapy.

2-16: Previous Studies:

No(1):
Clinical relevance of thyroid dysfunction in human haematopoiesis
Authors: Kawa M.P, Grymuła K, Paczkowska E, Baśkiewicz M.M, Dąbkowska E, Koziołek M, et al
Kawa M.P and et al in 2010 reported that RBC, HB and HCT in patients with hyperthyroidism were significantly higher than control groups while RBC and HB were decreased in hypothyroidism, and HCT was increased. They also showed that MCH and MCHC were lower in both groups in comparison with control group and MCV was increased in two groups of hypothyroidism and hyperthyroidism.\(^{(83)}\)

No(2):
Pancytopenia in untreated patients with Graves’ disease
Lima C.S and et al in 2006 described four patients with Graves’ disease who had severe pancytopenia. Finally they concluded that thyroid evaluation for all patients with pancytopenia should be performed even though no related symptoms were found.\(^{(84)}\)

No(3):
Role of red blood cell distribution width (rdw) in thyroid dysfunction
Authors: Geetha J, Srikrishna R.
Geetha J and Srikrishna R in 2012, red blood cell indices were compared in
patients with non-cancerous thyroid disorders and revealed that $RDW$ and $MCV$ in these two groups of patients in comparison to euthyroid individuals have statistically significant difference but other $RBC$ parameters like $HB$ and $HCT$ did not show any significant difference in comparison with euthyroid status but in our study, these parameters were statistically different between patients with non – cancerous thyroid disorders and control group except for $MCV$.\(^{(85)}\)

**No(4):**

**Effect of Thyroid Dysfunctions on Blood Cell Count and Red Blood Cell Indices**

**Authors:** Dorgalaleh A, Mahmoodi M, Varmaghani B.

The study performed on 102 patients with hypothyroid and hyperthyroid and 118 healthy individuals as control group. Initially patients TSH level of patients was determined by ELISA method, and then according to TSH ranges (0.3-5.5µIU/mL) patients were divided into two Hyperthyroidism (TSH<0.3µIU/mL) and hypothyroidism (TSH>5.5µIU/mL) groups. Then, complete blood count was measured by cell counter. Finally, obtained results were analyzed by SPSS software.

Analyzes of obtained data revealed statistically significant difference between two groups of patients in RBC count, MCH, MCHC, RDW, HB and HCT($P$-value<$0.05$), but the difference was not significant for WBC and PLT counts and MCV($P$-value>$0.05$).

In case of patients with unknown hematological dysfunctions must be evaluated for thyroid hormones.\(^{(92)}\)
No(5):
Coagulation profiles in hypothyroid and hyperthyroid female patients in Sudan.

Author: Mohamed-Ali-MS.

Abstract:
Objective: To evaluate disturbances in the coagulation system in female patients with thyroid disorders in order to assess the effects of thyroid diseases on coagulation parameters. This study was conducted in Khartoum state, the national capital of Sudan from February 2007 and February 2008. The study included 30 patients with clinical hypothyroidism, and 30 patients with sub-clinical hypothyroidism (21 of them were recruited before starting the treatment). Also, the study included 30 patients with clinical hyperthyroidism, 30 with sub-clinical hyperthyroidism, (37 of them were recruited before starting the treatment) and 30 normal individuals as the control group. Prothrombin time (PT), activated partial thromboplastin time, fibrinogen level, and platelets count were performed in patients and control samples. A significantly decrease in PT was observed in hypothyroid patients, and hyperthyroid patients compared to the control group. Activated thromboplastin time was significantly decreased only in hyperthyroid patients, compared to the control group. Moreover, fibrinogen level was significantly increased in hyperthyroid patients compared to hypothyroid patients. The study concluded that minor coagulation abnormalities were observed in both subclinical hypo- and hyperthyroidism compared to clinical hypo- and hyperthyroidism. Platelets count was also slightly decreased in both types of the disease. There was no significant effect of the treatment and age of such patients on the measured parameters. The study recommended screening female patients with hypo- and hyperthyroidism for coagulation defect, to avoid the risk of such
complications. (86)

No(6):
Correlation of prolactin and thyroid disorders in infertile women
Authors: Priyanka Sharma, Anita Pal, Rajeev Sood, Saroj Jaswal, Suman Thakur, Anupam Sharma.
Abstract
Background: The objective of the study was to review the impact of thyroid status on the fertility and to study the prevalence of hyperprolactinaemia in infertility. A total of (150) subjects were divided into (3) groups: (50) primary infertility, (50) secondary infertility and (50) controls. The incidence of hyperprolactinaemia and thyroid disorders was studied in all the three groups. The incidence of hyperprolactinaemia was (41%) in all infertile subjects (60% with primary and (22%) in secondary infertility) and (6%) in controls. The incidence of hypothyroidism was (17%) in infertility (18% in primary and 16% in secondary infertility) and (8%) in controls. In this study there is a positive correlation between increased prolactin levels and hypothyroidism and such patients’ exhibit ovulatory failure. All patients with infertility should undergo prolactin levels and thyroid profile. (90)

No(7):
Correlation of Prolactin and Thyroid Hormone Concentration with Menstrual Patterns in Infertile Women
Authors: Binita Goswami, Suprava Patel, Mainak Chatterjee, B.C. Koner, and Alpana Saxena.
In this study, we investigated 160 women with primary infertility who attended the Biochemistry department, Maulana Azad Medical College (MAMC), New Delhi for hormonal evaluations. Eighty fertile women with
similar age and socioeconomic status were enrolled as the controls. The association between thyroid dysfunction and levels of serum prolactin, LH and FSH as their menstrual status were reviewed. The majority of the infertile and fertile women were euthyroid. In infertile group, the crude prevalence of hypothyroidism was slightly higher in the infertile group in comparison with that of the general population. There was a positive correlation between serum TSH and prolactin levels in the infertile subjects. Menstrual disorders (mainly oligomenorrhgea), were reported by about (60% ) of the infertile women. Hyperprolactinemia was depicted in (41% ) of the infertile women while it was only (15%) in the control group. The infertile women with hypothyroidism had significantly higher prolactin levels when compared to the subjects with hyper- or euthyroidism. There was a significant association between abnormal menstrual patterns and anovulatory cycles, as observed on endometrial examination of infertile subjects with raised serum prolactin levels. There is a greater propensity for thyroid disorder in infertile women than the fertile ones. There is also a higher prevalence of hyperprolactinaemia in infertile patients.\(^{(91)}\)
Chapter Three

Materials and Methodology
3: Materials & Methods

3-1: Study design:
A cross-sectional, descriptive, hospital – based study, conducted at Almek Nimir University Hospital in females at reproductive age (18-48 years) with noncancerous thyroid disorders during the period from 2014 to 2017 to determine the haematological and hormonal changes.

3-2: Study area:
The study was conducted in Shendi town which is located (172Km) north to capital Khartoum, Southern part of River Nile State, and covering area about (30Km²).
There are several health centers for different services and purposes; also there is Shendi University with various faculties like faculty of medicine, faculty of Medical Laboratory Sciences & faculty of nurse. Shendi has three big hospitals, Elmek Nimir University Hospital, Shendi Teaching Hospital and Military Hospital; all of them have different departments which provide good health services for the population.

3-3: Study population & Sample size:
Venous blood samples were collected from (100 females) with thyroid disorders at reproductive age, (60 with hypothyroidism and 40 with hyperthyroidism) and (50) venous blood samples were obtained from healthy females as control group after they agreed to sign the consent form and to fill questionnaire.

Inclusion Criteria:
Females with thyroid disorders at reproductive age (18-48 years) & without treatment to know the effect of thyroid disorders on haematological parameters and hormones because the treatment may reveal these investigation to normal.
Exclusion Criteria:
Females with thyroid disorders at ages less than 18 or above 48 years, or under treatment (on warfarin, on corticosteroid) etc.

3-4: Sampling:
Six ml of venous blood was collected from each member (2.25 ml in trisodium citrate for coagulation study, (2.5 ml) in lithium heparin container for hormonal study and (1.25 ml) in EDTA container for CBC) then centrifuged immediately for (15 min) at (3000 rpm) to obtain plasma for estimating levels of PT and PTT (citrated plasma), the blood in lithium heparin also centrifuged to obtain plasma for estimating hormones levels (Prolactin & FSH).

3-5: Materials and instrument:

- Cotton.
- Syringe.
- Container with heparin as anticoagulant.
- EDTA container.
- Trisodium citrate container.
- Automatic pipettes.
- Tips (Yellow & Blue).
- Haematology analyzer (Mindray BC-5000).
- Centrifuge.
- Coagulyzer (Clot 2S).
- Automatic Immunoassay System (AIA 360).

3-6: Methods:

3-6-1: Complete blood count:
Complete blood count was performed using auto haematology analyzer.
Haematology Analyzer Automated Cell Counting Instrumentation BC-5000:
Definition: Haematology analyzers are computerized, highly specialized and automated machines that count the number of different kinds of white and red blood cells in a blood sample.

Electrical impedance method:
Principle of haematology analyzer: A stream of cells in suspension passes through a small aperture across which an electrical current is applied. Each cell that passes alters the electrical impedance and can thus be counted and sized. Particles such as blood cells are nonconductive but are suspended in an electrically conductive diluent. As a dilute suspension of cells is drawn through the aperture, the passage of each individual cell momentarily increases the impedance (resistance) of the electrical path between two electrodes that are located on each side of the aperture. A blood cell's size, surface charge, concentration of the cells, shape of cells can be determined. Impedance method for RBC and PLT counting Cyanide free reagent for hemoglobin test flow Cytometry (FCM) + Tri-angle laser scatter + Chemical dye method for WBCs

5. WBC differential analysis and WBC counting:
Parameters:
23 parameters: WBC, Lyme%, Mon%, Neu%, Bas%, Eos%, Lym#, Mon#, Neu#, Eos#, Bas#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, PDW, PCT,
(3) Histograms for WBC, RBC and PLT,
(3) Scatter grams for WBC differential,

Reagent:
Diluent, DIFF lyse, LH lyse, probe cleanser
Parameter Linearity Range:
WBC      (0-100×10^9/l).
RBC      (0-8×10^{12}/l).
HGB      (0-250g/l).
PLT      (0-1000×10^9/l).

Sample Volume:
Prediluted mode (20 μl).
Whole blood mode (15μ l).
Capillary whole blood mode (15 μl).

3-6-1-1: Principle of the method:

3-6-1-1-1: WBC measurement:

WBCs are counted and sized by the Coulter method. This method is based on the measurement of changes in electrical resistance produced by a particle, which in this case is a blood cell, suspended in a conductive diluent as it passes through an aperture of known dimensions. An electrode is submerged in the liquid on both sides of the aperture to create an electrical pathway. As each particle passes through the aperture, a transitory change in the resistance between the electrodes is produced. This change produces a measurable electrical pulse. The number of pulses generated signals the number of particles that passed through the aperture. The amplitude of each pulse is proportional to the volume of each particle. Each pulse is amplified and compared to the internal reference voltage channels, which only accepts the pulses of certain amplitude. If the pulse generated is above the WBC threshold, it is counted as a WBC.

3-6-1-1-2: HGB measurement:

HGB is determined by the colorimetric method. The WBC/HGB dilution is delivered to the WBC bath where it is bubble mixed with a certain amount of lyse, which converts haemoglobin to a haemoglobin complex that is measurable at
(525 nm). An LED is mounted on one side of the bath and emits a beam of light, which passes through the sample and a (525 nm) filter, and then is measured by a photo-sensor that is mounted on the opposite side. The signal is then amplified and the voltage is measured and compared to the blank reference reading understanding the system principles (readings taken when there is only diluent in the bath). The HGB is calculated per the following equation and expressed in (g/l).

\[ \text{HGB} \ (g/l) = \text{Constant Log 10 (Blank Photocurrent/Sample Photocurrent)} \]

**WBC** differential count with the help of the diluent and lyse, this analyzer can seize the white cells into three Sub-populations-lymphocytes, mid-sized cells (including monocytes, basophils and eosinophils) and granulocytes. Based on the **WBC** histogram, this analyzer calculates:

Lymph\%, Mid\% and Gran\% as follow and express the results in percents.

\[
\text{Lymph\%} = \frac{\text{PL} \times 100}{\text{PL} + \text{PM} + \text{PG}}
\]

\[
\text{Mid}\% = \frac{\text{PM} \times 100}{\text{PL} + \text{PM} + \text{PG}}
\]

\[
\text{Gran}\% = \frac{\text{PM} \times 100}{\text{PL} + \text{PM} + \text{PG}}
\]

Where PLT = particles in the lymphocyte region (10⁹ / l).

**PM** = particles in the mid size region (10⁹ / l).

**PG** = particles in the granulocyte region (10⁹ / l).

Having achieved the three parameters above, this analyzer proceeds to calculate the Lymph#, mid# and Gran# per the following equations and express them in (10⁹ / l).

\[
\text{Lymph}# = \frac{\text{Lymph\%} \times \text{WBC}}{100}
\]

\[
\text{Mid}# = \frac{\text{Mid}\% \times \text{WBC}}{100}
\]
Gran# = Gran% × WBC

\[
\text{Gran#} = \frac{\text{Gran} \% \times \text{WBC}}{100}
\]

3-6-1-1-3: RBC/PLT measurement:

RBCs/PLTs are counted and sized by the Coulter method. This method is based on
the measurement of changes in electrical resistance produced by a particle, which
in this case is a blood cell, suspended in a conductive diluent as it passes through
an aperture of known dimensions. An electrode is submerged in the liquid on both
sides of the aperture to create an electrical pathway. As each particle passes
through the aperture, a transitory change in the resistance between the electrodes is
produced. This change produces a measurable electrical pulse. The number of
pulses generated signals the number of particles that passed through the aperture.
The amplitude of each pulse is proportional to the volume of each particle. Each
pulse is amplified and compared to the internal reference voltage channels, which
only accepts the pulses of certain amplitude. If the pulse generated is above the
RBC/PLT lower threshold, it is counted as a RBC/PLT.

**Derivation of RBC-Related Parameters**

- **RBC:**
  RBC \((10^{12}/l)\) is the number of erythrocytes measured directly by counting the
  erythrocytes passing through the aperture.

- **MCV:**
  Based on the *RBC histogram*, this analyzer calculates the *mean cell volume (MCV)*
  and expresses the result in (fL).

This analyzer calculates the HCT (%), MCH (pg) and MCHC (g/l) as follows:

\[
\text{HCT} = \frac{\text{RBC} \times \text{MCV}}{10}
\]

\[
\text{MCH} = \frac{\text{HGB}}{\text{RBC}}
\]
\[ \text{MCHC} = \frac{\text{HGB} \times 100}{\text{HCT}} \]

- **RDW-CV:**
  Based on the *RBC histogram*, this analyzer calculates the *CV (Coefficient of Variation)* of the erythrocyte distribution width.

- **RDW-SD:**
  RDW-SD (RBC Distribution Width – Standard Deviation, fL) is set on the (20%) frequency level with the peak taken as (100%).

3.5.4 Derivation of PLT-Related Parameters:

- **PLT:**
  PLT \((10^9/l)\) is measured directly by counting the platelets passing through the aperture.

- **MPV:**
  Based on the *PLT histogram*, this analyzer calculates the *mean platelet volume* (MPV, fL).

- **PDW:**
  *Platelet distribution width (PDW)* is the *geometric standard deviation (GSD)* of the platelet size distribution. Each \(PDW\) result is derived from the platelet histogram data and is reported as \(10(GSD)\).

- **PCT:**
  This analyzer calculates the \(PCT\) and expresses it in (\%).

**3-6-2: Coagulation Profile:**

**Estimation of PT and APTT:**

**The Coagulyzer line:**

They combine the advantages of mechanical and photo-optical clot detection in the turbodensitometric principle.
**Reliable and accurate:**

- Results are automatically calculated in seconds, *INR* and *ratio*.
- Temperature controlled incubation block at (37.4 °C).
- Sample mixing by magnetic stir bar during measurement.
- Errors due to external light influence are excluded by the use of light protection caps.

**Agile and easy-to-use:**

- Automated cuvette detection and automated start function.
- Easy-to-use and straightforward operation via a membrane keypad
- Pre-programmed methods for *PT*, *APTT* and Fibrinogen efficient and affordable
- Memory function for reference curves.
- Ideal back-up solutions for the fully-automated Coagulyzer® 100.
- *PT*, *APTT* and Fibrinogen are already pre-calibrated (values are included in each LOT)

**Determination**  
**Short Information**

*PT recombinant* (ISI ~ 1.05), liquid stable

Prothrombin Time Human recombinant tissue factor.  
*APTT* Soy phospholipids, liquid stable.

Activated Partial Thromboplastin Time additionally required: calcium chloride  
**Calcium Chloride.**

For determination of *APTT* (0.025 M) calcium chloride.

**Controls:**

- **Control Plasma** N normal range, lyophilized  
- **Cuvette** CG0451 5x100 prefilled with mixer.
- **Cuvette** CG0452 1x500 separate mixers.
- **Cuvette** CG0454 5x500 separate mixers. **Teflon Mixer** for reagents CG0118 10 pcs.

**Preparation of blood sample:**
- Use freshly collected blood taken into (0.11 mol/l) trisodium citrate in the ratio (9) parts blood to (1) part anticoagulant.
- Centrifuged immediately in (5) minutes at RCF 1500-2000 g (approx.3000 rpm) and separate plasma into a clean test tube.
- Plasma should be tested within (3 hours).

**Procedure:**
1. Warm up the coagulation machine (at least 10 min before you begin the experiment switch on the machine).
2. Prepare all reagents (should have room temperature before use).
3. First place the special cuvette with a steel ball on the measuring positions in instrument related racks.

**Principle behind this technique:** Once the cuvette is kept in the rack it starts rotating and due to gravity the metal ball inside the cuvette always remains. When the plasma/blood is in solution the ball remains in the position and if the plasma/blood starts clotting the clot pulls the ball out of the basic position and the sensor detects the disturbance and measures the clotting time.
4. In order to measure the clotting times, add the reagents to the cuvette according to the schedule.

**3-6-2-1: Estimation of Prothrombin Time (PT):**
1. Add (100 µl) citrate plasma to the coagulometer and press incubation.
2. After (60 sec) incubation.
3. Add (200 µl) DiaPlastin: Calcium-Thromboplastin (rabbit brain) liquid. → then immediately press manual start button to measure coagulation.

**Interpretation of results:**
INR is calculated as follows:

\[
\text{Ratio} = \frac{\text{PT patient (second)}}{\text{PT FNP (second)}}
\]
INR= (Ratio) ^{ISI}

**Normal range:**
PT = (12-16 second).
INR= (0.64 to 1.17).

3-6-2-2: Estimation of Activated Partial Thromboplastin Time (APTT):

1- Add (100 µl) citrate plasma to the coagulometer and press incubation.

2- After (60 sec) incubation.

3-Add (100 µl) DiaCelin-L (Cephaloplastin, rabbit brain, with complexed kaolin), liquid.

4- Alter (200 sec) incubation, add (100 µl 0.02mol/l CaCl₂) → then immediately press manual start button to measure coagulation.

**Calculation and reporting of result:** (PTT)
a-The result can be directly reported in seconds.
b- Another reporting system is the PTT-ratio (R)

\[
R = \frac{\text{PTT patient plasma in seconds}}{\text{PTT (FNP) in seconds}}
\]

**Normal range:**
(25-40 seconds)

3-6-3: Estimation of Hormones:

3-6-3-1: Estimation of prolactin:

ST AIA-PACK PRL

For quantitative measurement of prolactin in heparinized plasma.

**Principle of the assay:**
The *ST AIA-PACK PRL* is a two-site immune-enzyme-metric assay which is performed entirely in the *ST AIA-PACK PRL* test cups. Prolactin present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase
and enzyme–labeled monoclonal antibody in the test cups. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate; 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the prolactin concentration in the test sample.

**Material provided:** (ST AIA-PACK PRL, at.No.0025255)

Plastic test cups containing lyophilized magnetic beads coated with anti-prolactin mouse monoclonal antibody and (100 µL) of anti-prolactin mouse monoclonal antibody conjugated to bovine alkaline phosphate with sodium azide as a preservative.

**Specimen collection:**

Heparinized plasma is required for assay. A venous blood sample is collected aseptically with designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.

The sample required for analysis is (30 µL).

**Procedure of method:**

For the AIA -360

1-**Reagent Preparation:**

a- **Substrate Solution:**

Bring all reagents to (18-25°C) before preparing the working reagent. Add the entire contents of the AIA-packs substrate reconstituent II (100 ml) to the lyophilized AIA -pack substrate reagent II and mix thoroughly to dissolve the solid material.

b- **Wash Solution.**

Add entire contents of the AIA-pack wash concentrate (100 ML) to approximately (2 L) of CAP Class 1 water or the clinical laboratory water. Mix well, and adjust the final volume to (2.5 L).
c- Diluent:
Add the entire contents of the AIA-pack diluent concentrate (100mL) to approximately (4 L) of CAP Class 1 water or the clinical laboratory reagent water, mix well, and adjust the final volume to (5 L).

**Calculation of Result:**
The TOSOH AIA System Analyzers perform all sample and reagent handling operations automatically. The TOSOH AIA System Analyzers read the rate of fluorescence produced by the reaction and automatically convert the rate to prolactin concentration in (ng/mL)

**Reference Range:**
Female: (4.1- 28.9 ng/ML)

3-6-3-2: Estimation of FSH:

*ST AIA-PACK PRL*

For quantitative measurement of *follicle-stimulating hormone (FSH)* in heparinized plasma.

**Principle of the assay:**
The ST AIA-PACK FSH is a two –site immune-enzymo-meteric assay which is performed entirely in the ST AIA-PACK FSH test cups. *FSH* present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme –labeled monoclonal antibody in the test cups. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate; 4-methylumbelliferyl phosphate (4MUP).The amount of enzyme- labeled monoclonal antibody that binds to the beads is directly proportional to the *FSH concentration* in the test sample.

**Material Provided** (ST AIA-PACK FSH, Cat.No.0025265):
Plastic test cups containing lyophilized magnetic beads coated with *anti-FSH* mouse monoclonal antibody and (100 µL of *anti-FSH*) mouse monoclonal
antibody conjugated to bovine alkaline phosphate with sodium azide as a preservative.

**Specimen collection:**
Heparinized plasma is required for assay. A venous blood sample is collected aseptically with designated additive. Centrifuged and separated plasma from the packed cells as soon as possible.
The sample required for analysis is (50 µl).

**Procedure of method:**
For the AIA -360

1-Reagent preparation:

a- Substrate Solution
Bring all reagents to (18-25°C) before preparing the working reagent. Add the entire contents of the AIA-pack substrate reconstituent II (100mL) to the lyophilized AIA pack substrate reagent II and mix thoroughly to dissolve the solid material.

b- Wash Solution:
Add entire contents of the AIA-pack wash concentrate (100ML) to approximately (2 L) of CAP Class 1 water or the clinical laboratory water. Mix well, and adjust the final volume to (2.5L).

c- Diluent:
Add the entire contents of the AIA-pack diluent concentrate (100mL) to approximately (4 L) of CAP Class 1 water or the clinical laboratory reagent water, mix well, and adjust the final volume to (5 L).

**Calculation of Result:**
The TOSOH AIA System Analyzers perform all sample and reagent handling operations automatically. The TOSOH AIA System Analyzers read the rate of
fluorescence produced by the reaction and automatically convert the rate to follicle-stimulating hormone concentration in mIU/mL

**Reference Range:**

**Ovulating Female:**

**Follicular Phase** = 4.5-11 mIU/mL  
**Mid-Cycle** = 3.6-20.6 mIU/mL  
**Luteal Phase** = 1.5-10.8 mIU/mL  
**Postmenopausal female** = 36.6-168.8 mIU/mL

**3-7: Ethical consideration:**

The entire sample collected for study population takes ethically after information about the study and ethical approvals, letter of the faculty, letter of the hospital and patient acceptance form.

**3-8: Data analysis:**

The gathered data was analyzed with Statistical Packages for Social Sciences (SPSS) soft ware version (20), Independent T-test was used for calculating degree of variation. P. value<0.05 was considered significant variation.
4: Results

The study includes (150) females at reproductive age from (18 to 48 years), (100) of them with thyroid disorders (60 with hypothyroidism and (40) with hyperthyroidism), and the other (50) are healthy females as control groups.

Table (4-1): Patients with thyroid disorders (%).

<table>
<thead>
<tr>
<th>Category</th>
<th>NO</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroidism</td>
<td>60</td>
<td>40%</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>40</td>
<td>27%</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>33%</td>
</tr>
</tbody>
</table>

Figure (4-1): Age of patients and control. The age groups (18-27), (28-37) and (38-48) years.
Chapter Four

Results
**Figure (4-2): Marital status of the patients and control**

The figure above showed the marital status of females under study and control, married were (44, 30 and 18) and single were (16, 10 and 32) in hypothyroidism, hyperthyroidism and control group respectively.
Table (4-2): Mean of RBCs count, Hb and RBCs indices in female with hypothyroidism and control group

<table>
<thead>
<tr>
<th></th>
<th>Hypothyroidism</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs x10¹² /L</td>
<td>4.2</td>
<td>4.3</td>
<td>0.19</td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>12</td>
<td>12.9</td>
<td>0.002</td>
</tr>
<tr>
<td>PCV %</td>
<td>37</td>
<td>38</td>
<td>0.02</td>
</tr>
<tr>
<td>MCV /fl</td>
<td>87</td>
<td>89</td>
<td>0.28</td>
</tr>
<tr>
<td>MCH/pg</td>
<td>29</td>
<td>30</td>
<td>0.054</td>
</tr>
<tr>
<td>MCHCg/dl</td>
<td>33</td>
<td>34</td>
<td>0.06</td>
</tr>
<tr>
<td>RDW %</td>
<td>14.8</td>
<td>13.9</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table (4-3): Mean of RBCs count, Hb and RBCs indices in female with hyperthyroidism and control group

<table>
<thead>
<tr>
<th></th>
<th>Hyperthyroidism</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs x10¹² /L</td>
<td>4.3</td>
<td>4.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>12.1</td>
<td>12.9</td>
<td>0.002</td>
</tr>
<tr>
<td>PCV %</td>
<td>36</td>
<td>38</td>
<td>0.007</td>
</tr>
<tr>
<td>MCV /fl</td>
<td>84</td>
<td>89</td>
<td>0.002</td>
</tr>
<tr>
<td>MCH/pg</td>
<td>28</td>
<td>30</td>
<td>0.002</td>
</tr>
<tr>
<td>MCHCg/dl</td>
<td>33</td>
<td>34</td>
<td>0.06</td>
</tr>
<tr>
<td>RDW %</td>
<td>14.9</td>
<td>13.9</td>
<td>0.02</td>
</tr>
</tbody>
</table>
### Table (4-4): Mean of Hb level (g/dL) according to Age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Hypothyroidism</th>
<th>Hyperthyroidism</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-27</td>
<td>12.1</td>
<td>11.9</td>
<td>12.8</td>
</tr>
<tr>
<td>28-37</td>
<td>12.4</td>
<td>12.8</td>
<td>12.9</td>
</tr>
<tr>
<td>38-48</td>
<td>11.9</td>
<td>11.8</td>
<td>13.3</td>
</tr>
</tbody>
</table>

### Table (4-5): Mean of TWBCs and differential count in female with hypothyroidism and control

<table>
<thead>
<tr>
<th></th>
<th>Hypothyroidism</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWBCs x10^6 /L</td>
<td>5.8</td>
<td>5.4</td>
<td>0.23</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>47</td>
<td>46</td>
<td>0.3</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>40</td>
<td>40</td>
<td>0.8</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>9</td>
<td>11</td>
<td>0.01</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>2.8</td>
<td>2.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Basophil %</td>
<td>0.9</td>
<td>1.1</td>
<td>0.03</td>
</tr>
</tbody>
</table>

### Table (4-6): Mean of TWBCs and differential count in female with hyperthyroidism and control

<table>
<thead>
<tr>
<th></th>
<th>Hyperthyroidism</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWBCs x10^6 /L</td>
<td>5.9</td>
<td>5.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>50</td>
<td>46</td>
<td>0.02</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>36</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>10</td>
<td>11</td>
<td>0.18</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>2.9</td>
<td>2.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Basophil %</td>
<td>0.9</td>
<td>1.1</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Table (4-7): Mean of Platelet count and MPV in female with hypothyroidism and control

<table>
<thead>
<tr>
<th></th>
<th>Hypothyroidism</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet</td>
<td>240</td>
<td>253</td>
<td>0.3</td>
</tr>
<tr>
<td>MPV</td>
<td>10.3</td>
<td>11</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table (4-8): Mean of Platelet count and MPV in female with hyperthyroidism and control

<table>
<thead>
<tr>
<th></th>
<th>Hyperthyroidism</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet</td>
<td>248</td>
<td>253</td>
<td>0.7</td>
</tr>
<tr>
<td>MPV</td>
<td>10.5</td>
<td>11</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Table (4-9): Mean of PT, INR and PTT in female with hypothyroidism and control

<table>
<thead>
<tr>
<th></th>
<th>Hypothyroidism</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>13.2</td>
<td>13.8</td>
<td>0.000</td>
</tr>
<tr>
<td>INR</td>
<td>1</td>
<td>1.1</td>
<td>0.002</td>
</tr>
<tr>
<td>PTT</td>
<td>33.3</td>
<td>35.5</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table (4-10): Mean of PT, INR and PTT in female with hyperthyroidism and control

<table>
<thead>
<tr>
<th></th>
<th>Hyperthyroidism</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>13.1</td>
<td>13.8</td>
<td>0.001</td>
</tr>
<tr>
<td>INR</td>
<td>1</td>
<td>1.1</td>
<td>0.003</td>
</tr>
<tr>
<td>PTT</td>
<td>33.4</td>
<td>35.5</td>
<td>0.12</td>
</tr>
</tbody>
</table>
Figure (4-3): Frequency of bleeding tendency and Coagulation disorders in female with thyroid disorders

Figure above reveal that (22, 13) females had abortion in hypothyroidism and hyperthyroidism respectively and one female with thrombosis and other one with menorrhagia in female with hyperthyroidism.
Table (4-11): Past history of bleeding & thrombotic complications in female with hypothyroidism

<table>
<thead>
<tr>
<th>Complication</th>
<th>Frequency</th>
<th>Percent%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion</td>
<td>22</td>
<td>36.7</td>
</tr>
<tr>
<td>No complications</td>
<td>38</td>
<td>63.3</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

Table (4-12): Past history of bleeding & thrombotic complications in female with hyperthyroidism

<table>
<thead>
<tr>
<th>Complication</th>
<th>Frequency</th>
<th>Percent%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion</td>
<td>13</td>
<td>32.5</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>No Complications</td>
<td>25</td>
<td>62.5</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>
Table (4-13): Mean of PT, PTT and platelet count in female with hypothyroidism with past history of bleeding & thrombotic complications

<table>
<thead>
<tr>
<th>Category</th>
<th>No</th>
<th>PT/sec</th>
<th>PTT/sec</th>
<th>Platelet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female with hypothyroidism &amp; abortion</td>
<td>22</td>
<td>13</td>
<td>32.3</td>
<td>244</td>
</tr>
<tr>
<td>Female with hypothyroidism &amp; without bleeding disorders</td>
<td>38</td>
<td>13.7</td>
<td>33.9</td>
<td>238</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>13.8</td>
<td>35.5</td>
<td>253</td>
</tr>
</tbody>
</table>

Table (4-14): Mean of PT, PTT and platelet count in female with hyperthyroidism with past history of bleeding & thrombotic complications

<table>
<thead>
<tr>
<th>Category</th>
<th>NO</th>
<th>PT/sec</th>
<th>PTT/sec</th>
<th>Platelet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female with hyperthyroidism &amp; history of abortion</td>
<td>13</td>
<td>12.9</td>
<td>31.5</td>
<td>215</td>
</tr>
<tr>
<td>Female with hyperthyroidism &amp; history of thrombosis</td>
<td>1</td>
<td>12.5</td>
<td>34.8</td>
<td>286</td>
</tr>
<tr>
<td>Female with hyperthyroidism &amp; history of menorrhagia</td>
<td>1</td>
<td>13.9</td>
<td>21.8</td>
<td>194</td>
</tr>
<tr>
<td>Female with hyperthyroidism &amp; without bleeding or coagulation disorder</td>
<td>25</td>
<td>13.4</td>
<td>34.8</td>
<td>266</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>13.8</td>
<td>35.5</td>
<td>253</td>
</tr>
</tbody>
</table>
Table (4-15): Mean of prolactin (ng/mL) according to thyroid disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mean</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroidism</td>
<td>18.2</td>
<td>14.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>10.3</td>
<td>14.4</td>
<td>0.004</td>
</tr>
</tbody>
</table>

The table above showed significant difference in prolactin between female with hyperthyroidism and control, but not significant with hypothyroidism

Table (4-16): Mean of FSH (mIU/mL) at different stage of menstrual cycle in female with hypothyroidism and control

<table>
<thead>
<tr>
<th>Phase</th>
<th>Normal Range</th>
<th>Hypothyroidism</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular</td>
<td>4.5-11</td>
<td>10.5</td>
<td>12.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Mid Cycle</td>
<td>3.6-20.6</td>
<td>5.9</td>
<td>9.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Luteal</td>
<td>1.5-10.8</td>
<td>30.8</td>
<td>7.6</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table (4-17): Mean of FSH (mIU/mL) at different stage of menstrual cycle in female with hyperthyroidism and control

<table>
<thead>
<tr>
<th>Phase</th>
<th>Normal Range</th>
<th>Hyperthyroidism</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular</td>
<td>4.5-11</td>
<td>31.3</td>
<td>12.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Mid Cycle</td>
<td>3.6-20.6</td>
<td>15.9</td>
<td>9.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Luteal</td>
<td>1.5-10.8</td>
<td>37.6</td>
<td>7.6</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Chapter Five

Discussion
Conclusion
Recommendations
5-1: Discussions:

Thyroid gland as the largest and the most important endocrine gland of human body with the secretion of two hormones, T3 and T4, has a major role in metabolism of cells and organs. Thyroid gland also has a crucial effect on erythropoiesis by induction of erythropoietin secretion and also proliferation of erythroid progenitors. (87, 88,89)

The most common thyroid dysfunctions, hypothyroidism and hyperthyroidism affect blood cells and cause anaemia with different severity. These thyroid disorders also cause thrombocytopenia, leukopaenia and even in rare cases cause pancytopenia (in hypothyroidism). Other blood indices including MCV, MCH, MCHC, Hb also could change during thyroid dysfunction. (83)

Thus, this study aimed to evaluate effects of thyroid dysfunctions on blood cells count and red blood cells indices, coagulation profile and hormonal abnormalities in female at reproductive age.

The study included (150) females (60) with hypothyroidism (40%), (40) females with hyperthyroidism (27%) and (50) females as control (33%) as shown in Table (4-1).

The age of the studied population range from (18-48 years) in the age group (18-27 years), (17,7,30) females with hypothyroidism, hyperthyroidism and control respectively, in the age group (28-37 years) (16,8,8) females with hypothyroidism, hyperthyroidism and control respectively, and in the age group (38-48 years) (27,25,12) females with hypothyroidism, hyperthyroidism and control respectively Figure-(4-1).
According to marital status (44, 30 and 18) were married and (16, 10 and 32) were single in females with hypothyroidism, hyperthyroidism and control groups respectively in figure (4-2)

According to data obtained in patients with hypothyroidism, although all parameters were decreased except RDW which increased, only Hb, PCV and RDW showed statistically significant difference between female with hypothyroidism compared to the control group (P-value <0.05). High RDW with the decreased MCV can be due to the slight anaemia seen in some groups (anaemia of chronic disorder) Table (4-4). The study agree with the study of Kawa MP and et al in 2010 who reported that RBC, HB, MCH and MCHC were decreased in hypothyroidism, while differ in HCT&MCV when they stated that they were increased. (83)(92)

In females with hyperthyroidism although all parameters were decreased except RDW which increased, only Hb, PCV, MCV, MCH and RDW were compared with the control group showed statistically significant difference (P-value <0.05) but the RBCs count and MCHC had no significant difference as indicated in Table (4-3). Also high RDW with the decreased MCV can be due to the slight anaemia seen in some groups (anaemia of chronic disorder) Table (4-4). This study didn’t coincide with the study of Kawa MP and et al in 2010 who reported that RBC, HB and HCT in patients with hyperthyroidism were significantly higher than control groups & this looks strange & may be due to the good medical supervision in those patients which was not available to the patients while they concluded that MCH and MCHC were lower in their study which agreed with the findings

Hb was low in females with these disorders in all age groups compared with control group. The Hb in age (18-27) were (12.1,11.9 and 12.8) and in age (28-37)
were (12.4, 12.8 and 12.9) and in age (38-48) were (11.9, 11.8 and 13.3) in hypothyroidism, hyperthyroidism and control groups as shown in Table (4-4), these results indicated that these disorders lead to decrease in Hb level & anaemia was seen in some patients.

**In hypothyroidism** the results showed statistically significant difference in monocyte and basophil count (P-value <0.05) but did not show statistically significant difference in WBC, neutrophil, lymphocyte and eosinophil count (P-value >0.05), Table(4-5).

**In hyperthyroidism** the neutrophil and basophil showed statistically significant difference compared with control group (P-value >0.05) but the WBC, lymphocyte, monocyte and eosinophil counts didn’t show significant difference (P-value >0.05) Table (4-6).

This study did not show statistically significant difference in PLT count and MPV in two groups of females and control (P-value >0.05), whereas; MPV had significant difference in hypothyroidism (P-value <0.05) Tables (4-7 &4-8).

In a study by Geetha J and Srikrishna R in 2012, red blood cell indices were compared with patients with hypothyroidism and hyperthyroidism revealed that RDW and MCV in these two groups of patients in comparison to euthyroid individuals were statistically significant difference but other RBC parameters like HB and HCT did not show any significant difference in comparison with euthyroid status but in this performed study, HB and PCV and RDW were statistically different between patients with hypothyroidism and hyperthyroidism and control group but RBCs and MCHC showed no difference and MCV and MCH were significantly different in hyperthyroidism. (85)
Lima C.S and et al in 2006 described four patients with Graves’ disease who had severe pancytopenia. Finally they concluded that thyroid evaluation for all patients with pancytopenia should be performed even though no related symptoms are found.\(^{(84)}\)

The results revealed that \(PT\), \(PTT\) & \(INR\) were decreased in the patients but it was statistically significant in \(PT\) and \(INR\) only \((P\text{-value} < 0.05)\) and not statistically significant in \(PTT\) Tables \((4-9 & 4-10)\).

To the study conducted by Mohamed-Ali MS, Ahmed RO (A significantly decrease in \(PT\) was observed in hypothyroid patients, and hyperthyroid patients compared with the control group, \(PTT\) was significantly decreased only in hyperthyroid patients compared to the control group.)\(^{(86)}\) But in this study the \(APTT\) were decreased in the two groups compared with control groups but they were not statistically significant as mentioned above.

**Figure (4-3)** revealed that, (22, 13) females had abortion in hypothyroidism and hyperthyroidism respectively and one female with \(DVT\) and other one with menorrhagia in female with hyperthyroidism.

In this study; (60) females with hypothyroidism (22) of them had a bleeding tendency (36.7%) such as abortion and (38) did not show bleeding tendency (63.3%) **Table (4-11)**. The hyperthyroidism status included (40) females (13) of them had abortion (32.5%), one female had \(DVT\) (2.5%), also another one female had menorrhagia (2.5%) and other (25) females did not show any disorders (62.5%) as mentioned in **Table-(4-12)**.

**Regarding the results obtained**: this study correlated between history of complications of pregnancy (bleeding tendency and coagulation disorders) by
estimation of $PT$, $PTT$ and platelet count our result show that $PT$ (13, 13.7 and 13.8), $PTT$ (32.3, 33.9 and 35.5) and platelet count was (244, 238 and 253) in females with abortion, without abortion in hypothyroidism and control respectively. The above results showed that $PT$ and $PTT$ in abortion was less than without abortion due to the disturbance in coagulation factor, but the Platelet count was more in the case of abortion because the bleeding induces bone marrow to produce more platelets, and there were low in $PT$, $PTT$ and Platelet count compared with control groups as appeared in Table-(4-13).

The above calculated results showed that the $PT$ (12.9, 12.5, 13.9, 13.4 and 13.8), $PTT$ (31.5, 34.8, 21.8, 33.8 and 35.5) platelet count (215, 286, 194, 266 and 253) in female with abortion, DVT, menorrhagia and had no disorders in hyperthyroidism and control respectively, this result showed $PT$ and Platelets were less in menorrhagia as presented in Table-(4-14).

There was statistically significant difference in prolactin level between hyperthyroidism and control ($P$-value < 0.05) but did not show significant statistical difference between hypothyroidism and control group ($P$-value > 0.05), as presented in Table (4-15). The prolactin level in hypothyroidism was high & indicates prolactinaemia. These results coincide with the study done by Priyanka Sharma which conducted in the Department of Obstetrics and Gynaecology, Kamla Nehru State Hospital for Mother and Child, Indira Gandhi Medial College, Sharma who concluded that there was a positive correlation between increased prolactin levels and hypothyroidism. Also the result was agree with the study conducted by Binita Goswami and et al, who conclude that There was a positive correlation between serum TSH and prolactin levels and Menstrual disorders.
In follicular phase *FSH level* in patients with hypothyroidism was within the normal range (10.5 and NR: 4.5-11), in mid cycle the level of *FSH* in hypothyroidism also within the normal range (5.9 and NR: 3.6-20.6). In luteal phase the level of *FSH* was high (30.8, and NR: 1.5-10.8) & these results showed significant difference in *FSH level* in luteal phase compared with control (*P*-value <0.05) **Table-(4-16).**

In females with hyperthyroidism although the level of *FSH* in follicular phase was high (31.3 and NR: 4.5-11) but it was not statistically significant compared with the control. In mid cycle the level of *FSH* in hyperthyroidism was within the normal range (15.9 and NR: 3.6-20.6). The *level of FSH* in luteal phase was high than normal (37.6 and NR: 1.5-10.8) & these results showed significant difference in *FSH level* in luteal phase when compared with control (*P*-value <0.05) **Table (4-17),** the *FSH* was high because there was chronic failure of ovulation due to thyroid disease as mentioned before.

**According to the cumulative data obtained:** it is suggested that all patients with hypothyroidism and hyperthyroidism should be periodically evaluated for probably haematological changes, coagulation disorders and hormones of reproduction.
5-2: Conclusion:

Thyroid noncancerous dysfunctions have a direct effect on most red blood cells indices, white blood cells, platelet count, coagulation profile and hormones of reproduction and these changes should be considered by medical care provider. The study concluded that:

► On complete blood count some females with thyroid disorders had $Hb$ less than the lower limit of normal range.

► White blood cells count did not affect by thyroid disorders but in differential count there were some variation between females with thyroid disorders and healthy females.

► Platelets count was also slightly decreased in both females with thyroid disorders compared with healthy females.

► Coagulation profile show decrease in $PT$ and $APTT$ in the two groups of females study in comparison to the healthy females.

► Minor coagulation abnormalities were observed in females with thyroid disorders that had past history of abortion or coagulation abnormalities.

► Females with thyroid disorders showed disturbances in $FSH$ which may lead to menstrual cycle abnormalities.

► Females with hypothyroidism had prolactin hormone level more than the control and that may lead to infertility.
5-3: Recommendations:

The study recommended that:

► Screening of females at reproductive age with hypothyroidism and hyperthyroidism for complete blood count to avoid the incidences of anaemia.

► Screening of females for coagulation defect, to avoid the risk of such complications (Bleeding tendency and thrombosis).

► Screening hormones of reproduction to avoid the disturbances in Prolactin and FSH levels; which lead to menstrual cycle disturbances and cause infertility.
Chapter Six

References

Appendix
6: References:


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University of Shendi
Faculty of Graduate Studies and Scientific Research

Questionnaire about:
Determination of Haematological and Hormonal changes in Females with Thyroid Disorders at Reproductive Age at Al Mak Nimir University Hospital - Shendi – River Nile State - Sudan
(For Study Groups)

1- Code

2- Name

3- Age

4- Marital status: married , not married

5- Do you currently have any of these symptoms?
Palpitations (rapid or forceful heart beat): Yes No
Poor concentration: Yes No
Difficulty sleeping: Yes No
Excessive need for sleep: Yes No
Fatigue: Yes No
Anxiety: Yes No
Nails changes: Yes [ ] No [ ]

Explained weight: Normal [ ] Increased [ ] Decreased [ ]

Vision disturbance: Yes [ ] No [ ]

Irregular menstrual periods: Yes [ ] No [ ]

Excessive menstrual flow: Yes [ ] No [ ]

Have you been pregnant or miscarried during the past 2 years?

a- Pregnant: Yes [ ] No [ ]

b- Miscarried: Yes [ ] No [ ]

6- if married is she fertile or infertile [ ]

7- Duration of married [ ]

8- Family history of thyroid disease? Yes [ ] No [ ]

If yes, please indicates the diagnosis which applies to them, if known.

a- Overactive thyroid gland [ ]

b- Underactive thyroid gland [ ]

9- Have you ever been diagnosed with a thyroid disease?

Yes [ ] No [ ]

If yes, please indicate:

a- Overactive thyroid gland [ ]

b- Underactive thyroid gland [ ]
10- Are you currently being treated for a thyroid disease?
Yes ☐ No ☐
If yes, please indicates:
  a- Thyroid hormone therapy (eg. Synthroid, Eltroxin, Levothyroxine) ☐
  b- Antithyroid drug therapy (eg. PTU, Methimazole) ☐
  c- Other ☐

11- Were you ever treated for a thyroid disease in the past?
Yes ☐ No ☐
If yes, please indicates all that apply: -
  a- Thyroid hormone therapy (eg. Synthroid, Eltroxin, Levothyroxine) ☐
  b- Thyroid surgery ☐
  c- Radioiodine therapy (not the diagnostic scan) ☐
  d- Antithyroid drug therapy (eg. PTU, Methimazole) ☐
  e- Other ☐

12- Do you complain of bleeding tendency: - Yes ☐ No ☐
If yes please indicate:
  a- The type of bleeding tendency: ……………
  b- The duration of bleeding tendency: ……………

13- Do you complain of thrombosis disorders: - Yes ☐ No ☐
If yes please indicates?
a- The type of thrombosis: ............
b- The duration of thrombosis: ............

**14- Date of menses**  .................................................................
Date: ......................  Signature: ...........................................

**Result:**

**Coagulation:**    Control =
PT =  INR=  PTT =  Ratio =

**Hormones:**
FSH =  Prolactin =

**Haematological Parameters:**
Hb =  Platelet=  TWBCs =
RBCs =  MPV =  N =
PCV =  PDW =  L =
MCV =  PCT =  M =
MCH =  E =
MCHC =  B =
RDW =
Appendix (3)

بسم الله الرحمن الرحيم

جامعة شندي

كلية الدراسات العليا والبحث العلمي

دراسة لنيل درجة الدكتوراة - لمعرفة التغيرات الدموية والهرمونية التي تحدث عند الإناث في فترة الإنجاب المصابات بمرض الغدة الدرقية

الإسم: ...........................................................................................................................................

سوف يتم أخذ عينة من الدم بحجم 6 مل من الوريدي بواسطة حقنة طحن معقمة وذلك بعد تعقيم منطقة أخذ العينة بواسطة مطهر. كل الأدوات المستخدمة لأخذ العينة معقمة ومتبع فيها كل وسائل السلامة العملية وليس هناك اثار جانبية للعملية ولكن ربما يحصل تورم بسيط واحمرار في منطقة أخذ العينة وسوف يزول بعد فترة قصيرة. الهدف من الدراسة هو معرفة تأثير مرض الغدة الدرقية على تعداد الدم الكامل (قياس الهيموغلوبين، تعداد كريات الدم الحمراء وعواملها وكيكيات الدم البيضاء والاعد التفريقي لها وتحديد الصفيحات الدموية)، وكذلك يتم إجراء بعض اختبارات التجلط (زمن البروثرمبين والثرومبلافاستين) وقياس الهرمونات الانتثوية (هرمون اللين والهرمون المنبه للجريب).

وافق أنا المذكورة أعلاه على أخذ عينة الدم لإجراء الدراسة.

الإض?action:..........................................................................................................................

التاريخ: ..........................................................................................................................