

بسم الله الرحمن الرحيم



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**Evaluation of some Haemostatic Parameter Among
hyperthyroidism and hypothyroidism Patients in Khartoum State.**

تقويم بعض قياسات تخثر الدم لمرضى الغدة الدرقية (الزيادة والنقصان في هرمون
الغدة الدرقية) في ولاية الخرطوم

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الآية

بسم الله الرحمن الرحيم

وَإِنْ يَمْسَسْكَ اللَّهُ بِضُرٍّ فَلَا كَاشِفَ لَهُ إِلَّا هُوَ ۖ وَإِنْ
يَمْسَسْكَ بِخَيْرٍ فَهُوَ عَلَىٰ كُلِّ شَيْءٍ قَدِيرٌ

صدق الله العظيم

سورة الأنعام الآية (17)

Dedication

To my teachers

Who gave me the gift of sharing their minds and experiences, so as to be a
creative one

To my father

Who gave me advices and support through the years. I am very grateful for
everything you have done for me

To my mother

Who is continuously encouraging me and guiding me toward success, made me
a man and learned the meaning of love

To my colleagues and dear friends

Who always being by my side through good and bad times

With love and best wishes

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By the grace of Allah and his help I completed this study, all praise to him,

I would like to express my appreciation to my advisory committee.

Thanks for giving me the opportunity to discuss this work

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Abstract

This is an analytical case control study ,it's aim to determine the changes induced by hyperthyroidism and hypothyroidism in platelet count , PT(INR) and APTT .It was conducted at the Radiation Isotope Center

Khartoum State during the period March to June 2014.The study population was 30 patients with hyperthyroidism , 30 patients with hypothyroidism and 30 normal individuals (control group) .

A questionnaire was used to obtain patients' information as age, gender, duration of diseases, treatment ,use of salt and family history.The participants were orally interviewed after an informed verbal consent was taken .

In both cases of hyperthyroidism and hypothyroidism the majority were females ,aged 31-45,more than 50% have family history of the disease, with a disease duration of less than one year in 90%of the cases and the majority did not use iodized salt .

Five ml of venous blood was collected from all the participants. Sysmex CA-50 automated blood coagulation analyze was used for measuring activated partial thromboplastin time(APTT) and prothrombin time(PT)and platelet(PLT) count was performed by Sysmex KX-21 hematological autoanalyzer.

APTT value was significantly lower ($P \leq 0.05$) in patients with hyperthyroidism (26.61 ± 2.86) than in the control group(33.2 ± 2.86). Hypothyroidism patient showed APTT values(33.84 ± 3.28) similar to that of the control group and it was significantly ($P \leq 0.05$)higher than that of hyperthyroid cases. PT values in both patients with hypothyroidism (10.20 ± 1.67) and hyperthyroidism (11.2 ± 1.95)were significantly lower ($P \leq 0.05$)than that of the control group (13.6 ± 1.23)

Hypothyroid patients showed a significantly ($P \leq 0.05$) higher reduction in PT value than hyperthyroid patients. No significant variation was observed in the PLT count among the study population.

It was concluded that both hypothyroidism and hyperthyroidism are associated with significant abnormalities in some of the coagulation parameters.

المستخلص

تهدف الدراسة إلى تحديد التغيرات الناجمة عن فرط نشاط الغدة الدرقية وخمول الغدة الدرقية في كل من اختبار عدد الصفائح الدموية، البروثرومبين (INR) PT و وقت التنشيط الجزئي لتجلط الدم APTT . أجريت الدراسة في مركز الطب النووي في ولاية الخرطوم خلال الفترة من مارس إلى يونيو 2014 . يشمل مجتمع الدراسة 30 مريضاً يعانون من فرط نشاط الغدة الدرقية ، و 30 مريضاً يعانون قصور في نشاط الغدة الدرقية و 30 شخصاً عادياً وهم عبارة عن المجموعة الضابطة . تم استخدام الاستبيان للحصول على المعلومات من المرضى كالعمر والجنس وفترة ظهور المرض، والعلاج، واستخدام ملح اليود و تاريخ المرض في الأسرة و أجريت المقابلات مع المشاركين من المرضى شفويًا بعد أخذ موافقتهم كتابةً ولفظاً.

في كلتا حالتَي قصور و فرط نشاط الغدة الدرقية كانت الغالبية من الإناث، وتراوحت أعمارهن ما بين 31-45، وكان أكثر من 50٪ لديهم تاريخ عائلي للمرض، وكانت مدة المرض أقل من سنة واحدة في 90٪ من الحالات، وكان الأغلبية ممن لا يستخدمون الملح المعالج باليود.

وقد تم جمع خمسة مل من الدم الوريدي من جميع المرضى وقد تم استخدام جهاز SYSMEX CA-50 لقياس زمن تخثر الدم في اختبار وقت التنشيط الجزئي لتجلط الدم (APTT) ووقت البروثرومبين (PT) وتم استخدام جهاز SYSMEX KX-21 المحلل التلقائي لعد الصفائح الدموية (PLT).

وكانت قيمة APTT أقل بكثير ($P \leq 0.05$) في المرضى الذين يعانون من فرط نشاط الغدة الدرقية (26.61 ± 2.86) مقارنة بالمجموعة الضابطة (33.2 ± 2.86). وأظهر مرضى قصور نشاط الغدة الدرقية قيمة مماثلة لتلك القيم المستخرجة من المجموعة الضابطة في اختبار وقت التنشيط الجزئي لتجلط الدم APTT وكانت قيمته (33.84 ± 3.28). وكانت قيمة APTT أعلى في حالات زيادة نشاط الغدة الدرقية مقارنة بحالات قصور نشاط الغدة ($P \leq 0.05$). كما كانت قيم وقت البروثرومبين PT في كل من المرضى الذين يعانون من قصور الغدة الدرقية (10.20 ± 1.67) و في مرضى فرط نشاط الغدة الدرقية كانت قيم وقت البروثرومبين PT (11.2 ± 1.95) وهي أقل بكثير من تلك القيم المستخرجة من مجموعة السيطرة (13.6 ± 1.23) وكانت ($P \leq 0.05$). كما أظهر مرضى قصور الغدة الدرقية وبشكل ملحوظ أعلى انخفاض في قيمة PT من الذي كان في مرضى زيادة نشاط الغدة . الدرقية ($P \leq 0.05$). وقد لوحظ عدم وجود اختلاف كبير في عدد الصفائح الدموية PLT بين مرضى الحالتين في الدراسة.

نخلص إلى أن كلا من قصور و فرط نشاط الدرقية ترتبط بتغيرات غير طبيعية في بعض مؤشرات قياس تخثر الدم .

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Abbreviations

| | |
|------------|---|
| ADP | : Adenosine diphosphate |
| ATP | : Adenosine triphosphate |
| APTT | : Activated Partial Thromboplastin Time |
| β TG | : B-thromboglobulin |
| CBC | : Complete Blood Count |
| DC | : Direct Current |
| DNA | : Deoxyribonucleic acid |
| EDTA | : Ethylene diamine tetra acetic acid |
| Fbg | : Fibrinogen |
| GP 1b | : Glycoprotein 1b |
| INR | : International Normalise Ratio |
| Na/ I | : Sodium iodide |
| PAI-1 | : Plasminogen activator inhibitor-1 |
| PDGF | : Platelet-derived growth factor |
| PF4 | : Platelet factor 4 |
| Pg12 | : Prostaglandin 12 |
| PLT | : Platelets count |
| PPP | : Platelet poor plasma |
| PT | : Prothrombin Time |
| SD | : Stander Deviation |
| T3 | : <u>Triiodothyronine</u> |
| T4 | : Tetraiodothyronine |
| Tg | : Thyroglobulin |
| TPO | : Thyroid peroxidase |
| TF | : Tissue factor |
| TFPI | : Tissue factor pathway inhibitor |
| TNF | : Tumour necrosis factor |
| t-PA | : Tissue plasminogen activator |
| TSH | : Thyroid stimulating hormone |
| TxA2 | : Thromboxane A2 |
| VWF | : von Willebrand factor |

Chapter one

Introduction and Literature review

1.1.Introduction:

Thyroid gland produce T3 and T4 these hormone intervention in number of vital function in the body such as metabolism including regulation of lipid , CHO ,protein and mineral metabolism and also have role in normal growth and maturation of the skeleton (Henry et al,1992) (Anothonia and sonny ,2011).

Thyroid dysfunction is group of disorders that affect the thyroid some of them have accompanion change in structure and function, others have no effect.

Thyroid disease, which is observed spread in Sudan include hypothyroidism, thyrotoxicosis (which could be from hypothyroidism or non thyroid causes), thyroid malignancies and iodine deficiency disorder(Anothonia and sonny ,2011).

More than one billion persons are at risk of iodine deficiency worldwide and 200 millions have goitre (Elnour *et al.*, 2000)., the additional role of goitrogens has been shown or suspected in areas such as Sudan (Osman and Fatah, 1981; Elmahdi *et al.*, 1983), in which goitre is endemic. In Sudan, endemic goitre and iodine deficiency disorders are serious health problems in many areas. The prevalence of goitre among school children was estimated to be 85% in Darfur region in western Sudan, 74% in Kosti area in the centre of Sudan, 13.5% in Port-Sudan in eastern Sudan, and 17% in the capital, Khartoum (Eltom, 1984), 22.3% in southern Blue Nile area of Sudan (Elnour *et al.*, 2000)., Thyroid hormones in relation to iodine status had been studied in a group of Sudanese pregnant women with goitre in Central Sudan (Eltom et al., 1999). Little is known about the prevalence of thyroid status and goitre in Kordofan region in western Sudan.

Most of coagulation abnormalities associated with thyroid disorder are consequence of the direct action of thyroid hormones on synthesis of various haemostatic factor, or derangement of immune function however, these abnormalities suggest that hyper-coagulable state is present in hyperthyroid patient, while patient suffering from moderate hypothyroidism are at increased risk of thrombosis contrasting with bleeding tendency of those presenting severe hypothyroidism (Squizzato et al., 2005)

1.2 Literature review

1.2.1 Normal hemostatic mechanisms:

The haemostatic mechanisms have several important functions: (a) to maintain blood in a fluid state while it remains circulating within the vascular system; (b) to arrest bleeding at the site of injury or blood loss by formation of a haemostatic plug; and (c) to ensure the eventual removal of the plug when healing is complete. (Lewis *et al.*, 2006).

1.2.1.1 Hemostatic system:

The hemostatic system consists of blood vessels, platelets, and the plasma coagulation system including the fibrinolytic factors and their inhibitors. When a blood vessel is injured, three mechanisms operate locally at the site of injury to control bleeding: (1) vessel wall contraction, (2) platelet adhesion and aggregation (platelet plug formation), and (3) plasmatic coagulation to form a fibrin clot. It is customary to divide hemostasis into two stages (i.e., primary and secondary hemostasis). Primary hemostasis is the term used for the instantaneous plug formation upon injury of the vessel wall, which is achieved by vasoconstriction, platelet adhesion, and aggregation. Primary hemostasis is only temporarily effective. Hemorrhage may start again unless the secondary hemostasis reinforces the platelet plug by formation of a stable fibrin clot. Finally, mechanisms within the fibrinolytic system lead to a dissolution of the fibrin clot and to a restoration of normal blood flow (Munker *et al.*, 2007).

1.2.1.2 Endothelium and the vascular system:

Normal, intact endothelium does not initiate or support platelet adhesion and blood coagulation. Endothelial thromboresistance is caused by a number of antiplatelet and anticoagulant substances produced by the endothelial cells. Important vasodilators and inhibitors of platelet function are prostacyclin (prostaglandin I₂, PgI₂) and nitrite oxide (NO). The thrombin-binding protein thrombomodulin and heparin-like glycosaminoglycans exert anticoagulant properties. Thrombomodulin not only binds thrombin, but also activates protein C as a thrombin–thrombomodulin complex. Endothelial cells also synthesize and secrete tissue factor pathway inhibitor (TFPI), which is the inhibitor of the extrinsic pathway of blood coagulation. In addition, tissue plasminogen activator (t-PA) and its inhibitor plasminogen activator inhibitor-1 (PAI-1), which modulate fibrinolysis, are secreted by endothelial cells. Endothelial cells also possess some procoagulant properties by synthesizing and secreting von Willebrand factor (VWF) and PAI-1. Following injury, these procoagulant factors and tissue factor (TF) activity are induced. This leads to adhesion and activation of platelets and local thrombin generation. The hemostatic properties of the endothelial cells are modulated by cytokines such as endotoxin, interleukin (IL)-1, and tumor necrosis factor (TNF), resulting in an increased TF activity and downregulated thrombomodulin. Small blood vessels comprise arterioles, capillaries, and venules. Only arterioles have muscular walls, which allow changes of the arteriolar caliber. Upon contraction, arterioles contribute to hemostasis, thus temporarily preventing extravasation of blood. Platelet secretion of thromboxane A₂, serotonin, and epinephrine promotes vasoconstriction during hemostasis (Munker *et al.*, 2007).

1.2.1.3 Platelet production (Thrombopoiesis):

Platelets are produced predominantly by the bone marrow megakaryocytes as a result of budding of the cytoplasmic membrane. Megakaryocytes are derived from the haemopoietic stem cell, which is stimulated to differentiate to mature megakaryocytes under the influence of various cytokines, including thrombopoietin. Once released from the bone marrow young platelets are trapped in the spleen for up to 36 hours before entering the circulation, where they have a primary haemostatic role (Provan, 2003).

1.2.1.3.1 The role of platelets:

Platelets are anuclear cells released from megakaryocytes in the bone marrow. Their life span in the peripheral blood is approximately 9 days. The average platelet count in peripheral blood ranges from 150,000 to 400,000 per microliter. The exterior coat of platelets is comprised of several glycoproteins, including integrins and leucine-rich glycoproteins. They mediate platelet adhesion and aggregation as receptors for agonists such as adenosine diphosphate (ADP), arachidonic acid, and other molecules. Electron microscopic examination shows the presence of many cytoplasmic bodies such as α -granules and dense bodies. α -Granules are the storage site of β -thromboglobulin, platelet factor (PF) 4, platelet-derived growth factor (PDGF), VWF, fibrinogen, factor V, PAI-1, and thrombospondin. Dense bodies contain ADP, ATP, calcium, and serotonin. Platelets also contain actin filaments and a circumferential band of microtubules, which are involved in maintaining the shape of the platelets. The open canicular system has its role in the exchange of substances from the plasma to the platelets and vice versa. Whereas normal platelets circulate in the blood and do not adhere to normal vasculature, activation of platelets causes a number of changes resulting in promotion of hemostasis by two major mechanisms:

1. Formation of the hemostatic plug at the site of injury (primary hemostasis).
2. Provision of phospholipids as a procoagulant surface for coagulation process.

The formation of the initial platelet plug can be divided into separate steps, which are very closely interrelated in vivo: platelet adhesion, shape change, the release reaction, and platelet aggregation. Within seconds after endothelial injury, platelets attach to adhesive proteins, such as collagen, via specific glycoprotein surface receptors (platelet adhesion). In this context, VWF serves as a bridge that first adheres to collagen fibers and then changes its confirmation. This is followed by the binding of platelets to VWF via the platelet membrane glycoproteins (GP) Ib and IX. Following adhesion, platelets undergo a shape change from a disc shape to a spherical shape and extend pseudopods. Almost simultaneously the release reaction occurs by which a number of biologically active compounds stored in the platelet granules are secreted to the outside. These released substances, which include ADP, serotonin, Thromboxane A₂ (TxA₂), β TG, PF₄, and VWF, accelerate the reaction of plug formation and also initiate platelet aggregation, i.e., the adhesion of platelets to each other. As a result of platelet aggregation, the platelet plug increases in size and a further release of granular contents is initiated in order to induce more platelets to aggregate. The prostaglandins play an additional role in mediating the platelet-release reaction and aggregation. Thromboxane A₂ is a very potent inducer of platelet secretion and aggregation. It is formed from arachidonic acid by the enzyme cyclooxygenase. Arachidonic acid is liberated from the platelet membrane by phospholipases following activation of the platelets by collagen and epinephrine. At the end of the aggregation process, the hemostatic plug consists of closely packed degranulated platelets.

Fibrinogen is required for platelet aggregation binding to specific glycoprotein receptors (GP IIb/IIIa) (Munker *et al.*, 2007).

Many patients with uremia have a bleeding diathesis characterized by a prolonged bleeding time and abnormal platelet adhesion, aggregation, secretion, and platelet procoagulant activity. The pathogenesis of the platelet defect is not clear. Abnormalities in plasma VWF, reduction in GP Ib, and a decreased adhesion via GP IIb/IIIa have been reported. Uremic platelets exhibit, when stimulated, a reduced release of arachidonic acid from membrane phospholipids. The bleeding diathesis and the prolonged bleeding time in uremia often improve with dialysis (Munker *et al.*, 2007).

1.2.1.4 blood coagulation:

The fibrin clot is the end product of complex reactions of coagulation or clotting factors. Most of the clotting factors are zymogens of serine proteases and are converted to active enzymes during the process of blood coagulation. The six serine proteases are the activated forms of the clotting factors II, VII, IX, X, XI, and XII. Factors V and VIII are co-factors, which, after activation, modify the speed of the coagulation reaction. The reactions of the coagulation factors take place on the surface of phospholipids that become exposed on the activated platelet surface. The plasmatic coagulation has been divided into two different pathways—the intrinsic and

extrinsic pathway. The principal initiating pathway of in vivo blood coagulation is the extrinsic system. The critical component is TF, an intrinsic membrane component expressed by cells in most extravascular tissues. TF functions as a co-factor of the major plasma component of the extrinsic pathway, factor VII. A complex of these two proteins leads to the activation of factor VII to factor VIIa, which then converts factor X to factor Xa, the identical product as formed

by the intrinsic pathway. As factor Xa levels increase, however, factor VIIa-TF complex is subject to inhibition by factor Xa-dependent TFPI.

The early part of the intrinsic pathway (contact phase) is carried out by factor XII (contact factor), prekallikrein, and high-molecular weight kininogen (HMWK). In vitro contact phase is initiated by the binding of factor XII to negatively charged surfaces, such as glass or kaolin. This leads to the formation of the enzyme factors XIIa and kallikrein, and the release of bradykinin from HMWK. Factor XIIa then activates factor XI. The resulting factor XIa converts factor IX to factor IXa, a reaction that requires the presence of calcium. Factor IXa then forms a complex with its co-factor protein factor VIIIa on a negatively charged membrane surface. This enzymatic complex (tenase complex) converts factor X to factor Xa. After both the extrinsic and intrinsic pathways have resulted in the formation of factor Xa, the ensuing reactions of the coagulation pathway are the same and are referred to as the common pathway (Munker *et al.*, 2007).

1.2.1.5 Formation of Fibrin:

Fibrinogen is a large plasma protein with a molecular weight of 340 kDa. It is synthesized in the liver and its concentration in normal individuals is in the range of 200 to 400 mg/dl. The half-life of fibrinogen is about 4 to 5 days. It is a dimeric protein of extremely low solubility composed of two pairs of three nonidentical polypeptide chains, designated as A- α , B- β , and γ (A- α -2, B- β -2, γ -2). The chains are covalently linked together by disulfide bonds. The conversion of fibrinogen to fibrin proceeds in three stages. In the first step, thrombin cleaves four small peptides from the fibrinogen molecule, resulting in the formation of a new molecule called fibrin monomer. The release of these fibrinopeptides exposes sites on the A- α and the B- β chains that seem to be essential for the polymerization of the fibrin. In the second step, polymerization

occurs spontaneously to form fibrin polymers which are easily dissolved in denaturing agents such as urea or monochloroacetic acid. In the third step, a resistant and stable fibrin molecule is formed by the action of factor XIII (fibrin-stabilizing factor) and calcium ions. Factor XIII must first be activated by thrombin before it becomes a transglutaminase capable of crosslinking fibrin polymers by forming covalent bonds (γ -glutamyl/ epsilon-lysyl). The fibrin gel is now stabilized and insoluble in urea or monochloroacetic acid (Munker *et al.*, 2007).

1.2.1.6 heamostatic test

The two commonly used coagulation tests, the activated partial thromboplastin time (APTT) and the prothrombin time (PT) have been used historically to define two pathways of coagulation activation: the intrinsic and extrinsic paths, respectively. (Lewis *et al.*, 2006).

1.2.1.6.1 platelets count:

For CBC typically, EDTA anticoagulated blood is obtained for analysis in an automated particle counter. The reported platelet count is usually quite precise (CV ~ 5%).

In asymptomatic patients in whom thrombocytopenia is reported, the possibility of pseudo- thrombocytopenia or EDTA-induced thrombocytopenia should be considered, especially in patients without a history of bleeding. This phenomenon occurs in 0.1–1% of normal people; it results from EDTA modifying platelet membrane proteins which then react with preexisting antibodies present in patient blood that recognize the modified platelet proteins, producing platelet clumping or satellitism. It should be routine laboratory policy for technical personnel to review peripheral blood smears of patients with newly diagnosed thrombocytopenia to determine whether the thrombocytopenia is true or false. If EDTA-induced thrombocytopenia is suspected, the CBC should be

repeated using blood collected in a citrate or Acid-Citrate-Dextrose collection tube. In terms of hemostasis evaluation, one limitation of the CBC is that even though it is usually a reliable indicator of the platelet count, the CBC does not measure platelet function. The bleeding time test was originally thought to perform this function, but it is not uniformly reliable in assessing platelet function (Bennett *et al.*, 2007).

1.2.1.6.2 Prothrombin time test (PT):

The PT assay has two purposes: to screen for inherited or acquired deficiencies in the extrinsic and common pathways of coagulation and to monitor oral anticoagulant therapy. The PT is affected by decreased levels of fibrinogen, prothrombin, factors V, VII, or X. Since 3 of the 5 coagulation factors measured by the PT are vitamin K-dependent proteins (prothrombin, factors VII and X), the PT assay is useful in detecting vitamin K deficiency from any cause including liver disease, malnutrition, or warfarin therapy. The PT does not measure factor XIII activity or components of the intrinsic pathway¹. The PT assay is performed by mixing patient plasma with thromboplastin, which is a commercial tissue factor/phospholipid/calcium preparation. The tissue factor binds factor VII in patient's plasma to initiate coagulation. The clotting time is measured in seconds using instruments with mechanical or photo-optical endpoints that detect fibrin formation. Thromboplastin preparations can vary in their sensitivities, resulting in different clotting times. A typical PT reference range is 10–15 sec. In general, the PT assay is more sensitive in detecting low levels of factors VII and X than low levels of fibrinogen, prothrombin or factor V. In particular, different thromboplastin reagents may exhibit variable sensitivities to these factor deficiencies. Mild factor deficiency (i.e., 40–50% of normal) may not be detected by many thromboplastin reagents (Bennett *et al.*, 2007).

1.2.1.6.3 Activated partial thromboplastin time test(APTT):

The APTT assay is useful for three reasons – as a screening test for inherited or acquired deficiencies of the intrinsic pathway, to detect inhibitors, and to monitor heparin therapy. Factors VII and XIII are not measured by the PTT assay. To perform the PTT assay, patient plasma is preincubated with the PTT reagent (crude phospholipid and a surface-activating agent such as silica or kaolin). This preincubation initiates contact activation (intrinsic pathway activation) in which factors XII and XI are activated in the presence of cofactors, prekallikrein and high-molecular-weight kininogen. Factor XIa then converts factor IX to IXa. Calcium is then added to the preincubation mixture; this results in factors IXa/VIII activation of factor X, then factor Xa/V-mediated activation of prothrombin to thrombin followed by conversion of fibrinogen to soluble fibrin that polymerizes into fibrin strands, the endpoint of the PTT assay. The usual PTT reagent is less sensitive to factor IX than to factors VIII, XI, and XII. The PTT may be affected by high levels of factor VIII, an acute-phase response protein; high factor VIII levels may mask co-existing mild intrinsic coagulation deficiencies. A typical PTT reference range is 25-36 sec. (Bennett *et al.*, 2007).

1.2.2 Anatomy of thyroid gland:

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 fifth or sixth tracheal ring (Yalçin and Ozan,2006).

1.2.2.1 Physiology of thyroid:

The primary function of the thyroid is production of the hormones T3, T4 and calcitonin. Up to 80% of the T4 is converted to T3 by organs such as the liver, kidney and spleen. T3 is several times more powerful than T4, which is largely a prohormone, perhaps four (Ekholm and Bjorkman,1997) or even ten times more active (Bianco et al,2002).

1.2.2.2 T3 and T4 production

The synthesis of the thyroid hormones T3 and T4Synthesis of the thyroid hormones, as seen on an individual thyroid follicular cell (Jansen et al,2005)

- 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.
- Meanwhile, a sodium-iodide (Na/I) symporter pumps iodide (I-) actively into the cell, which previously has crossed the endothelium by largely unknown mechanisms.
- This iodide enters the follicular lumen from the cytoplasm by the transporter pendrin, in a purportedly passive manner (Jansen et al,2005).
- In the colloid, iodide (I-) is oxidized to iodine (IO) by an enzyme called thyroid peroxidase.
- Iodine (IO) is very reactive and iodinates the thyroglobulin at tyrosyl residues in its protein chain (in total containing approximately 120 tyrosyl residues).
- In conjugation, adjacent tyrosyl residues are paired together.
- The entire complex re-enters the follicular cell by endocytosis.

- Proteolysis by various proteases liberates thyroxine and triiodothyronine molecules, which enters the blood by largely unknown mechanisms.

Thyroxine (T₄) is synthesised by the follicular cells from free tyrosine and on the tyrosine residues of the protein called thyroglobulin (Tg). Iodine is captured with the "iodine trap" by the hydrogen peroxide generated by the enzyme thyroid peroxidase (TPO) (Walter,2010) and linked to the 3' and 5' sites of the benzene ring of the tyrosine residues on Tg, and on free tyrosine. Upon stimulation by the thyroid-stimulating hormone (TSH), the follicular cells reabsorb Tg and cleave the iodinated tyrosines from Tg in lysosomes, forming T₄ and T₃ (in T₃, one iodine atom is absent compared to T₄), and releasing them into the blood. Deiodinase enzymes convert T₄ to T₃. (Yamamoto et al,1988).

1.2.2.3 Physiological effect of T₃ and T₄

Thyroid hormone secreted from the gland is about 80-90% T₄ and about 10-20% T₃. (Bianco et al,2002)

Cells of the developing brain are a major target for the thyroid hormones T₃ and T₄. Thyroid hormones play a particularly crucial role in brain maturation during fetal development (Patrick,2008) .

A transport protein that seems to be important for T₄ transport across the blood–brain barrier (OATP1C1) has been identified. A second transport protein (MCT8) is important for T₃ transport across brain cell membranes (Hidaka et al,1993).

Non-genomic actions of T₄ are those that are not initiated by liganding of the hormone to intranuclear thyroid receptor. These may begin at the plasma membrane or within cytoplasm. Plasma membrane-initiated actions begin at a receptor on the integrin α V β 3 that activates ERK1/2. This binding

culminates in local membrane actions on ion transport systems such as the Na(+)/H(+) exchanger or complex cellular events including cell proliferation. These integrins are concentrated on cells of the vasculature and on some types of tumor cells, which in part explains the proangiogenic effects of iodothyronines and proliferative actions of thyroid hormone on some cancers including gliomas. T4 also acts on the mitochondrial genome via imported isoforms of nuclear thyroid receptors to affect several mitochondrial transcription factors. Regulation of actin polymerization by T4 is critical to cell migration in neurons and glial cells and is important to brain development.

T3 can activate phosphatidylinositol 3-kinase by a mechanism that may be cytoplasmic in origin or may begin at integrin alpha V beta3.

alone, in pairs or together with the retinoid X-receptor as transcription factors to modulate DNA transcription (Yalçın and Ozan,2006).

1.2.2.4 Abnormality of T3 and T4 secretion

1.2.2.4.1 Hyperthyroidism

Hyperthyroidism, or overactive thyroid, is due to the overproduction of the thyroid hormones T3 and T4, which 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, diarrhea, weight loss, muscle weakness and unusual sensitivity to heat. The appetite is often increased (Massimo et al, 2010).

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 (often one pill every 8 hours) 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[clarification needed] and inconvenience of such drug regimens, some patients choose to undergo radioactive iodine-131 treatment. Radioactive iodine is administered in order to destroy a portion 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(Patrick ,2008).

1.2.2.4.2 Hypothyroidism

Hypothyroidism is the underproduction of the thyroid hormones T3 and T4.

Hypothyroid disorders may occur as a result of congenital thyroid abnormalities (Thyroid deficiency at birth. See congenital hypothyroidism),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 (Bifulco and Cavallo, 2007).

Iodine deficiency is the most common cause of hypothyroidism and endemic goiter worldwide (Garber et al., 2012). In areas of the world with sufficient dietary iodine, hypothyroidism is most commonly caused by the autoimmune disease Hashimoto's thyroiditis (chronic autoimmune thyroiditis) (Garber et al., 2012). Hashimoto's may be associated with a goiter. It is characterized by infiltration of the thyroid gland with T lymphocytes and autoantibodies against specific thyroid antigens such as thyroid peroxidase, thyroglobulin and the TSH receptor (Garber et al., 2012).

1.3 Previous Studies:

Zeynep et al.,(2003). Reported that Platelet count, PTT, PT and INR were not different in hypothyroid patients .

Ford and Carter 1990. reported that coagulation factor VII,IX,XI,XII are decreased in hypothyroid patients and platelet are not effect.

Mohammed and Rogia(2008).A significant decrease of PT in both hypothyroid and hyperthyroid patients when compared with control group ,APTT was decreased significantly in hyperthyroid patients and no significant effect in APTT in hypothyroidism patients. while A platelet count was found slightly low in hyperthyroidism without statistical difference and normal platelet count in hypothyroidism patients.

Sguizzato et al(2007) .reported a shortening of PT,APTT in hyperthyroid patients

Massimo et al,(2010) documented a significant reduction in coagulation factors VIII, IX, and XI activities in hypothyroid patient with lower levels of plasma coagulation factors VII, X, and XII

Also shown that in a sample of 1329 unselected adult outpatients, those with hyperthyroidism had shortened APTT and higher plasma fibrinogen levels when compared with euthyroid patients, whereas no significant differences were observed between euthyroid patients and those with hypothyroidism.

Rationale

A strong relationship between thyroid hormones and the coagulation system has been established since the last century. Thyroid disorders are one of the high risk factors for bleeding and venous thromboembolism. People with hypothyroidism are more susceptible to bleeding while hyperthyroidism subjects are at a high risk of thrombosis. In Sudan thyroid disorders incidence are increasing subjecting the patients to a wide range of cardiovascular abnormalities.

In Sudan there is a paucity of data regarding the disorders of coagulation system in people suffering from hyperthyroidism or hypothyroidism. So this study was undertaken to assess the coagulation system state during hypothyroidism and hyperthyroidism.

1.4 Objectives

1.4.1 General Objective:

To evaluate some haemostatic parameter among patients with hyperthyroidism or hypothyroidism attended to Radiation Isotope Center Khartoum State.

1.4.2 Specific objectives:

1-To measure prothrombin time, activated partial thromboplastin time and platelet counts among the study group and compare them with healthy individuals.

Chapter Two

2. Materials and methods

2.1 Study design

It is a case-control and analytical study.

2.2 Study area and line

This study was carried out during the period March to June 2014 in Radiation and Isotopes Center Khartoum Khartoum state.

2.3 Study population:

patients with thyroid disease at different ages, and sexes

2.4 selection criteria:

2.4.1 Inclusion criteria:

All patients with hypothyroidism and hyperthyroidism diagnosed by treated or not treated

Healthy people as control

2.4.2 Exclusion criteria:

Patients with other disease which may interfere with result

2.5 Demographic Data:

A questionnaire was filled for each patient by direct interviewing and obtain the following variable

Age :measure by years

Gender :described by male and female

Duration of disease :described by years

2.6 Specimen collection:

Five ml of venous blood samples was collected using disposable syringes without stasis, divided equally into two containers previously labeled. The first one contained 0.25 ml of 0.38% tri-sodium citrate to which 2.5 ml of blood was added slowly, gently mixed and then separated immediately at 3000 rpm for 15 min, after which platelet poor plasma (PPP) was collected in plain containers for performing PT, APTT, and INR assays. The second one contained K₃-EDTA in a concentration of 1.50 ± 0.25 mg/ml of blood for determinant of platelet count

2.7 Complete Blood Count (CBC):

Sysmex KX-21 hematological autoanalyzer of S. N: A 1967 (Japan by Toa Medical Electronics CO., LTD) was used to measure PLT count. PT and APTT were measured by Sysmex CA-50 automated blood coagulation analyzer

Principle:

Sysmex KX-21 performs blood counts by direct current (DC) detection method in which blood sample was sucked measured to a predetermined volume, diluted at the specified ratio, and then fed into transducers. The transducer chamber has a minute hole called aperture. On both sides of the aperture there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing electric changes, the blood cell size is detected as electric pulses.

Blood cell count is calculated by counting the pulses, and a histogram of blood cell size is plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data

2.7.1 Sample material: EDTA blood

2.7.2 Test procedure:

Performed as described in the operator's manual of Sysmex KX-21

2.8 Laboratory test

PT and APTT were measured by Sysmex CA-50 automated blood coagulation analyzer of S. N: A 1321

2.8.1 Detection principle for coagulation method by using CA 50:

After the incubation of a fixed quantity of citrated plasma for certain period of time, reagent was added, then exposed to light of wavelength of 660 nm, and the turbidity of the plasma during the coagulation process was detected as a change in scattered light intensity.

From this change in scattered light intensity, a coagulation curve was prepared and the coagulation time was found by means of Percentage

2.8.2 Detection Method.

The CA-50 utilizes the optical detection method and thus detecting the change in turbidity of blood plasma during the coagulation process as the change in scattered light intensity. Light that is emitted from a light source reaches the sample. The generated scattered light is received by a photodiode, which converted a light into electrical signals. These signals are stored and calculated by a microcomputer in order to find the coagulation time.

2.8.3 PT assay principle:

The PT test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. The test is also depend on reactions with factors V, VII, and X and on the fibrinogen concentration of the plasma.

Test procedures:

Performed as described in the operator's manual of Sysmex CA-50

2.8.4 PT procedure:

-0.05 ml of PPP was applied into a reaction tube and incubated for 3 minutes.

-0.1 ml of pre warmed PT reagent was applied and clotting time detected.

-Steps were repeated for a duplicate and the average obtained.

-Reference values: PT: 11---16 seconds

2.8.5 APTT assay principle:

The test measures the clotting time of plasma after the activation of contact factors but without added tissue thromboplastin and so indicates the overall efficiency of the intrinsic pathway. To standardize the activation of contact factors, the plasma is first preincubated for a set period with a contact activator such as kaolin. During this phase of the test factor XIIa is produced, which cleaves factor XI to factor XIa, but coagulation does not proceed beyond this in the absence of calcium. After recalcification, factor XIa activates factor IX and coagulation follows. A standardized phospholipid is provided to allow the test to be performed on PPP. The test depends not only on the contact factors and on factors VIII and IX, but also on the reactions with factors X, V, prothrombin, and fibrinogen. It is also sensitive to the presence of circulating anticoagulants (inhibitors) and heparin.

Sample material: Citrated platelet poor plasma (PPP).

Reagents: Spectrum reagents were used for the determination of PT and APTT

2.8.6 APTT procedure:

-0.05 ml of PPP was applied into a reaction tube and incubated for 1 minute.

-0.05 ml of prewarmed (at 37°C) APTT reagent was applied and incubated for 3 minutes.

-0.05 ml of prewarmed CaCl_2 was applied and clotting time detected.

-Steps were repeated for a duplicate and the average obtained.

-Reference values: APTT: 26---40 seconds.

2.9 Validity of reagents and instruments:

All reagents used for PT, APTT were examined against commercial normal control.

A set of blood samples were analyzed manually and the results were compared to the results obtained by automated Sysmex instruments for the same samples.

The results were accepted only when the difference between the two values was less than 2 SD.

2.10 Ethical consideration:

It was taken according to The College of Medical Laboratory Sciences – SUST. All patients were informed about the aim of the study, and a verbal consent was obtained from the respondent patients.

2.11 Statistical analysis:

Data were collect manually in a master sheet and comparison among the study groups was performed by analysis of variance using computerized SPSS program (version 15.5)

Chapter Three

Results

3.1.Characteristic of the studied patients :

3.1.1 The demographic characteristics of 30 hyperthyroidism patients attended Radiation Isotope Center Khartoum

The studied patients characteristics are presented in figures 1-6. Females represent (90%) while males represent(10%) the lowest frequency of hyperthyroidism. The highest occurrence of hyperthyroidism (43%) was found among those aged less 31-45 years and the least rate (3%) was found in those over sixty years old. More than half of the patients (60%) have family history of hyperthyroidism. About(97%)of patients do not use iodized salt while (10%) are under treatment. Darfour State represent the lowest percentage (2 %) of the studied patients. Duration of the disease among the studied population was found to be less than a year (90%) ,from 2-4 years (6.6%) and only(3.4%) had a disease duration of more than five years.

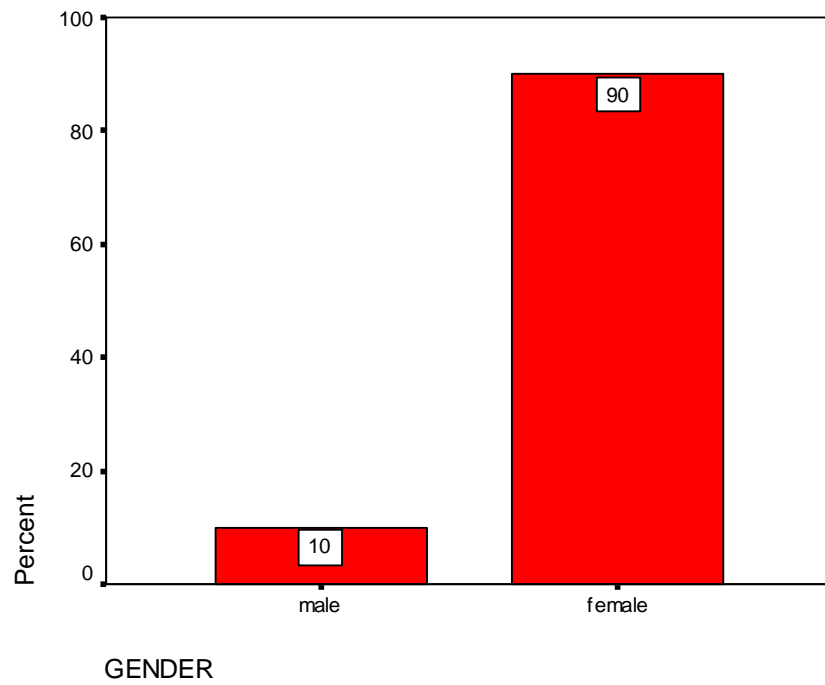


Figure.1.Distrbution of hyperthyroidism according to gender

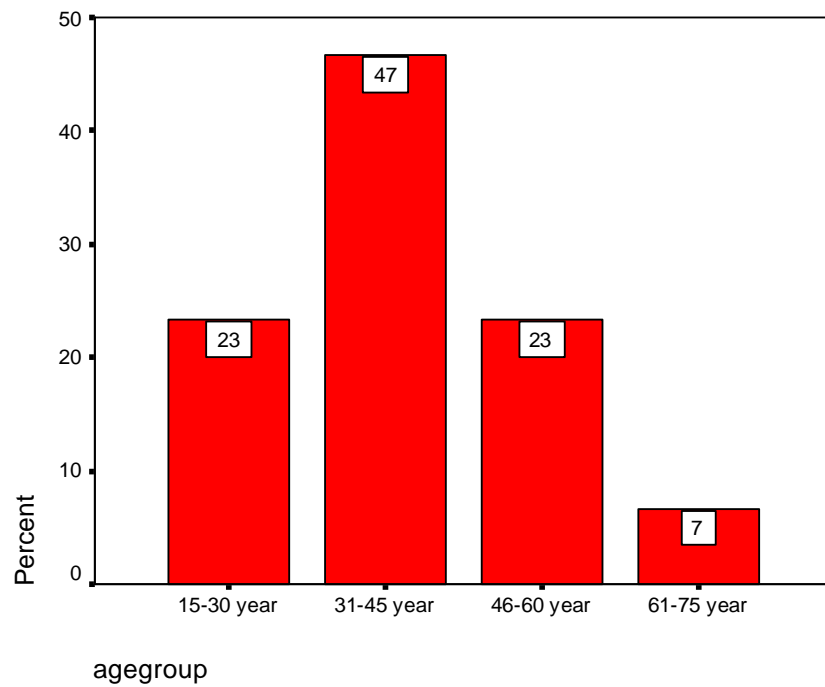


Figure.2: .Distribution of hyperthyroidism patients according to age

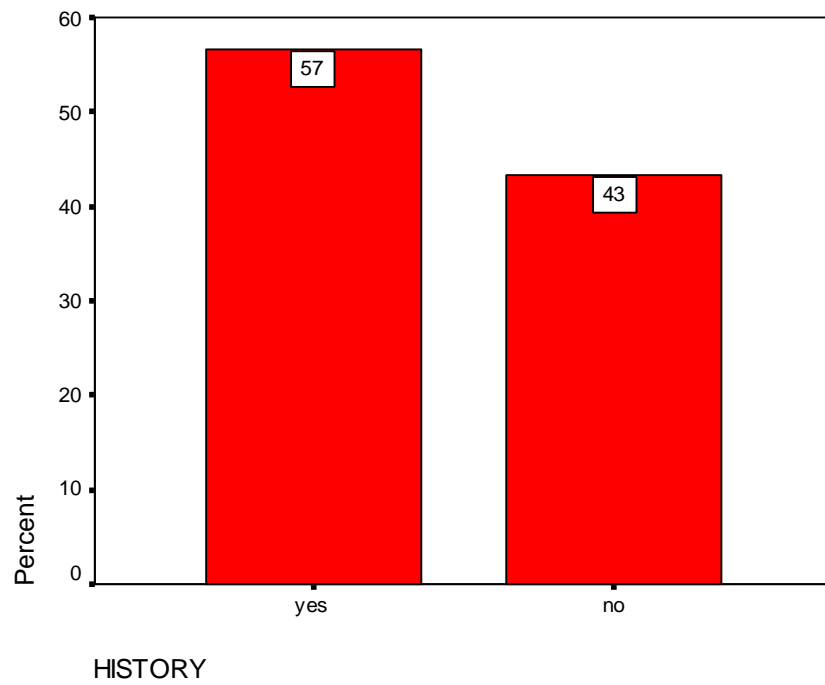


Figure.3: .Distribution of hyperthyroidism cases according to family history

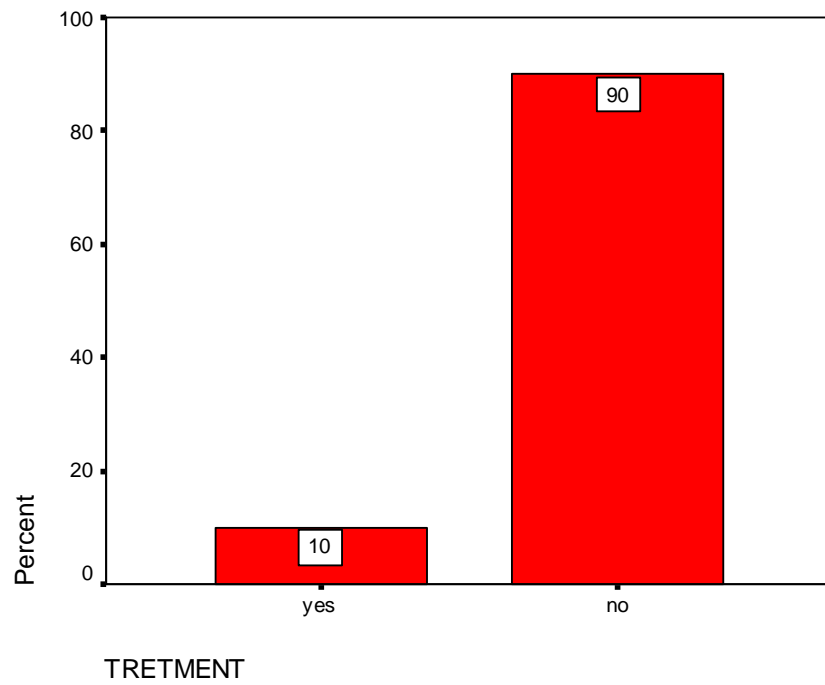


Figure.4: Distribution of hyperthyroidism according to treatment use

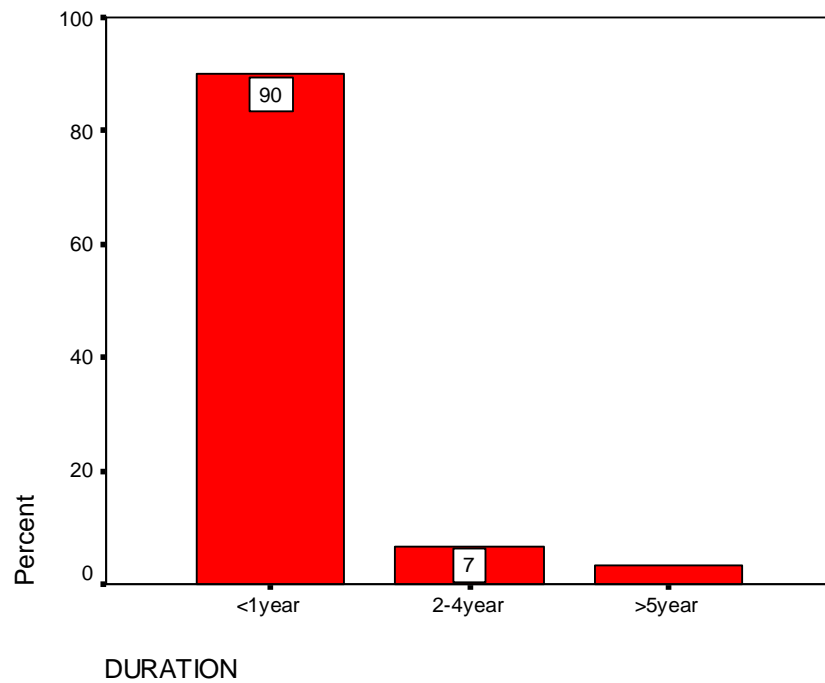


Figure.5: Distribution of hyperthyroidism cases according to duration of disease

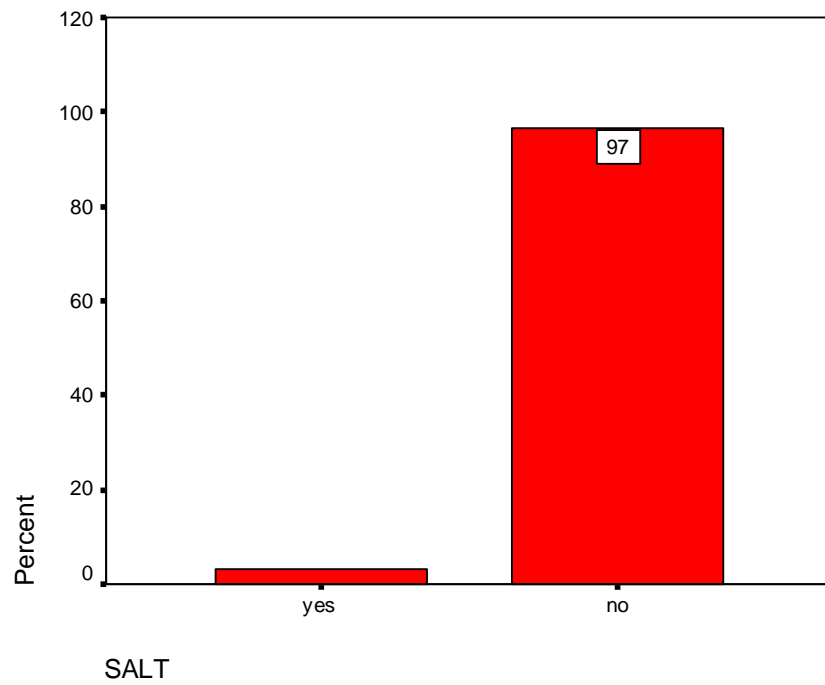


Figure.6: Distribution of hyperthyroidism cases according to salt use

The demographic characteristics of 30 hypothyroidism patients attended Radiation Isotope Center Khartoum

The studied patients characteristics are presented in figures 8-14. Females represent (97%) of hypothyroidism cases. The highest occurrence of hypothyroidism (70%) was found among those aged 31-45 years and the least rate (30%) was found in those 46-70 years old. More than half of the patients (63%) have family history of hypothyroidism. Patients who use iodized salt (10%) while (13%) are under treatment. Patients from Darfour State represent the lowest percentage (2 %) of the studied patients. Duration of the disease among the studied population was found to be less than a year (90%), from 2-4 years (10%) .

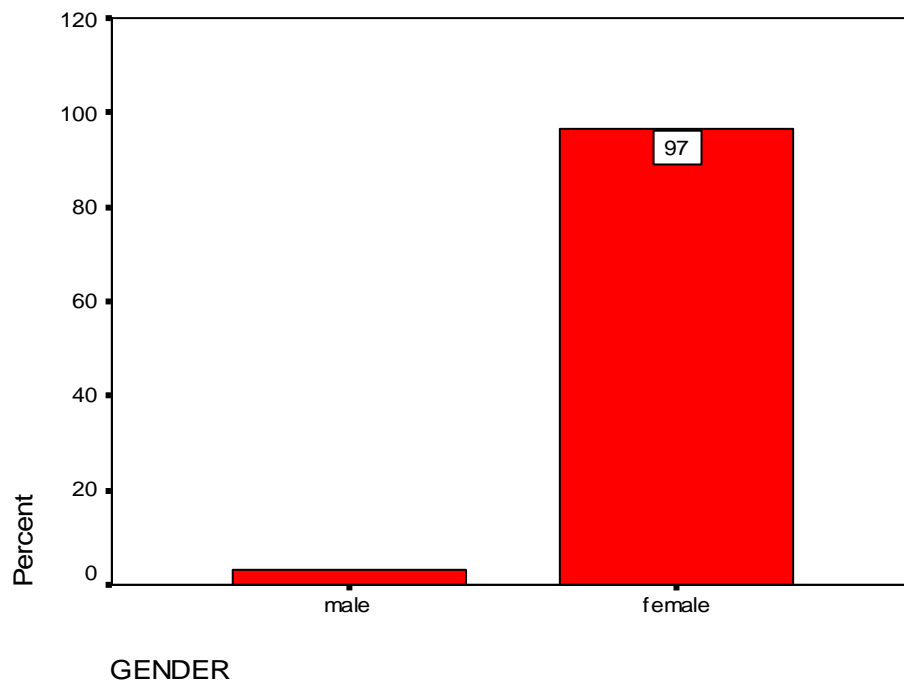


Figure.7: Distribution of hypothyroidism according to gender

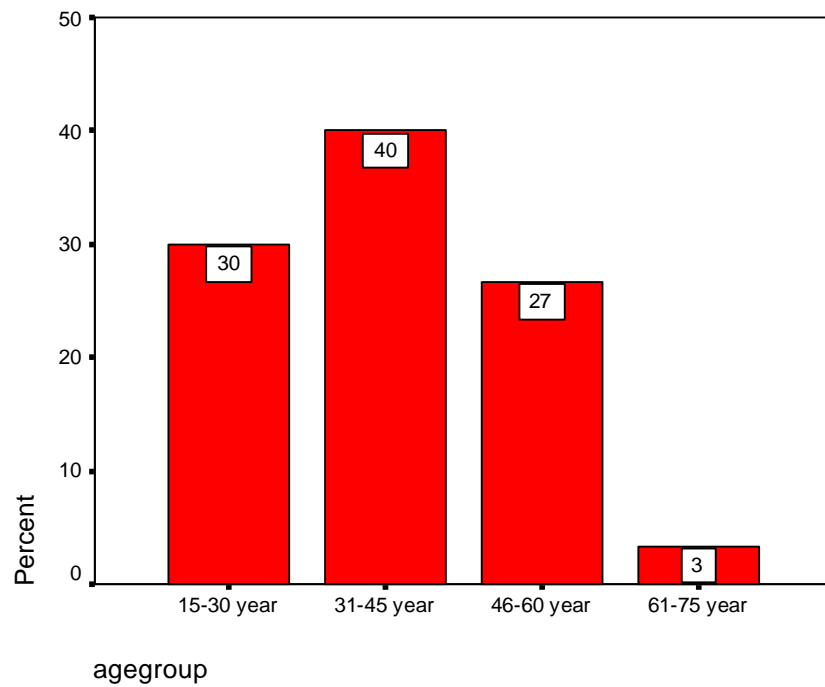


Figure.8: Distribution of hypothyroidism according to age

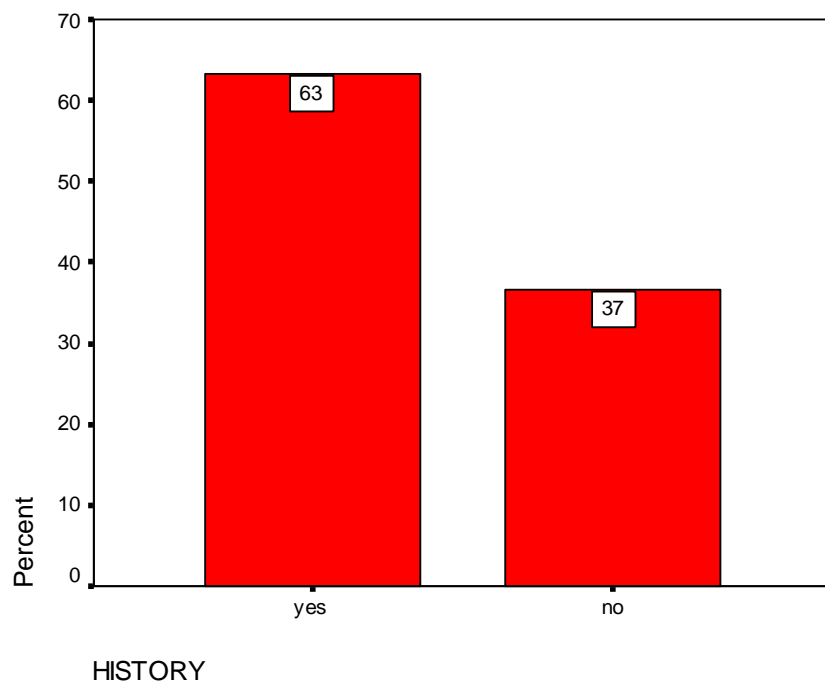


Figure.9: Distribution of hypothyroidism according to family history

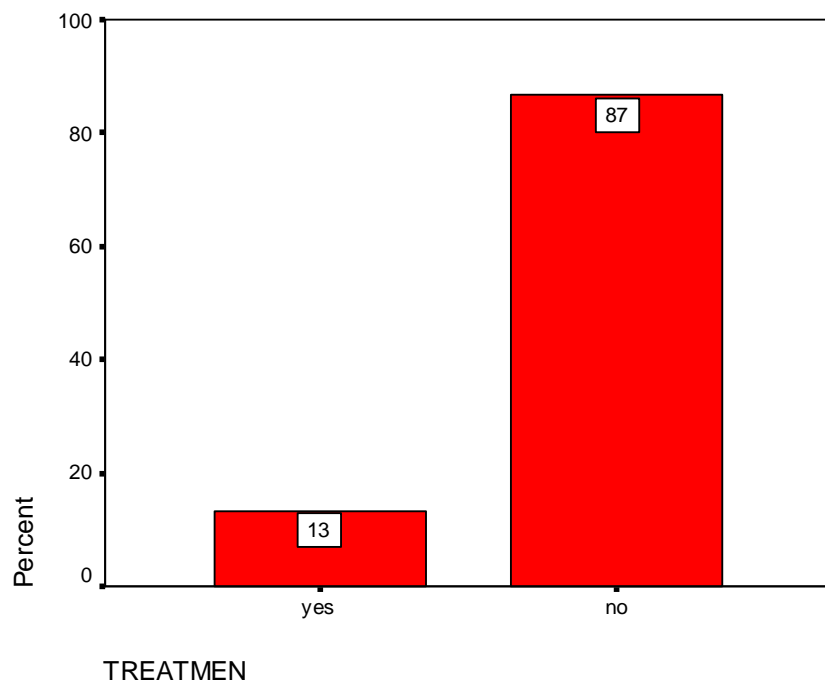


Figure.10: Distribution of hypothyroidism according to treatment use

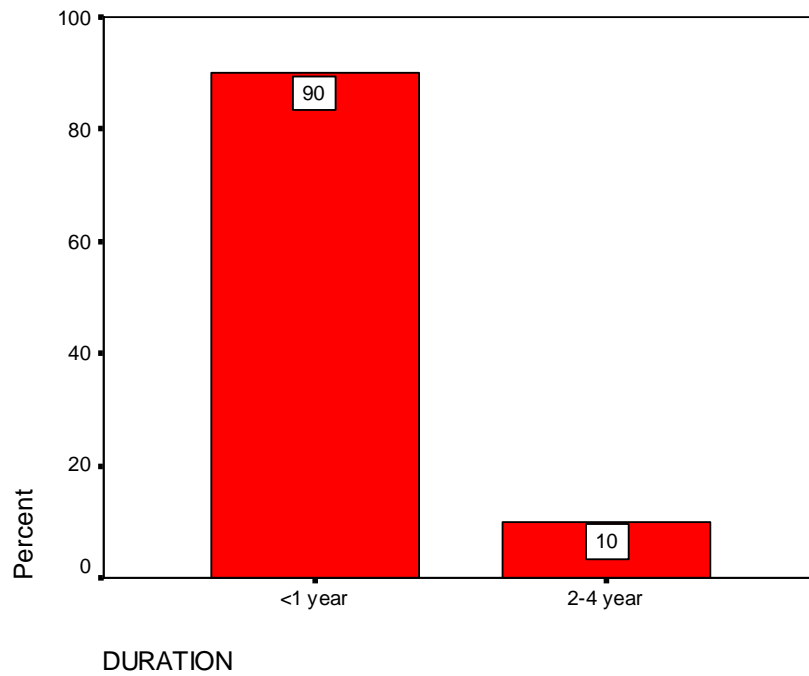


Figure.11: Distribution of hypothyroidism according to duration of disease

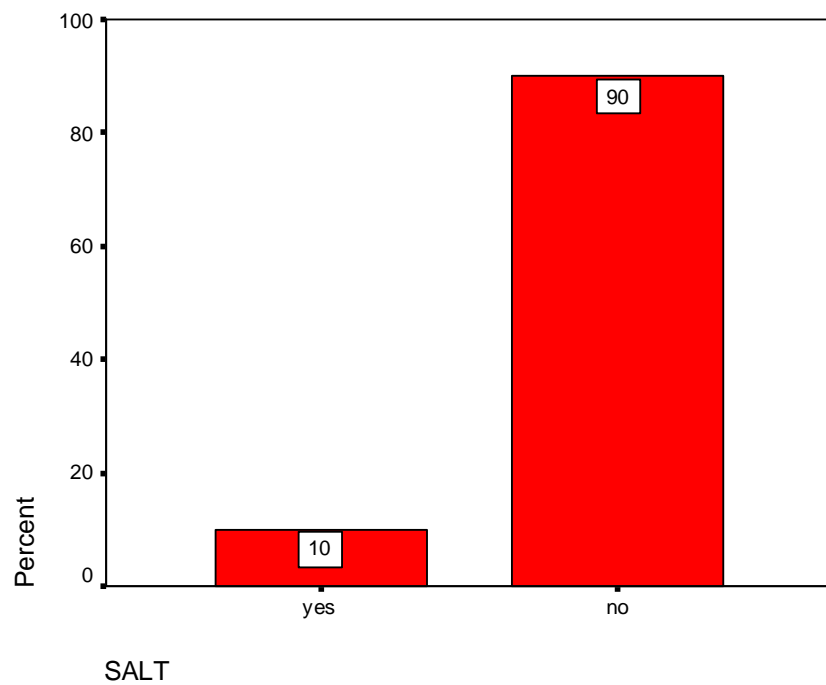


Figure.12: Distribution of hypothyroidism according to duration of disease

3.1.3.Coagulation tests among the study population:

Table(1) Shows the results of some coagulation tests among hyperthyroidism patients , hypothyroidism patients and the control group.

Hypothyroidism patients registered numerically lower platelet count than the control and hyperthyroidism patients. No significant variation in platelets count was found between the control group and hyperthyroid patients .

In both patients with hypothyroidism and hyperthyroidism PT values are significantly lower ($p \leq 0.05$) than that of the control group. Hypothyroid patients showed a significantly ($p \leq 0.05$) higher reduction in PT value than hyperthyroid patients

APTT is significantly decreased ($P \leq 0.05$) in patients with hyperthyroidism compared to the control group , while hypothyroidism patients exhibited insignificant($p \leq 0.05$) increase in APTT values compared to the control group .A statistically significant ($p \leq 0.05$) higher APTT value was registered in hypothyroid patients compared to hyperthyroid ones.

INR values are significantly decreased ($p \leq 0.05$) in patients with hypothyroidism and hyperthyroidism compared to control group. No significant variation was observed between hypothyroid patients and hyperthyroid patients .

Table(1) Mean of some coagulation parameters among hypothyroidism and hyperthyroidism patients and the control group

| Population Variables | Hypothyroidism Mean±SD | Hyperthyroidism Mean±SD | Control Mean±SD |
|-----------------------------|-----------------------------------|------------------------------------|----------------------------|
| PLT | 259.13±.63.3 ^a | 273.23±76.26 ^a | 270±50.47 ^a |
| PT | 10.20±1.67 ^c | 11.2±1.95 ^b | 13.6±1.227 ^a |
| APTT | 33.84±3.28 ^a | 26.61±2.86 ^c | 33.2±2.86 ^b |
| INR | .80±.12 ^c | .86±.151 ^b | 1.06±.14 ^a |

PLT=Platelet count x 10³ cell/ mm³

PT= Prothrombin time/second

APTT=Activated partial thromboplastin time/second

INR=International normalize rate

Chapter Four

Discussion, Conclusion and Recommendation

4.1 Discussion

Many factors are responsible for maintaining the hemostatic balance ;among them, hormones directly influence both primary and secondary hemostasis Squizzato et al.,(2006).The link between the hemostatic system and thyroid diseases has been known since the beginning of the past century.

Therefore, the purpose of this study was to investigate the effects of excess or deficiency of thyroid hormone on the coagulation .

According to the demographic data of the study population it was clear that females are more prone to thyroid disorders than males. It seems that family history of thyroid disorders and lack of iodized salt are risk factors for both hyperthyroidism and hypothyroidism.

In the present study the decrease of PT(INR) and APTT of hyperthyroid patients agree with that of **Mohammed and Rogia (2008) ; who** found a significant reduction in the values of PT (12.02 ± 1.29) and APTT (28.52 ± 6.27) in Sudanese patients with hyper activity of the thyroid gland .Also **Squizzato, et al(2007)** reported a shortening of PT,APTT values in hyperthyroid patients. The current work is on line with the two previous studies. These results suggest that hyperthyroidism is associated by mild to moderate hypercoagulable state (Lippi, et al ,2009).

Platelet count was numerically lower in hyperthyroidism patients compared with the control group this is on line with that reported by **Mohammed and Rogia,(2008)** who reported a platelet count(192 ± 52.53) which is slightly lower in hyperthyroidism than euthyroidism (200.85 ± 61.77)without statistical difference.

The PT(INR) value was found to be significantly low in hypothyroid patients when compared with the control group in the present study which accords with the findings (11.98 ± 1.16)of **Mohammed and Rogia,(2008)**.Higher values of PT(INR)(1.13 ± 0.41) and lower values APTT (29.6 ± 3.4 sec.) than of the present study were found by Zeynep, *et al* (2003) in women with subclinical hypothyroidism, this variation can be attributed to the severity of the disease or interlaboratory analytical variations. **Ford and Carter(1990)** and **Zeynep *et al*,(2003)** reported normal platelet count in hypothyroid patients which is fortified by the results of the current work. However there have been several controversial results in the literature to the findings of this work. Stern and Altschule (1936) found a significant reduction in platelets count after induction of hypothyroidism, while van Doormall *et al* (1987) reported an unexplained increased platelet count in hypothyroidism

Squizzato *et al* (2007) reviewed thyroid function and effects on coagulation and they stated that clinically overt hypothyroidism and hyperthyroidism modify the hemostatic balance in opposite directions and they find this supporting the assumption that thyroid hormone excess and deficit are the main mechanisms of a hypercoagulable and hypocoagulable state , respectively. The conclusion of the previous authors fortify the findings of the present study.

4.2 Conclusion

-PT is decreased significantly in both hypothyroidism and hyperthyroidism when compared to the control group.

-APTT is significantly decreased in patients with hyperthyroidism and increased in hypothyroidism compared to control group.

- Platelet count was not affected by neither hypothyroidism nor by hyperthyroidism.

4.3 Recommendation:

- Haemostatic balance should be assessed routinely in patients with thyroid disease.
- Further studies with larger sample size should be done to determine the effect of thyroid disease on coagulation and fibrinolysis.
- Further studies are recommended to include adult males and children with thyroid disease.
- Epidemiological data should be done to document the prevalence of hypothyroidism and hyperthyroidism among Sudanese people and to determine the risk factors exposing to them.

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Appendix(1)

Questionnaire

Date: / / 2014

Patient No

Name:

Age: yrs

Gender **Male**

☐ **Female**

☐

Duration of disease yrs

Treatment use

Family history.....

Salt use

Appendix (2): Sysmex KX-21 Hematology Analyzer



Sysmex KX-21 Hematology Analyzer

The compact Sysmex KX-21 hematology analyzer offers fully automatic sample aspiration and dilution and can provide 18 parameter test results. The KX-21 analyzer can run samples in whole blood mode and predilute mode and has a throughput capacity of 60 samples per hour. In addition, the KX-21 features automatic self-testing startup, on board storage for 240 test results, and single start button operation.

Appendix (3): Sysmex CA-50



Sysmex CA-50

Fully Automated Blood Coagulation Analyzer