بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Assessment of Changes in Coagulation Profile and Platelets Count in Sudanese Patients with Hypothyroidism and Hyperthyroidism in Khartoum State .

تقييم التغيرات في اختبار السيولة و عدد الصفائح الدموية في المرضى السودانيين المصابين بقصور الغدة الدرقية وفرط نشاط الغدة الدرقية في ولاية الخرطوم

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الآيـــــة

بـسم الله الـرحمن الـرحيم

وإِن يَمْسَسْكَ اللَّهُ بِضُرٍّ فَلَا كَاشِفَ لَهُ إِلَّا هُوَ ۖ وَإِن يُرِدْكَ بِخَيْـــرٍ فَلَـــا رَادَّ لِفَضْلِهِ ۚ يُصِيبُ بِهِ مَن يَشَاءُ مِنْ عِبَادِهِ ۚ وَهُوَ الْغَفُورُ الرَّحِيمُ (107)

سورة يونس

Dedication

TO my Father who gave me advice and supporting through the years

My mother who is continuously encouraging me and guiding me to Success

TO MY best sister AMIRA YASSIR

TO MY FriendsWho always being by my side through good and bad times

WITH LOVE AND BEST WISHES

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First all thanks toAllah for completing this work in this satisficed way

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Abstract

Thyroid hormones have a crucial role in metabolism , Thyroid dysfunction induces different effect on coagulation system and platelet count **.**

. This was retrospective analytical case control study was conducted at the Radiation Isotope center in Khartoum state during the period of August to December 2019,aimed to investigate the association between thyroid disease and changes in coagulation profile and platelet count . The study populations were 33 patients with hypothyroidism, 17patients with hyperthyroidism and 50 healthy volunteers as control matched age and sex .5 ml of blood was collected from all participants automated blood coagulation analyzer was used for measuring activated partial thromboplastin time (APTT)and prothrombin time (PT)and. APTT value was significantly lower (p value less than .05) in patients with hyperthyroidism (26.52 sec) than in control group (32.43 sec). Hypothyroidism patients showed APTT values (33.66 sec), PT valuesin both patients with hypo thyroidism (10.77second)and hyperthyroidism (11.28 sec)were significantly lower (p value 0.00) than that of the control group(13.79 sec)

. Hypothyroid patients show a significantly(p value less than 0.05) higher reduction in PT value than hyperthyroid patients. Sysmex hematological autoanalyzer used for measured of total platelet count . Data were analyzed by SPSS computer soft program version 20 , this study included 10% male and 90% female. No significant variation was observed in platelet count among the study population. Hypothyroidism(256),hyperthyroidism (267),and platelet counts in control group was (252).

In conclusion; both hypothyroidism and hyperthyroidism were associated with significant abnormalities in some coagulation parameters. So coagulation profile in patients with thyroidism must be evaluated routinely to avoid their complications**.**

المستخلص

هرمونات الغدة الدرقية لها دور حاسم في عملية التمثيل الغذائي ، ويؤدي اختلال وظائف الغدة الدرقية إلى تأثير مختلف على نظام التخثر وعدد الصفائح الدموية.

.تم إجراء دراسة تحليلية للتحكم في الحالات بأثر رجعي في مركز النظائر المشعة في ولاية الخرطوم خلال الفترة من أغسطس إلى ديسمبر 2019 ، بهدف التحقق من العلاقة بين مرض الغدة الدرقية والتغيرات في ملف التخثر وعدد الصفائح الدموية. كان مجتمع الدراسة 33 مريضا يعانون من قصور قصور الغدة الدرقية ، و 17 مريضا يعانون من فرط نشاط الغدة الدرقية و 50 متطوعًا صحيًا مع التحكم في العمر والجنس. تم جمع 5 مل من الدم من جميع المشاركين تم استخدام محلل تخثر الدم الآلي لقياس الوقت المنشط للجلطات الدموية الجزئية (APTT) ووقت البروثرومبين (PT) و. كانت قيمة APTT أقل بكثير (قيمة p أقل من 0.05) في المرضى الذين يعانون من فرط نشاط الغدة الدرقية (26.52 ثانية) عن المجموعة الضابطة (32.43 ثانية). أظهر مرضى قصور الغدة الدرقية قيم (33.66 APTT ثانية) ، وكانت قيم PT في كل من المرضى الذين يعانون من قصور الغدة الدرقية (10.77 ثانية) وفرط نشاط الغدة الدرقية (11.28 ثانية) أقل بكثير (قيمة (0.00 p من مجموعة التحكم (13.79 ثانية)

.يظهر مرضى الغدة الدرقية انخفاضًا ملحوظًا (قيمة p أقل من 0.05) في قيمة PT مقارنة بمرضى فرط الدرقية. محلل دموي Sysmexيستخدم لقياس إجمالي عدد الصفائح الدموية. وقد تم تحليل البيانات بواسطة SPSS برنامج الكمبيوتر نسخة 20 ، وشملت هذه الدراسة 10 ٪ من الذكور و 90 ٪ من الإناث. لم يلاحظ أي اختلاف كبير في عدد الصفائح الدموية بين مجتمع الدراسة. كان قصور الغدة الدرقية (256) ، فرط نشاط الغدة الدرقية (267) ، وعدد الصفائح الدموية في المجموعة الضابطة (.252)

فى الختام؛ ارتبطت كل من قصور الغدة الدرقية وفرط نشاط الغدة الدرقية مع تشوهات كبيرة في بعض المعلمات التخثر. لذلك يجب تقييم الملف التخثر في المرضى الذين يعانون من الغدة الدرقية بشكل روتيني لتجنب مضاعفاتهم.

List of contents

Chapter III: Materials and Methods

List of figures:

Abbreviations

PT: Prothrombin time

APTT: Activated partial thromboplastin time

PLT: platelets

PPP: platelet poor plasma

CBC: Complete Blood Count

SD: Stander deviation

T3: Triiodothyronine

T4: Tetraiodothyronine

VWF: von Willebrand factor

TF: Tissue factor

TSH: Thyroid Stimulating Hormone

TPO: Thyroid Peroxidase

Tg: Thyroglobulin

Gp:Glycoprotein

TFPI: Tissue Factor Pathway Inhibitor

INR : International Normalization Ratio

OH: Overt Hypothyroidism

SH : Sever Hypothyroidism

TNF: Tumor Necrosis Factor

t.PA: tissue Plasminogen Activator

TxA2: Thromboxane A2

Pg12:prostaglandin 12

PF4:Platelet Factor 4

PDGF: Platelet derived Growth Factor

PAI-1:Plasminogen Activator Inhibitor 1

Fbg:Fibrinogen

GP1b: Glycoprotein 1b

\DC: Direct Current

BTG : B-.thromboglobulin

ADP: Adenosine diphosphate

ATP: Adenosine triphosphate

Chapter I

Introduction

1.1 Introduction:

Thyroid is a small,butterfly –shaped gland located at the base of the neck .it is part of network of glands called the endocrine system. The endocrine system is responsible for coordinating many of the body activity,Thyroid gland manufactures hormones (T3 and T4) that regulate the body metabolism (regulation lipid,CHO, Protein,and mineral metabolism)and also have role in normal growth maturation of skeleton.Thyroid dysfunction is group of disorders that affect the thyroid some of them have acompanion 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 million have goiter (Elnour*et al.*,2000); the additional role of goitrogens has been shown or suspected in areas such as Sudan (Osman and Fatah, 2001;Elmahdi*et al.*, 2003),in which goitre is endemic. In Sudan, endemic goitre and iodine deficiency disorders are serious health problems in many areas. Theprevalence of goitre among school children was estimated to be 85%in Darfur region in western Sudan, 75%in kosti area in the center of sudan,13.5% in port-Sudan in eastern Sudan, and 17%in the capital, Khartoum(Eltom, 2002), 22.3% in southern blue Nile area of Sudan (Elnour*et al.*, 2000),.thyroid hormones in relation to iodine status had been studied in group of Sudanese pregnant women with goitre in central Sudan (Eltom *et al*.,2004). Little is known about the prevalence of thyroid status and goitre in Kordofan region in western Sudan.Most of coagulation abnormalitiesassociated with thyroid disorder are consequence of the direct action of thyroid hormones on synthesis of various hemostatic factor, or derangement of immune function however, these abnormalities suggest that hyper-co-agulable state is present in hyperthyroidpatient, while patient suffering from moderate hypothyroidism are at increased risk of thrombosis contrasting with bleeding tendency of those presenting sever hypothyroidism (Squizzato *et al*., 2005).

Both abnormal thyroid function and autoimmunity may interact in the pathogenesis of bleeding disorders that may be observed in thyroid diseases ,

Platelet count is usually normal in hypo and hyperthyroid patients, in rare cases megakaryocytopoiesis may be severely inhibited .Alow platelet count may also develop in

hypothyroidism as the result of an autoimmune peripheral platelet consumption (idiopathic thrombocytopenic purpura or ITP).

Thyroid disease is one of the most common diseases in Sudanese population in different area of Sudan. Thyroid gland disturbances make individual susceptible for bleeding tendency and venous thrombo-embolism. People with hypothyroidism are most susceptible to bleeding while hyperthyroidism subject are at high risk of thrombosis. Thyroid disorders incidence are increasing of cardiovascular abnormalities risk.

Thyroid dysfunction , mostly hypothyroidism ,is a frequent disorder in the general population

Especially among women ,hypothyroid patients may have several hemostatic abnormalities such as modification of the coagulation proteins and bleeding tendency .A coagulation disorder

Resembling von wellibrand disease has been reported in patients with overt hypothyroidism ,but the influence of hypothyroidism on hemostasis remains controversial since ,in addition to

These hypocoagulable states, hypercoagulable states have also been reported .The mechanisms relating to the alterations in the coagulation system in hypothyroidism are not very well established but direct effects of thyroid hormones have been proposed .

1.2 Objectives

1.2.1 General objective:

To assess the change in coagulationprofile(PT ,APTT) and platelets count in patients with hypo and hyperthyroidism.

1.2.2 Specific objective:

-To measure Prothrombin time (PT) among the study population .

-To measure activated partial thromboplastin time (APTT)among the study population.

-To perform platelets count among the study population .

-To compare coagulation profile (PT–APTT) and plateletscount between hypo and hyperthyroidism and normal control group.

1.3 Rationale

Thyroid disease is one of the most common diseases in Sudanese population in different area of Sudan**.**

. In Sudan there is a paucity of data regarding the disorder of coagulation system in people with hyperthyroidism and hypothyroidism .so this study undertaken to filling this gap.

Chapter II

Literature Review

2.1Literature review

2.1.1 Anatomy of thyroid gland:

The thyroid gland is butterfly-shaped organ and is composed of two cons-like lobes or wings, lobusedexter (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 (YalÇinand Ozan,2006).

2.1.2 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, whch is largely a**.**prohormone, perhaps four (Ekholmand Bjorkman,2003) or even ten times more active (Bianco *etal*., 2002).

2.1.3 T3 and T4 production:

The system of thyroid hormones T3 and T4 synthesis of the thyroid hormones, as seen on an individual thyroid follicular cell (Jansen *etal*., 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 (I0) by an enzyme called thyroid peroxidase.Iodine (I0) 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 synthesisedby 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 tyrosine 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 (Yamomoto *etal*.,2006).

2.1.3.1Physiological effect of T3 and T4:

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

2.1.3.2 Abnormality of T3 and T4 secretion:

2.1.3.2.1 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 production which simulate 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 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(Massimotal, 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 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 cell 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).

2.1.3.2.2Hypothyroidism:

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 week 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 etal,.2012). Hashimoto's may be associated with a goiter. It is characterized by infiltration of the thyroid gland with T lymphocytes and autoantibodiesagainst specific thyroid antigens such as thyroid peroxidase, thyroglobulin and the TSH receptor (Garber *et al*., 2012).

2.2 Normal hemostatic mechanisms:

The haemostatic mechanisms have several important function: (a) to maintainblood 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 of the plug when healing is completed (Lewis *et al.*,2006).

2.2.1 Hemostatic system:

The hemostatic system consists of blood vessels, platelets, and the plasma coagulation system including the fibrinolytic factor 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 with in the fibrinolytic system lead to a dissolution of the fibrin clot and to a restoration of normal blood flow (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).

2.2.1.1. Blood coagulation:

The central event in the coagulation pathways [8] is the production of thrombin, which acts upon fibrinogen to pro-duce fibrin and thus the fibrin clot. This clot is further strengthened by the crosslinking action of factor XIII, which itself is activated by thrombin. 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 pathway, respectively. However, this bears only a limited relationship to the way coagulation is activated in vivo. For Example, Deficiencies of factor XII or of factor VIII both produce marked prolongation of the APTT, but only deficiency of the latter is associated with a hemorrhagic tendency. Moreover, there is considerable evidence that activation of factor IX (intrinsic pathway)by factor VIIa (extrinsic pathway) is crucial to establishing coagulation after an initial stimulus has been provided by VIIa-tissue factor (TF)activation of factor X. Investigation of the coagulation system centres on the coagulation factors, but the activity of these proteins is also greatly dependent on specific surface receptors and phospholipids largely presented on the

surface of platelets and also by activated endothelium. The necessity for calcium in many of these reactions is frequently used to control their activity in vivo.

2.2.1.1.2 Hemostatic test:

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

2.2.1.1.2.1. 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 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 time, A typical PT reference range is 10-15 sec.in general, the PT assay is more sensitive in detecting low levels is 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(Benntt *et al.*,2007).

2.2.1.1.2.2Activated 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 PPT assay , patient plasma is pre incubated with the PTT reagent (crude phospholipid and a surface-activating agent such as

silica or kaolin).this preincubatin initiates contact activation(intrinsic pathway activation)in which factor XII and XI are activated in the presence of cofactors, prekalikrein 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 stands, the endpoint of the PTT assay. The usual PTT reagent is less sensitive to factor IX then to factor VIII, XI, and XII. The PTT may be affected by high level 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).

2.2.2 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 result from EDTA modifying platelet membrane proteins which then react with preexisting antibodies present in patient mood that recognize the modified platelet protein, producing platelet clumping or flarirsm.it should be routine laboratory police for technical personnel to review peripheral blood smears of patient 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 uniformly reliable in assessing platelet function (Bennett *et al.,* 2007).

2.3 Previous studies:

Mohamed and Rogia (2008) founded a significant decrease of PT in both hypothyroid and hypothyroid patients when compared with control group, APTT was decreased significant in hypothyroid 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 hypothyoidism patients.

Zynep*et al.* (2003) reported that; platelet count, PTT, PT and INR were not different in hypothyroid patient.

Ford and Carter (2005) reported that; coagulation factors VII,IX,XI,XII are decreased in hypothyroid patients and platelet are not effect.

Squizzato*etal*. (2007) reported a shortening of PT, APTT in hyperthyroid patients.

Study does by Mohamed S.Mohamed-Ali and Rogia O. Ahmed in Al-NeelainUniversity received 15th April 2008; recruited 150 female patient .of 60 patient with hypothyroidism 29 were under treatment and 21 were recruited prior to treatment .60 patients with hypothyroidism 23 under treatment and 37 prior to treatment and 30 normal individual. Bleeding was observed in 8 patients with clinical hypothyroidism, one patient with sub clinical hypothyroidism, and in one patient with clinical hyperthyroidism. Bleeding is absent with sub clinical hyperthyroidism. PT is significant decrease in hypo and hyperthyroidism. While APTT increased in hypo and significant decreased in hyperthyroidism. Fibrinogen level increased while platelets decreased in hypo and-hyperthyroidism.

In other study in Ibn-I sina Hospital, sihhiye 06 100, Ankara, Turkey in 15 patients with SH(TSH levels 5-10 mU/I),15 patients with OH and 15 euthyroidcontrolsin. Hemostatic parameters of the control subjects and patients with subclinical and overt hypothyroidism, Result are presented as means ±s.d. or median(range).Factor VIII and VWF activities were significantly decreased in the patients with SH compared with the control group ($P<0.01$). Patients with OH showed significantly elevated bleeding time, prothrombin time, APTT and clotting time, and significantly decreased factor VIII activity, VWF levels and platelet count compared with the controls. VWF and factor VIII activities were lower and APTT duration was higher in the patients with OH when compared with the patients with SH.

This study takes from several studies in Servizio di Immunoematologia e Trasfusione-Centro Emofilia, AziendaOspedaliera di Verona, Verona, Italy; reported hemostatic abnormalities, both in terms of bleeding or thrombosis, in patients with various thyroid dysfunctions. The aim of this

review is to briefly discuss the relationship between thyroid disorders and hemostasis (i.e. primary hemostasis, coagulation factors and fibrinolytic system). From the analysis of the more recent literature data, it appears evident that most of the coagulation abnormalities associated with thyroid disorders is a consequence of a direct action of thyroid hormones on the synthesis of various hemostatic factors or a derangement of immune function. On the while, these data suggest that a hypercoagulable state is present in hyperthyroid patients, while patients suffering from moderate hypothyroidismare at increased risk of thrombosis contrasting with the bleeding tendency of those presenting severe hypothyroidism.

There was an observational cross sectional study in Hematology, Cliniques Universities Saint-Luc, Universities Catholique de Louvain, Avenue Hippocrate, 10, B-1200 Brusseld, Belgium. In patients hypothyroidism induced several hemostatic disturbances, VWF:CBA, VWF:Ag, and F VIIL:C levels were significantly decreased (P-<0.001), while APTT was significantly increased (P<0.001)in severe hypothyroidism, compared with euthyroidism. None of the patients with severe hypothyroidism developed acquired von Willebrand disease. There was no significant difference with respect to the platelet count, prothrombin time, thrombin time, between severe Hypothyroidism and euthyroidism.

Chapter III

Materials and Methods

3: Materials and Methods

3.1 Study design:

The study utilizes descriptive case control study.

3.2 Study area:

The study will be conducted in the radiation and isotopes center Khartoum hospital.

3.3 Study duration: The study was conducted in Khartoum state from August to December 2019.

3.4Study population:

Study populations included patients with thyroid diseases (Hypo and hyperthyroidism) of different age groups and both sexes.

3.5 Inclusion criteria:

All patients with hypothyroidism and hyperthyroidism ,their age range from (20-70)years .healthy people as control.

3.6 Exclusion criteria:

Patients with other disease and patients using anticoagulated drug which may interfere with result.

3.7 Ethical consideration:

All patients were informed about the aim of the study ,and averable consent was obtained from respondent patients.

Information associated with patients was kept as highly securable data and result was not permitted for any person.

3.8 Sampling:

The sample was selected by simple random sampling method.The study sample size was set 50 patients with hypo and hyperthyroidism against 50 healthy individual as control .Data collected by using questionnaire and laboratory investigation to obtain coagulation profile and platelet count.

3.8.1 Sample size:

33 patients with hypothyroidism and 17 patients with hyperthyroidism were selected for the study ,and 50 normal individuals as control group with matched ages .

3.8.2 Sample collection:

About 1.8 ml of tri sodium citrate anti coagulated blood for prothrombin time and activated partial thromboplastin time. In addition to 2.5ml EDTA anti-coagulated whole blood was drawn from each participant for platelet count.

3.9 Data collection:

Data collected by using questionnaireand laboratory investigation to obtain coagulation profile and platelet count

3.10 Laboratory test:

PT and APTT were measured by sysmex CA-50 automated blood coagulation analyzer.

3.10.1 Detection principle for coagulation method by using CA-50:

After the incubation of fixed quantity of citrated plasma for certain period of time reagent was added then exposed to light of wave length of .660 nm and the turbidity of the plasma during the coagulation process was detected as change in scattered light intensity. From this change in scattered light intensity coagulation carve was prepared and the coagulation time was found by means of percentage.

3.10.1.1 Prothrombin (PT) assay principle:

The PT test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract [thromboplastine] and indicates the overall efficiency of extrinsic clotting system. The test also depends on reactions with factor V, VII, X and fibrinogen concentration of the plasma.

3.10.1.2- Prothrombin (PT) procedure:

- 0.1ml of PPP was applied into reaction tube and incubated for 3 minutes.
- 0.2ml of pre-warmed PT reagent was applied and clotting time was detected.
- Steps were repeated for duplication and the average was obtained.
- Reference value: PT: 11-16 seconds.

3.10.2 -Activated partial thromboplastin time (APTT) assay principle:

The test measures the clotting time of plasma after the activation of contact factors but without added tissue thromboplastine and indicates the overall efficiency of the intrinsic pathway. To standardize the activation of contact factors the plasma was first pre-incubated for set period with contact activator such as kaolin. During 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 re-calcification, factor Xia activates factor IX and coagulation follows. Standardized phospholipids were provided to allow the test to be performed on platelet poor plasma. The test depends not only on the contact factors and on factors VIII and IX but also on reactions with factors X, V, prothrombin and fibrinogen.

3.10.3 -Activated partial thromboplastin time (APTT) procedure:

0.1ml of PPP was applied into reaction tube and incubated for 1 minute. 0.1ml of pre-warmed [37 temp] APTT reagent was applied and incubated for 3 minutes. 0.1ml of pre-warmed calcium chloride was applied and clotting time was detected. Steps were repeated for duplication and the average was obtained.

Reference values:

APTT: 26-40 seconds

Complete Blood Count {CBC):

Sysmex kx-21 hematological analyzer of S.N.A 1967(japan by Toa Medical Electronics CO ,LTD)was used to measure platelet count .

Sysmex Principle:

Sysmex kx-21 performed blood counts by direct current detection method in which blood sample was sucked measured to predetermined volume ,diluted at the specified ratio ,and then fedinto 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 determined the pules size .Also analyzing a histogram makes it possible to obtain various analysis data.

3.11 Quality control of reagents and instrument:

All reagents used for PT and APTT were examined against commercial normal control. A setoff blood samples were analyzed manually and the results were compared to the result obtained by automated Sysmex instruments for the same samples. The results were accepted only when the difference between the two values less than two SD.

3 .12 Data analysis:

Data was analyzed to obtain the mean .standard deviation for patients with thyroidism and normal control using statistical package for social science program version20,Independent T test was used to calculate the p.value of mean differences between different groups the level of significant was set p.value less than 0.05.

Chapter IV

Results

Results

Demographic data of studied population:

The study was done on 100 thyroid patients (33hypothyriodism)and (17 hyper thyriodism)and compared with 50 normal individual as control group.PTmeans in hypo and hyperthyroidism was(10.77 sec and 11.28 sec respectively)were lower than control group mean (13.79 sec) are significant decrease while APTT mean in hypo and hyperthyroidism were (33.66 sec and 26.52 sec respectively) were increased than control group mean (32.43 sec). Platelet count was not significantly differs among control group, hypothyroidism and hyperthyroidism patients.

Table 4.1 show means and SD of PT, APTT, and platelet count among hypo and hyperthyroidism. There is gradual reduction in PT in both compared with normal control. Also there is reduction in APTT only in hyperthyroidism while platelets show no obvious variation among the all groups.

Table4.1: Comparison of mean and SD of PT, APTT, and platelet count of PT, APTT and platelet count among normal control, hypo and hyperthyroidism

Variable	Mean			SD		
	PT	APTT	Plts	PT	APTT	Plts
Hypothyroidism	10.7	33.6	255	1.5072	2.6436	38.485
Control	13.7	32.4	252	1.2521	1.5675	.3625
Hyperthyroidism	11.3	26.5	267	1.2517	1.5675	49,849

Table 4.2 show significant mean difference of PT level between different groups normal control ,hypo and hyperthyroidism (p-value less than .005)

Table (4.2) Comparison of means differences of PT level between normal control ,hypo and hyperthyroidism.

Groups	PT	
	MD(95%CI)	P- value
Control and hyper	2.5578	.000
Control and hypo	2.9672	.000
Hypo and hyper	-.4094-	.423

One way ANOVA test was applied followed by post –hoc comparison (CI :confidence interval ,MD: mean difference)

Table 4.3 show significant mean difference of APPT level between different groups normal control , hypo and hyperthyroidism (p-value .000,.028,.000)

Groups	APTT	
	MD(95%CI)	p-value
Control and hyper	5.9086	.000
Control and hypo	-1.2317-	.028
Hypo and hyper	7.1403	.000

Table 4.3 : comparison of mean difference of platelet count between different groups .these results indicate that there are no effect of thyroids on platelet count .

Groups	PLTS	
	MD(95%CI)	p-value
Control and hyper	-14.966-	.285
Control and hypo	-4.230-	.704
Hypo and hyper	-10.736-	.470

4.4:Distribution of mean PT according to age group

The result show no significant difference in PT level among study age groups (figure 4.1)

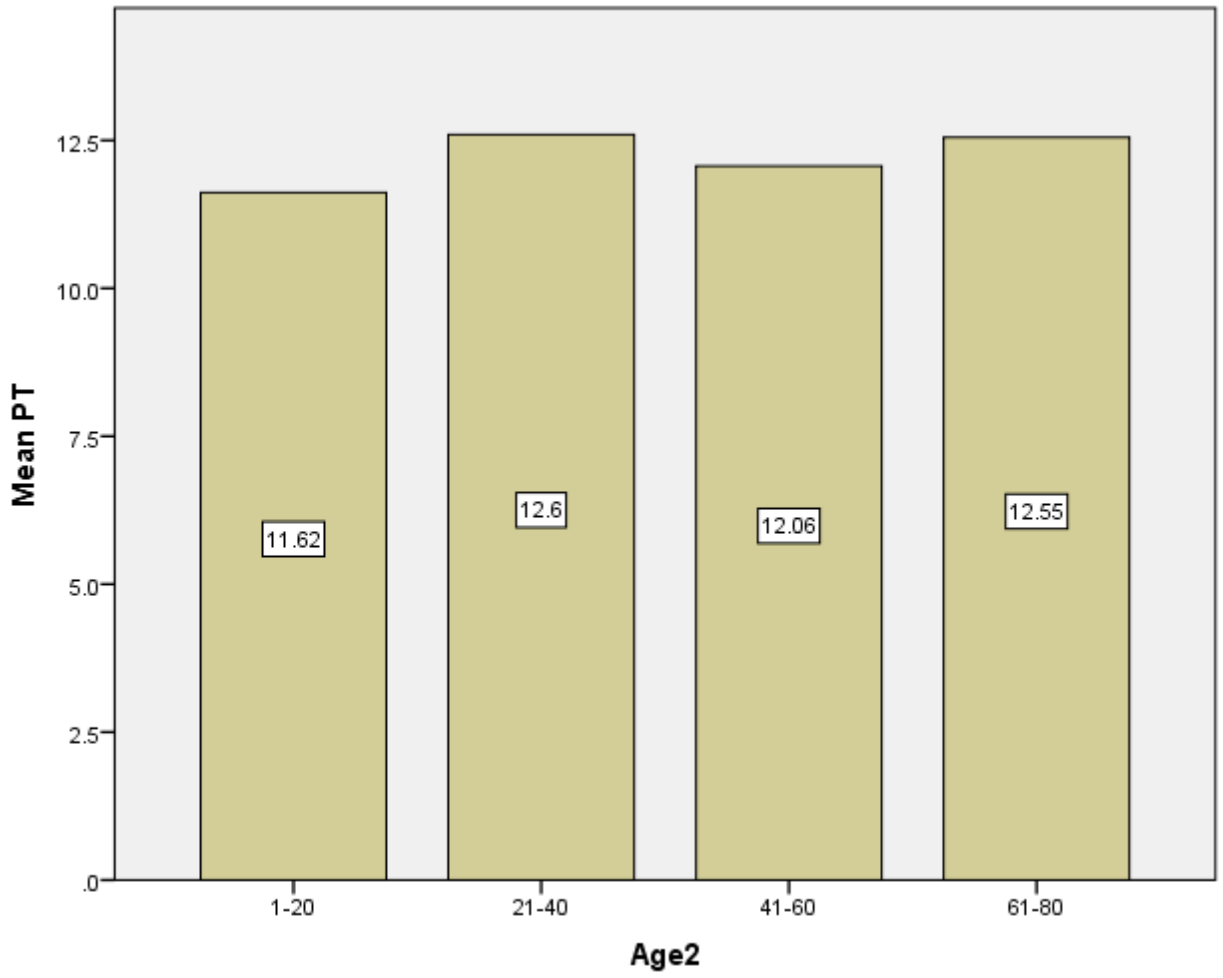

Figure (4.1): Distribution of mean PT according to age group

4.5: Distribution of mean PT among study population (gender)

This result show distribution of PT level among male and female of study population with no significant difference between the two groups

(Figure 4.2).

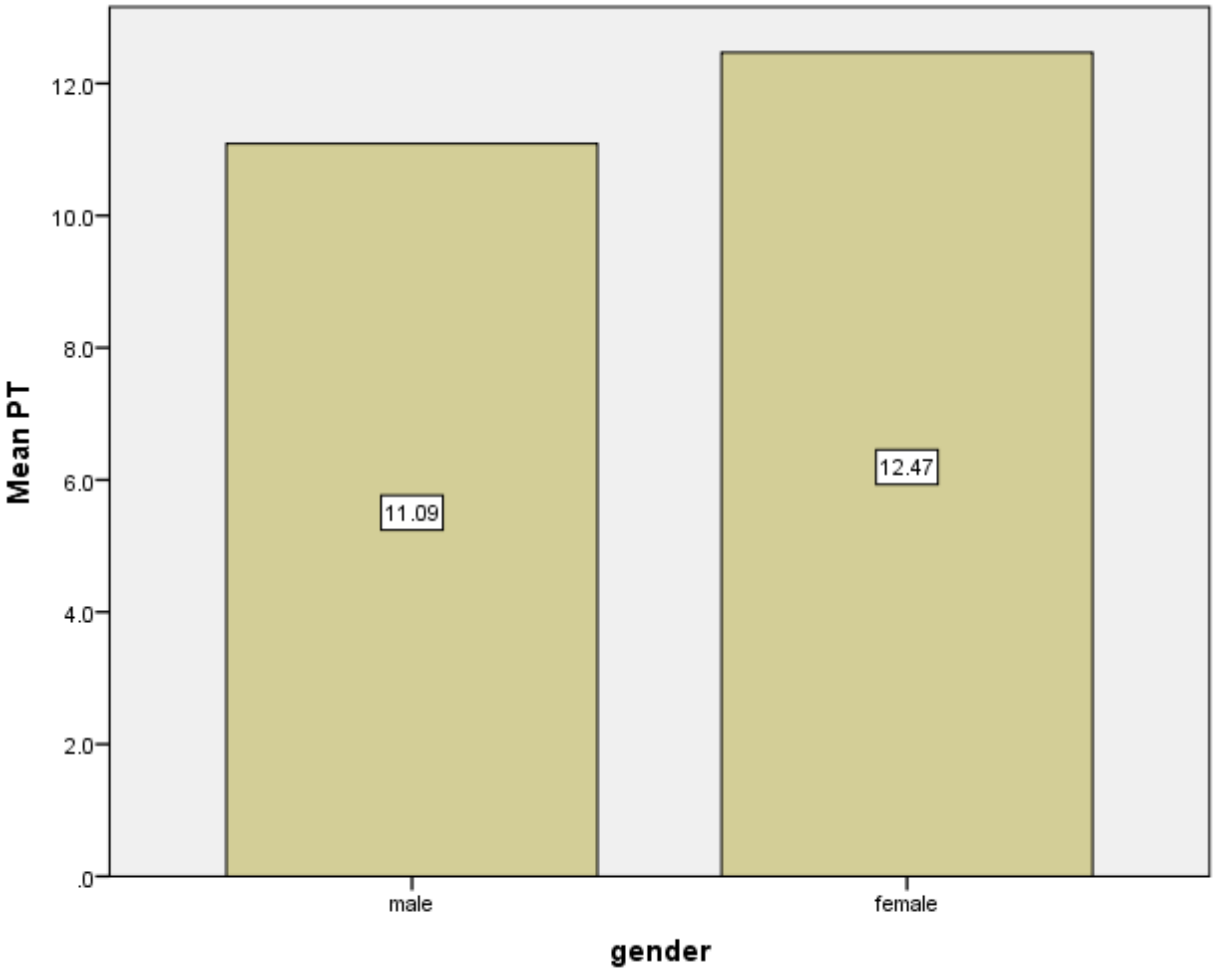

Figure (4.2): Distribution of mean PT according to gender

4.6 Distribution of mean APTT among age group

This result show distribution of APTT level among age group of study population. Level of APTT decrease with age

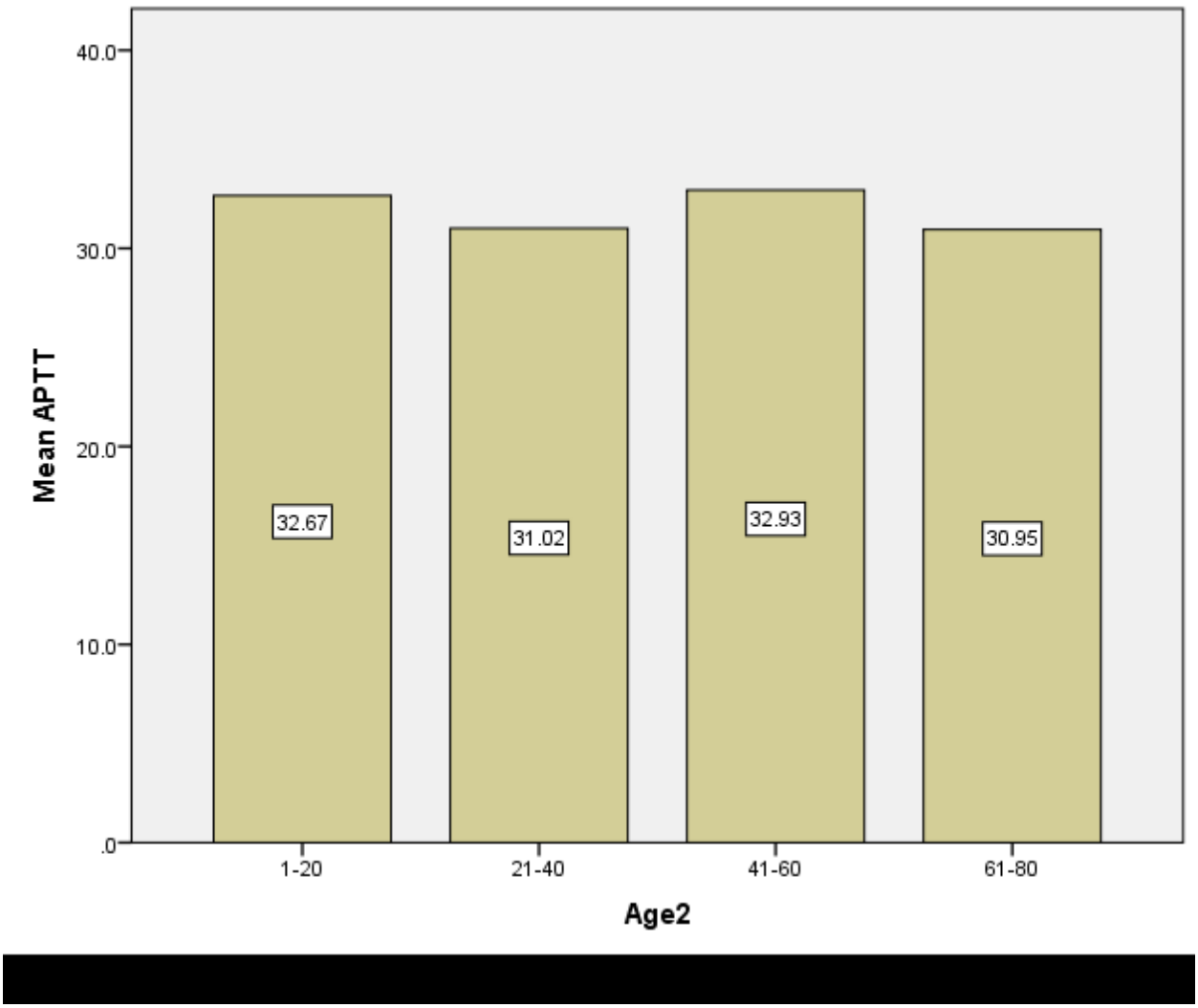

Figure 4.3:Distribution of mean APTT among age group

CHAPTER V:

Discussion, conclusion and Recommendation

CHAPTER V:

Discussion, conclusion and recommendation

5.1 Discussion:

This study aimed to evaluate effect of thyroid dysfunction (hypothyroidism and hyperthyroidism) on coagulation profile. Many factors are responsible for maintaining the hemostatic balance among them hormone directly influence both primary and secondary hemostasis.. Squizzato *etal*,.(2006).The link between the hemostatic system and thyroid diseases has been known since the beginning of the pastcentury .

In the present study the decrease of PT and APTT of hyperthyroid patients is agreement with Mohammed and Rogia (2008).They found a significant reduction in the values of PT and APTT in Sudanese patients with hyperthyroidism. Also Squizzato,*et al*. (2007)study which reported shortening of PT and APTT values in hyperthyroid patients.In this study the platelet count in hyperthyroidism slightly high compared to normal control and hypothyriodism this study dis agreement with Mohammed and Rogia study (2008) that reported the platelet count is slightly lower in hyperthyroid patients compare to hypothyroid patients.

The PT value was found to be significantly lower in hypothyroid patients when compared with the control group in the present study which agreement with Mohammed and Rogia ,(2008).Higher values of PT and lower value of APTT than of the present study were found by Zeynep, et al (2003)in female with subclinical hypothyroidism .

Squizzato et al (2007)reviewed thyroid dysfunction and effect on coagulation and they stated that clinically overt hyperthyroidism and hypothyroidism modify the hemostatic balance in opposite directions and they find this supporting the assumption that thyroid hormone excess and deficit are the main mechanism of hypercoagulable and hypocoagulable state ,respectively .PT level of female patients were significantly higher from male patients ,also PT level was increase in elder patients compared to young patients .

APTT levels did not differ between genders in total participants .

5.2 Conclusion:

The study concluded:

Thyroid dysfunction have effect on coagulation profile these changes should be evaluated to avoid their complications.

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

APTT is significantly decreased only in patients with hyperthyroidism and normal in hypothyroidism compared with normal control group.

Platelet count was not affected by hypothyroidism and hyperthyroidism.

5.3Recommendation

At the bases of this study ,recommended :

- Patients should take their treatment regularly to avoid the risk of thrombosis and bleeding tendency that associated with hypo and hyperthyroidism.
- Haemostatic balance should be assessed routinely tin patient with thyroid disease .
- Increase sample size and study population to get more reliable and applicable result.
- Performing more studies in thyroid disease to focus the defect in coagulation changes.

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Appendices

Appendix I:Coagulometer

Appendix II:SysmexKX-21 Hematology analyzer


sysmex
XP-300

Appendix III

Assessment of Changes in Coagulation Profile and Platelets Count in Patientswith Hypothyroidism and Hyperthyroidism in Khartoum state.

Questionnaire

Hospital:

Name:

Age:

Gender:

Male: Female:

Occupation:

Location of residence:

Duration of disease:

Thyroid hormone status:

Hypothyroidism:

Hyperthyroidism:

Euthyroidism:

Plan of management: