Sudan University of Science and Technology

College of Graduate Studies

Assessment of Thyroid Hormones and C - reactive protein Among Sudanese Males Tobacco Users Reside in the United Arab Emirates

تقييم مستوي هرمونات الغدة الدرقيه وبروتين C التفاعلي لدي الذكور السودانين مستخدمي (التبغ) المقيمين بدولة الامارات العربيه المتحدة

A dissertation submitted for partial fulfillment of requirement of the degree of M.Sc. in Clinical Chemistry

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الأية

وَقُلِ اعمَلوا فَسَيَرَى اللّـهُ عَمَلَكُم وَرَسولُهُ وَالمُؤمِنونَ وَسَتُرَدّونَ إِلى عالِمِ الغَيبِ وَالشَّهادَةِ فَيُنَبِّئُكُم بِما كُنتُم تَعمَلونَ ﴿١٠٥﴾

صدق الله العظيم
[سورة التوبة: 105]

بسم الله الرحمن الرحيم

وَقُل رَبِّ أَدخِلني مُدخَلَ صِدقٍ وَأَخرِجني مُخرَجَ صِدقٍ وَاجعَل لي مِن لَدُنكَ سُلطانًا نَصيرًا ﴿٨٠﴾

صدق الله العظيم

[سورة الإسراء :80]

Dedication

I dedicate this to my mother, father, my supportive husband and our kids.

Iman

Acknowledgement

With a deep gratitude I acknowledge the support of my supervisor Dr. Tarig Karar, Dr. Nuha Elgaili, Abdalla Elmosharf, Ahmed Ali, Esraa Abdin, Nahl aAwad and Maha Abdin without Their efforts this work will never be achieved.

Abstract

The thyroid gland is one of the most important endocrine gland responsible for the secretion of thyroxine (T4) and triiodothyronine (T3) the two major thyroid hormones, are the regulators of growth, development, and basal metabolic rate, and both hormones modulate energy utilization and heat production and facilitate growth as well. Globally, tobacco use is one of the commonest licit substances of abuse and is projected to kill 50% more people than HIV/AIDS by 2015, and to be responsible for 10% of all deaths by 2030. This case control study was done for the estimation of thyroid hormone and C- reactive protein in Sudanese males Tobacco users and its clinical correlation. It was conducted in the United Arab Emirates Sudanese social clubs of the Emirates of Ajman and Dubai, from January 2015 to December 2015. A questionnaire was designed to study person's age, tobacco using, type of Tobacco, clinical problem such as diabetes and hypertension, after signing the consent form a total of 100 Samples were collected from Sudanese males, age ranged from 22-66 years. The blood draw as a venipuncture in a plain gel separator tube red capped and centrifuge speed was 3000/rbm10 minutes. Serum obtained for the analysis was done in Cobas E411 from Roche for the thyroid hormones, CRP analyzed in Cobas Integra 4000 plus. All the results were filled out in the data collection sheet.

Tobacco users were 51 persons (51%) which (smokers and tobacco chewers) and non-tobacco users 49 (49%). These tobacco users there further divided into smokers only 19 persons (37%), chewing tobacco only 14 (27%) and individuals who were both chewers and smokers 18 persons (36%).The study concluded that there is a tobacco effect on the thyroid hormones level

especially reduction in T4 while other findings although reflective for tobacco effect compared to normal, were markedly not significant except the reduction ofT4.The C-reactive protein was significant among chewers and both chewer and smokers group with p= 0.0001.As opposed to other studies, we found CRP to be reduced among chewers and chewers and smokers group, with its reduction may indicate that chewing may halt inflammatory response.

مستخلص الدراسة

الغدة الدرقية هي واحده من أهم الغدد الصماء المسؤولة عن إفراز هرمونات الغدة الدرقيةالثيروكسين (T4) وثُلاثيُ يودو تيرونين (T3)هذه الهرمونات الرئيسية هي المنظمه للنمو ، ، ومعدل الأيض القاعدي ، وكلاهما ينظم استخدام الطاقة وإنتاج الحرارة وتيسير النمو أيضا. وعلي الصعيد العالمي ، فان تعاطي التبغ هو أحد أشيع المواد المشروعة التي يساء استخدامها ، ومن المتوقع ان يقتل 50 في المائة من الأشخاص أكثر من فيروس نقص المناعة البشرية/الإيدز بنسبه 2015 ، وان يكون مسؤولا عن 10 في المائة من جميع الوفاات وقد أجريت هذه الدراسة المتعلقة بمراقبه الحالات لتقدير هرمون الغدة الدرقية والبروتين C-المتفاعل في السودانيين الذكور من متعاطي التبغ وارتباطه السريري. وقد أجريت في الانديه الاجتماعية السودانية للامارات العربية المتحدة في أماره عجمان ودبي ، في الفترة من كانون الثاني/يناير 2015 إلى كانون الأول/ديسمبر 2015. وقد صمم استبيان لدراسة عمر الشخص ، وتعاطي التبغ ، ونوع التبغ ، والمشكلة السريرية مثل مرض السكري وارتفاع ضغط الدم ، وبعد التوقيع علي استمارة الموافقة تم جمع ما مجموعه 100 عينه من الذكور السودانيين ، وتراوحت أعمارهم بين 22-66 سنه. تم سحب الدم من الوريد ووضعه في انبوب خالي من مواد منع التجلط وتم فصل المصل بواسطةجهاز الطرد المركزي بسرعة rbm10/3000 وقد تم اختبار وتحليل العينات باستخدام جهز كوباس E411 من روش لهرمونات الغدة الدرقية ، وتحليل بروتين C التفاعلي باستخدام جهاز Cobas Integra 4000. وقد تم ملء جميع النتائج في ورقه جمع البيانات.

وكان مستخدمو التبغ 51 شخصا (51 في المائة) (المدخنون والسجائر التبغ) والمستخدمون من غير التبغ 49 (49 في المائة). وانقسم هؤلاء المستخدمون إلى المدخنين في 19 شخصا فقط (37 في المائة) ، ومضغ التبغ 14 (27 في المائة) فقط ، والافراد الذين كانوا علي حد سواء من الشياش والمدخنين 18 شخصا (36 في المائة). وخلصت الدراسة إلى ان هناك تاثيرا علي التبغ علي مستوي هرمونات الغدة الدرقية ولا سيما الانخفاض في مستوي الثيروكسينفي حين ان النتائج الأخرى علي الرغم من انها تعكس تاثير التبغ مقارنه بالمعتاد ، فانها ليست كبيره بشكل ملحوظ باستثناء الحد من الثيروكسين. وكانت نتيجة بروتين Cالتفاعلي ذات درجه مئويه كبيره الدرجة من ناحية القيمه Pلدي فئه المدخنين وماضغي التبغ ولدي ماضغين التبغ فقط من غير المدخنينمع $p = 0.0001$. وعلي العكس من الدراسات الأخرى ، وجدنا هذا الانخفاض الذي يشير ان المضغ قد يوقف الاستجابة التهابيه.

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List Abbreviations

CNS	Central Nervous System
CRP	C-Reactive Protein
DPG	2,3-diphosphoglycerate
EDTA	Ethylenediaminetetraacetic acid
FSH	Follicular Stimulating Hormone
HCG	Human Chorionic Gonadotrophin
IL	Interleukin
LH	Luteinizing Hormone
SKH	Sheikh Khalifa Bin Zayed Hospital
T4	Thyroxine and
T3	Triiodothyronine
TBG	Thyroxine Binding Globulin (TBG),
TRH	Thyrotropin-Releasing hormone
TSH	Thyroid Stimulating Hormone

Chapter 1

Introduction and Literature Review

Chapter One

Literature review

1. Literature review

1.1.1. Thyroid Anatomy, Histology and Physiology

The thyroid gland is one of the most important endocrine gland responsible for secretes thyroxine (T4) and triiodothyronine (T3) two major thyroid hormones, a regulator of growth, development, and basal metabolic rate, and both hormones modulate energy utilization and heat production and facilitate growth as well and the other hormone is calcitonin, a regulator of calcium homeostasis. In effect, T3 is the actual hormone, since T3 has a much higher affinity for the thyroid hormone receptor than does T4; T4 is thought to act primarily a precursor for T3 (Mark **Brandt,** 2002).

The English name thyroid gland is derived from Latin glandulathyreoidea. Thyreoidea named after the thyroid cartilage i.e in Greek means shield--like/shield-shaped. (Anderson, 2000).

1.1.2. Endocrine Development and Anatomy

The thyroid is the first endocrine gland to develop in the embryo about 24 days after fertilization from a median endodermal thickening in the floor of the primordial pharynx. This thickening soon forms 'the thyroid primordium'. As the embryo and tongue grow, the developing thyroid gland descends in the neck, passing ventral to the developing hyoid bone and laryngeal cartilages. Then the gland assumes a definitive shape and reaches its final site in the neck and the thyroglossal duct has disappeared remaining

the muscular part of the distal end known as pyramidal lobe (Moore, Persaud, 2003).Parathyroid glands; group of glands most commonly 4 in number arise from 3rd branchial pouch (Inferior parathyroids) and 4th branchial pouch (Superior parathyroids). Parathyroid gland is responsible of parathyroid hormone production and consisted of neuroendocrine epithelial cells (Miller, 2003).

Anatomically, the thyroid gland is located lower down at the front of neck weighing about 25 g. The gland has two lobes; the right lobe is often larger than left and joined together by the Ithmus. A small portion of gland substance, pyramidal lobe often projects upwards from isthmus, generally to the left of midline. The gland has its own capsule and is also enclosed by an envelope of pretracheal fascia which is thickened posteriorly and attached to the cricoid cartilage and upper tracheal rings known as suspensory ligament of Berry. This fixation and investment of gland by pretracheal fascia are responsible for the gland moving up and down with larynx during swallowing **(**Sinnatamby, 1999).

In histology, the thyroid consists of large number of closed follicles lined by a single layer of epithelial cells. Colloid is made up of proteins, especially the iodinated glycoprotein, thyroglobulin, a 19S protein to which tyrosine residues is bound and in which thyroid hormone synthesis and storage takes place (store or reserve for hormones). The follicles are bound together in groups to form lobules each supplied by an end artery. There are para follicular cells constituting < 2% of total cells which are scattered on outer aspects of the follicles and secrete calcitonin. The thyroid gland is highly vascular and is the only endocrine gland to store its secretion outside the cells (Sinnatamby, 1999).

1.1.3. Endocrine Effect

The principal hormones secreted by the thyroid are thyroxine (T4) and triiodothyronine (T3). T3 is also formed in the peripheral tissues by deiodination of T4. Both hormones are iodine containing amino acids. T3 is more active. Naturally occurring forms are L–Isomers (levo isomers) (Ganong, 2005). The hormones are bound to thyroglobulin within the colloid. Synthesis in the thyroglobulin complex is controlled by several enzymes such as thyroid peroxidase, in distinct steps; these include tapping of inorganic iodide from the blood, followed by oxidation of iodide to iodine, through binding of iodine with tyrosine to from iodotyrosines and finally coupling of mono-iodotyrosines and diiodotyrosines to form T3 and T4. By the aid of feedback mechanism from the pituitary, and when hormones are required, the complex is resorbed into the cell and thyroglobulin is broken down. T3 and T4 are liberated and enter the blood, where they are bound to serum proteins: albumin, thyroxine binding globulin and thyroxine binding prealbumin. The small amount which remains free is biologically active. T3 is the more important physiological hormone and is quick acting (within a few hours), whereas T4 acts slowly (4-14 days) (Krukowski, 2004). This process also involves Iodine metabolism which is a raw material essential for thyroid hormone synthesis. Minimum daily required intake to maintain normal thyroid function is 150 μg. Normal plasma Iodine level is 0.3 μg/dl. Normal diet contains about 500mcg Iodine (Ganong, 2005). The normal level of Thyroid hormones depends on methods used and the manufacturer is roughly T3 - 70-190ng/dl (1.1 – 2.9 nmol/L), T4 - 5-12 mcg/dl (64 –154 nmol/L) [17] Normal daily secretion: T3 - 4 μg (7 nmol), T4 - 80 μg (103 nmol**) (**Ganong, 2005).

Thyroid hormones enter cells and T3 binds to thyroid receptors in the nucleus. T4 can also bind, but not as avidly. The hormone-receptor complex then binds to DNA via Zinc fingers and alters the expression of a variety of different genes that code for enzymes which regulate cell function.

Thyroid function is regulated primarily by variations in the circulating level of pituitary thyroid stimulating hormone (TSH). In addition to TSH receptors the thyroid cells contain receptors for IGF-1, EGF, gamma interferon and TNF-alfa, growth factors. IGF – 1 and EGF promote growth whereas gamma interferon and TNF-alfa inhibit growth. Thyroid stimulating hormone: Glycoprotein secreted by anterior pituitary. Normal level: 0.4-5.0 microIU/ml (0.4-5.0mu/L) (Jameson and Weeman, 2005), Normal daily secretion is 110 μg. Half life is 60 minutes.TSH secretion increased by TRH and by cold and decreased by increased free T4 and T3 and by stress and heat.

Effects of TSH on thyroid generally increases iodide binding, Increases synthesis T3, T4 and iodotyrosines, Increases secretion of thyroglobulin into colloid and also endocytosis of colloid, Blood flow increases, the cells hypertrophy and weight of gland increases. And in turn for The pituitary thyroid Axis secretion of TSH depends upon the level of circulating thyroid hormones and is modified in a classic negative feedback manner**(**Khatawkar and Awati , 2015**).**

1.1.4. Biochemistry of Thyroid Hormones:

As T4 is considered to be a prohormone, therefore its metabolism occurs peripheral in liver, kidney, & other tissues in two ways: outer ring deiodination by the enzyme 5'-D, which yields T3, and inner ring deiodination by the enzyme 5-D, which yields rT3, for which there is no

known biologic function. In humans, deiodination is the most important metabolic pathway of the hormone, not only because of its dual role in the activation and inactivation of T4, but also in quantitative terms: 87% of T3 in circulation is formed from T4. Degradative metabolism of the thyroid hormones, apart from peripheral deiodination, occurs mainly in the liver, where both T3 and T4 are conjugated to form either glucuronide (mainly T4) or sulfate (mainly T3) through the phenolic hydroxyl group. The resulting iodothyronine conjugates are excreted via the bile into the intestine, where a portion is hydrolyzed by bacteria.

It also undergoes marginal enterohepatic circulation and is excreted unconjugated in feces. T4 is conjugated with sulfate in kidney and liver, and the T44'-O-sulfate, an excellent substrate for 5'- D, is believed to play a role in the regulation of T4 metabolism.

Additional metabolism, involving side-chain degradation, proceeds by transamination, oxidative deamination, and decarboxylation to yield thyroacetic acid and thyroethanediol; also, cleavage of the diphenyl ether linkage has been detected both in vitro and in vivo.

Thyroid hormones regulated through Thyroid-stimulating hormone (TSH) from the anterior pituitary increases all known activities of the thyroid gland to increase release of thyroid hormone. TSH is controlled by the hypothalamic peptide, thyrotropin-releasing hormone (TRH), following release into the hypothalamic portal system. Thyroid hormone release is controlled by an inverse feedback system on TRH and TSH release (see Figure 7). TSH is two subunit (alpha and beta) glycoprotein (211 AA). Its alpha subunit is identical to other pituitary hormones (FSH, LH, etc.) encoded on chromosome 6 and tohCG.

The TSH beta subunit unique to TSH encoded on chromosome 1. TSH has a half-life = 60 minutes and typical plasma level are 0.4 - 4.8 mU/L in those with normal thyroid function. TSH binds to TSH-receptor (TSH-R) on the thyroid cell membrane and receptor is coupled to a Gprotein system coupled to adenylate cyclase. Thus stimulation of this receptor results in increased cAMP formation which mediates increases in uptake and transport of iodide, iodination of thyroglobulin, and synthesis of iodotyrosines. TSH binding to TSH-R also stimulates phospholipase C leading to thyroid cell hypertrophy. Chronic TSH stimulation causes entire gland to hypertrophy causing a goiter.

The regulation of thyroid hormone production as well as the physiologic actions and disease states associated with thyroid overproduction (hyperthyroidism) or underproduction (hypothyroidism) are discussed in more detail in the chapter "Thyroid Disorders" in the Pharmacotherapy: a Pathophysiologic Approach.

Thyroid hormones, especially T3, enter tissue cells by diffusion or specific transport where they bind to two different receptors nuclear receptors then binds DNA via “zinc fingers” and this produces a change in the expression of a variety of genes that encode enzymes that control cellular metabolism and function. Thyroid hormones effect normal growth and development (particularly in bone and CNS), help regulate lipids (adipose tissue), increase absorption of carbohydrates from intestine, increase protein breakdown in muscle, increases dissociation of O2 from hemoglobin by increasing RBC 2,3-diphosphoglycerate (DPG). They also stimulate increased O2 consumption and metabolic rate in most metabolically active tissues (exceptions are brain, testes, uterus, lymph nodes, spleen and anterior

pituitary).Thus the thyroid hormones increase cellular respiration and thereby increase the basal metabolic rate (BMR).

1.1.5 Pathophysiology

Thyroid hormones increase basal metabolic rate by increasing oxygen consumption and heat production inseveral body tissues. Thyroid hormones also have specific effects on several organ systems. These effects are exaggerated in hyperthyroidism and lacking in hypothyroidism, accounting for the wellrecognized signs and symptoms of these two disorders. Total serum T4 and T3 measure the total amount of hormone bound to thyroid-binding proteins by radioimmunoassay. Total T4 and total T3 levels are elevated in hyperthyroidism and low in hypothyroidism. Increase in TBG (as with pregnancy or estrogen therapy) increases the total T4 and T3 measured in the absence of hyperthyroidism. Similarly, total T4 and T3 are low despite euthyroidism in conditions associated with low thyroid-binding proteins (e.g., cirrhosis or nephritic syndrome). Thus, further tests to assess the free hormone level that reflects biologic activity must be performed.

Free T4 level can be estimated by calculating the free T4 index or can be measured directly by dialysis. The free T4 index is an indirect method of assessing free T4. It is derived by multiplying the total T4 by the T3 resin uptake, which is inversely proportional to the available T4 binding sites on TBG. Free T4 can be measured directly by dialysis or ultrafiltration. This is more accurate and is preferred to the free T4 index.Serum TSH is measured by a third-generation immunometric assay, which employs at least two differentmonoclonal antibodies against different regions of the TSH molecule, resulting in accurate discriminationbetween normal TSH levels and levels below the normal range. Thus, the TSH assay can diagnose

clinicalhyperthyroidism (elevated free T4 and suppressed TSH) and subclinical hyperthyroidism (normal free T4and suppressed TSH). In primary (thyroidal) hypothyroidism, serum TSH is supranormal because of diminished feedback inhibition. In secondary (pituitary) or tertiary (hypothalamic) hypothyroidism, the TSH is usually low but may be normal. Serum thyroglobulin measurements are useful in the follow-up of patients with papillary or follicular carcinoma. After thyroidectomy and iodine-131 (131I) ablation therapy, thyroglobulin levels should be less than 2mg/L while the patient is on suppressive levothyroxine treatment. Levels in excess of this value indicate the presence of persistent or metastatic disease. Calcitonin is produced by the medullary cells of the thyroid. Calcitonin measurements are invaluable in the diagnosis of medullary carcinoma of the thyroid and for following the effects of therapy for this entity (Friedman and Bonert, 2016).

1.1.6 General Aspects of Tobacco Effects on the Thyroid

Cigarettes are considered as the commonest source of toxic chemical exposure and chemically mediated illness in humans. Globally, tobacco use is one of the commonest licit substances of abuse and is projected to kill 50% more people than HIV/AIDS by 2015, and to be responsible for 10% of all deaths by 2030. Of great concern is the fact that more than 80% of these deaths are expected to occur in low- and middle-income countries this is attributed to poor medical care (Mathersand Loncar, 2006). The first Global Adult Tobacco Survey of 2010 for neighboring countries as example Egypt, nearly 20% of the Egyptian population uses some form of tobacco product. Of this percentage, about 16% smoke cigarettes, 3.3% smoke shisha and 2.6% use smokeless (chewed) tobacco. The percentage of the population using any tobacco product increases to around 23% and nearly 26% among

the productive age groups 25–44 and 45–64 years, respectively. The prevalence of using any tobacco product among all university graduates is about 16%. The percentage of using any tobacco product among those with no formal education or those with some primary level education was higher at around 21% and nearly 26%, respectively (WHO, 2010). Additionally, prevalence of tobacco use is high among people seeking help for use of other psychoactive substances as well based on Indian studies (Jhanjee at *al.*, 2009). In view of the magnitude of the problem, it becomes necessary for us to understand the effect of tobacco on various body systems.

1.1.7. Chemical Agents in Tobacco Smoke Affecting Thyroid:

Tobacco smoke contains around 7000 chemical compounds of which at least 158 compounds have been reviewed in scientific literature as harmful, carcinogenic, and/or potentially affecting physiological functions. Of these combustion products, nitroso compounds, polycyclic hydrocarbons, aromatic amines, and aldehydes are most commonly implicated for toxicity (Fowles andDybing, 2003). The effect of cigarette smoke on thyroid is believed mostly to be due to the compound ‘‘Thiocyanate,’’ a derivative of hydrogen cyanide with a half-life >6 days. Thiocyanate hampers thyroid functioning by at least three distinct pathways. Thiocyanate inhibits iodine uptake by the thyroid, thereby substances of abuse in return have multiple effects on the hypothalamic-pituitary-thyroid axis. Indeed, ample evidence now documents the effect of nicotine and tobacco smoke on human endocrine system. Of the multitude of drug-endocrine interactions reported, this review focuses on the effect of tobacco on the hypothalamo-pituitary-thyroid axis. A major shortcoming of the current literature is that most of the information is in context of smokable forms of tobacco. The impact on hypothalamic-

pituitary-thyroid (HPT) axis of smokeless tobacco, the commoner form of abuse in India, largely remains unexplored. The paper is presented as a critical overview for readers and is by no means an exhaustive systematic review because of wide scope of the subject. Inhibition of iodide transport by thiocyanate is independent of TSH concentration but competitive with iodine concentration (Fukayama, 1992). Because of this competitive inhibition, iodine deficiency enhances the antithyroid action of thiocyanate, whereas iodide excess diminishes its harmful effect. Thereby, thiocyanate may be responsible for the goitrogenic effect of cigarette smoking seen at least in iodine deficient areas. Variations in iodine intake might also modulate the response to smoking, the predominant action of smoking being antithyroid when iodine intake is low and immunogenic when it is adequate. Whether thiocyanate affects the peripheral action of T3 or T4 is currently not known (Tziomalos and Charsoulis, 2004).

Studies show that nicotine and cotinine, the major psychoactive compounds of cigarette do not have any direct detrimental effect on the thyroid gland. However, nicotine has been implicated to be a thyroid stimulant by its action through the HPA. As early as in 1989, Balfour showed that nicotine was a potent activator of the HPA. Nicotine mimics the effects of acetylcholine at selected central nicotinic acetylcholinergic receptors, thereby causing sympathetic activation, which can increase thyroid secretion (Matta *et al.*, 1998). Thus, tobacco smoke might have a dual mode of action on the thyroid gland, one of direct suppression by thiocyanate along with indirect activation through the Health Protection Agency. The activation-suppression model might be an oversimplification of the effects of tobacco smoke, as tobacco smoke is known to contain thousands of active chemicals. Though evidence currently favors to the role of thiocyanate as the predominant thyroid

suppressor, multiple other components of smoke, like hydroxypyridine metabolites and benzpyrenes, are also being researched for their potential to interfere with thyroid function. 2,3-hydroxypyridine have been found to inhibits thyroxin deiodination by reducing iodothyronine deiodinase activity. Several other mechanisms have been forwarded to explain the effect of tobacco smoke on thyroid. Tobacco smoke can cause hypoxia and formation of oxygen-free radicals which may result in free radical injury. In addition, tobacco glycoprotein promotes formation of interleukin (IL)-1 in in vitro experiments (Ericssonand Lindgärde, 1991). Thus, smoking may promote inflammatory processes via an increase in IL-1 in humans which may contribute to autoimmune thyroid diseases. In support of this theory, higher concentration of IL-1a, IL-1ß, and soluble IL-1RA (sIL-1RA) have been demonstrated in serum of smokers compared to nonsmokers with active autoimmune thyroid disease.

1.2 Rationale:

As documented as several studies investigated the relationship between tobacco users and clinically apparent goiter. Two population-based surveys of patients with a clinical diagnosis of goiter reported that the prevalence of goiter was 50 to 100 percent higher among women smokers than among women nonsmokers (Christensen, 1984; Ericsson and Lindgärde, 1991). We referred to several studies some in hospital employees found that the prevalence of goiter among cigarette smokers was 10 times that among nonsmokers when analyzed combined data for women and men. In our study we focused on the effect of tobacco use and changes in thyroid hormones and the changes in C - reactive protein. In several studies the changes are correlated whether hypo or hyperthyroidism or even proliferative and cancerous changes. Or support to our study and in a series of studies, mostly clinic based, has reported that cigarette smokers have a higher risk for Graves' disease with ophthalmopathy (eye involvement) than do nonsmokers. (Hägg and Asplund, 1987; Bartalena, 1989). We have found that various analyses were presented in these studies, and some made no adjustment for age and gender. Nonetheless, these findings consistently suggest that smoking and tobacco as general modestly increases the risk for Graves' hyperthyroidism and greatly increases the risk for Graves' disease with ophthalmopathy. The rationale also focused on the fact data on the association of smoking with other thyroid disorders was limited.

As general role that tobacco is among the leading causes of death in most countries. Using data published by the United States Centre for Disease Control (CDC), it was calculated that in the year 2000 18.1% of deaths in the USA were attributable to smoking tobacco. We believe that cessation of smoking reduces the risk of smoking associated cardiovascular disease and

cancer substantially, and quitting tobacco has been vigorously promoted to prevent disease and death.

1.3 Objectives

1.3.1 General Objective:

To assess thyroid hormones among Tobacco users.

1.3.2 Specific Objectives:

- To measure T3, T4, TSH andC- reactive protein to determine if there is a difference between smoking and chewing.
- To find a correlation between the Thyroid Hormones serum level and the duration of smoking.

Chapter Two

Materials and Methods

2.1 Materials

2.1 .1 Study design:

This was a case control study.

2.1.2 Study area and period:

This study was done in Sheikh Khalifa Bin Zayed Hospital (SKH), Ajman, UAE during the period January 2015 to Decmber 2015.

2.1.3 Study population:

The study involved adult males smokers, tobacco chewers or both and non tobacco users as a control group.

2.1.4 Inclusion criteria:

Adults smokers, chewers or both or non chewers healthy male adult

2.1.5 Exclusion Criteria:

Tobacco users and non tobacco users with known thyroid disease or other apparent complications.

2.1.6 **Ethical consent:**

An ethical consent form had been read, agreed and signed by any one of the participants .

2.1.7 Specimen collection:

After reading the consent form and signing it by the research subjects they filled in the questionnaires. The blood draw as vein puncture, is performed I asked them to sit in a comfortable chair. Then byusing a tourniquet,veinous blood was collectedin plain gel separator tube red capped and centrifuged i for 3000/rbm 10 minutes. Serum obtained wasanalyzedbyCobas E411 from Roche for the thyroid hormones, CRP analysis in cobas Integra 4000 plus. All the results were filled out in the data collection sheet.

2.6. Procedure:

Specimen collection and preparation:

For specimen collection and preparation only use suitable tubes or collection containers. Only the specimens listed below were tested and found acceptable. Serum: Collect serum using standard sampling tubes. Serum is the specimen of choice. Plasma: Li-heparin or oxalate plasma. Do not use EDTA or fluoride plasma. The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Specimens should not be repeatedly

frozen and thawed. Thawed specimens should be inverted several times prior to testing. Stability: 8 hours at 15-25 °C up to 48 hours at 2-8 °C longer periods at (-20) °C or below Centrifuge samples containing precipitates before performing the assay.

System information Test TSH, T3 and T4, test ID 0-900.

Intended use In vitro test for the quantitative determination of TSH, T3 and T4 (free and protein bound) in human serum or plasma prepared with heparin or oxalate on COBAS INTEGRA systems. Summary Thyroxine (T4) is the most abundant of the iodinated hormones secreted by the thyroid gland.1,2 most of the serum T4 is bound to proteins, primarily thyroxine binding globulin (TBG), which transport the hormone to its sites of action.3,4 A small fraction of T4 is free in the circulatory system and is thought to be the biologically active material that can enter cells as needed to maintain a balanced tissue-bound fraction of T4. T4 secretion is stimulated by thyroid stimulating hormone (TSH), and hormone levels are regulated by a negative feedback system to the pituitary gland.5 Abnormalities in T4 secretion can have profound effects in energy production or fat or protein metabolism. Clinical symptoms such as seen in Graves's disease are associated with hyperthyroidism, and hypothyroidism can be associated with goiters and cretinism. Changes in TBG concentration can make thyroid assessment difficult. Such changes may occur, for example, with pregnancy, estrogen treatment, and usage of drugs such as phenytoin and salicylates which compete for T4 binding sites on serum proteins. Under these conditions it is best to use the total T4 assay in conjunction with other thyroid function tests. For example, a total T4 run together with T3 uptake will generate a free thyroxine index (FTI).

Test principle Fluorescence polarization. COBAS INTEGRA Total T4 (Thyroxine) measurements are made on COBAS INTEGRA systems using the principle of fluorescence polarization. When a fluorescent molecule, or fluorophore, is irradiated with light of the proper wavelength (the excitation wavelength) some of the light is absorbed. Within a few nanoseconds the absorbed light is emitted, although at a longer wavelength (the emission wavelength). Whether or not the emitted light is polarized depends on the freedom of the fluorophore to rotate in solution. A small molecule, such as fluorescein, can rotate rapidly before light emission occurs, resulting in depolarization of the emitted light. In contrast, a fluorescent macromolecule, such as a fluorescein-labeled protein, will rotate much more slowly. Thus, in the time frame between excitation and emission, the macromolecule will have rotated only very slightly and the emitted light will be polarized. Fluorescence polarization is a reproducible function of the hormone concentration, and is suitable for the quantitative determination of total T4 concentrations in serum for the purpose of thyroid monitoring. Surface active agents are used to ensure dissociation of the hormone from serum proteins and to prevent nonspecific binding of the tracer.

2.7 Data analysis:

Data entering and checking and analyzed using Statistical Package for the Social Sciences version 20. Data represented by mean ±STD frequency, *P-value* significant at level ≤0.05

Chapter Three

Results

Total of one hundred were collected from two groups for this study. There were tobacco users 51 persons (51%) which (smokers and tobacco chewers) and non tobacco users 49 (49%). These tobacco users there further divided into smokers only 19 persons (37%), chewing tobacco only 14 (27%) and individuals who were both chewers and smokers 18 persons (36%). All patients enrolled in this study age range from 25 yrs to 66 yrs average 44 years males table 1 & Fig 1. Age groups were further divided as follows; non smokers and none chewing range (23 and 66), smokers (23 and 65), chewing (33 and 59), and chewing and smokers (22 and 66). Other variables included in this study were whether these persons were alcoholic or not and whether they were diabetics or hypertensive. For all these cases samples were tested for thyroid hormones assessment including TSH, T3, T4 and C - reactive protein.

TSH

Table(1,2 and 3) shows no significance difference between mean of TSH levels in test group of cigarette smokers, tobacco chewers only or both on smoking and hewing and control group (1.9+0.8) vs (1.9+1.2) p= 1.0000, (1.7+ 0.9) vs (1.9+1.2) p= 0.5657 and (2.0+ 1.4) vs (1.9+1.2) p= 0.7735 respectively.

T3

Table (1,2 and 3)shows no significance difference between mean of T3 levels in test group of cigarette smokers and tobacco chewers only (5.1+0.7) vs (5.2+ 0.6) p= 0.5341 and (5.5+ 1.0) vs (5.2+ 0.6) p= 0.1651 respectively,

while slightly significant for those both on smoking and hewing and control group (5.5+ 0.6) vs (5.2+ 0.6) p= 0.0743

T4

Table(1,2 and 3) shows no significance difference between mean of T4 levels in test group of cigarette smokers, tobacco chewers only or both on smoking and hewing and control group (17.1+2.1) vs (16.3+3.0) p= 0.2535, (17.1+1.6) vs (16.3+3.0) p= 0.3429 and (16+2.0) vs (16.3+3.0) p= 0.6960 respectively.

CRP

Table(1) shows no significance difference between mean of CRP levels in test group of cigarette smokers (2.9+ 2.4) vs (4.8+1.2) p= 0.2255.

Table 2 and 3 show extremely significant difference between mean of CRP levels in test group of tobacco chewing and both smokers and tobacco chewing (2.0+1.3) vs (4.8+1.2) p= 0.0001 and (2.6+2.4) vs (4.8+1.2) p= 0.0001 respectively.

Table (1) Comparison of means plasma levels of TSH, T3, T4 and CRP for smokers and control group.

Variable/Smokers	Test(n=23)	Control Group (n=49)	P .value
TSH	(1.9±0.8)	(1.9±1.2)	**1.0000**
T3	(5.1±0.7)	(5.2± 0.6)	**0.5341**
T4	(17.1±2.1)	(16.3±3.0)	**0.2535**
CRP	(2.9± 2.4)	(4.8±1.2)	**0.2255**

- The table shows the mean ± STD, range in brackets and probability (P).
- Independent t-test was used for comparison
- P-value ≤ 0.05 is considered significant

Table (2) Comparison of means plasma levels of TSH, T3, T4 and CRP for tobacco chewing and control group.

Variable/Chewing	Test Group (n=14)	Control Group (n=49)	P .value
TSH	(1.7± 0.9)	(1.9±1.2)	**0.5657**
T3	(5.5± 1.0)	(5.2± 0.6)	**0.1651**
T4	(17.1±1.6)	(16.3±3.0)	**0.3429**
CRP	(2.0±1.3)	(4.8±1.2)	**0.0001**

- The table shows the mean± STD deviation, range in brackets and probability (P).
- Independent t-test was used for comparison
- P-value ≤ 0.05 is considered significant
- The table shows the mean± STD, range in brackets and probability (P).
- Independent t-test was used for comparison

Table (3) Comparison of means plasma levels of TSH, T3, T4 and CRP for tobacco chewing and control group.

Both/Chewing and Smokers	Test Group (n=18)	Control Group (n=49)	P .value
TSH	(2.0± 1.4)	(1.9±1.2)	**0.7735**
T3	(5.5± 0.6)	(5.2± 0.6)	**0.0543**
T4	(16±2.0)	(16.3±3.0)	**0.6960**
CRP	(2.6±2.4)	(4.8±1.2)	**0.0001**

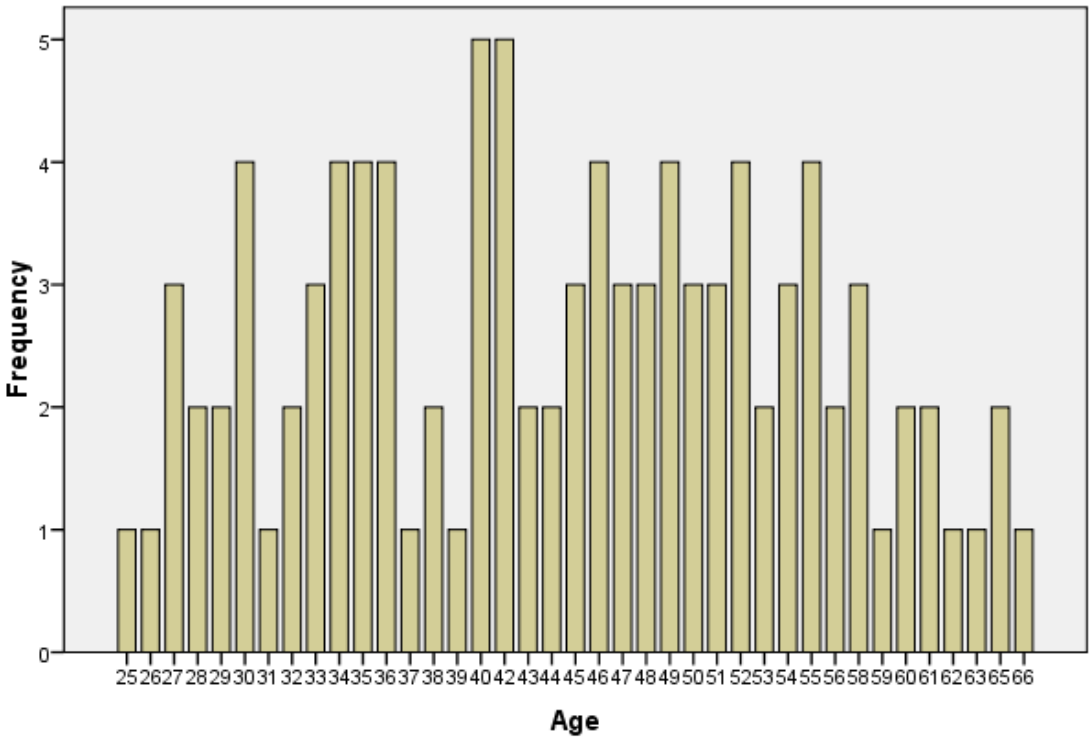

Fig (1): Frequency of age among study population

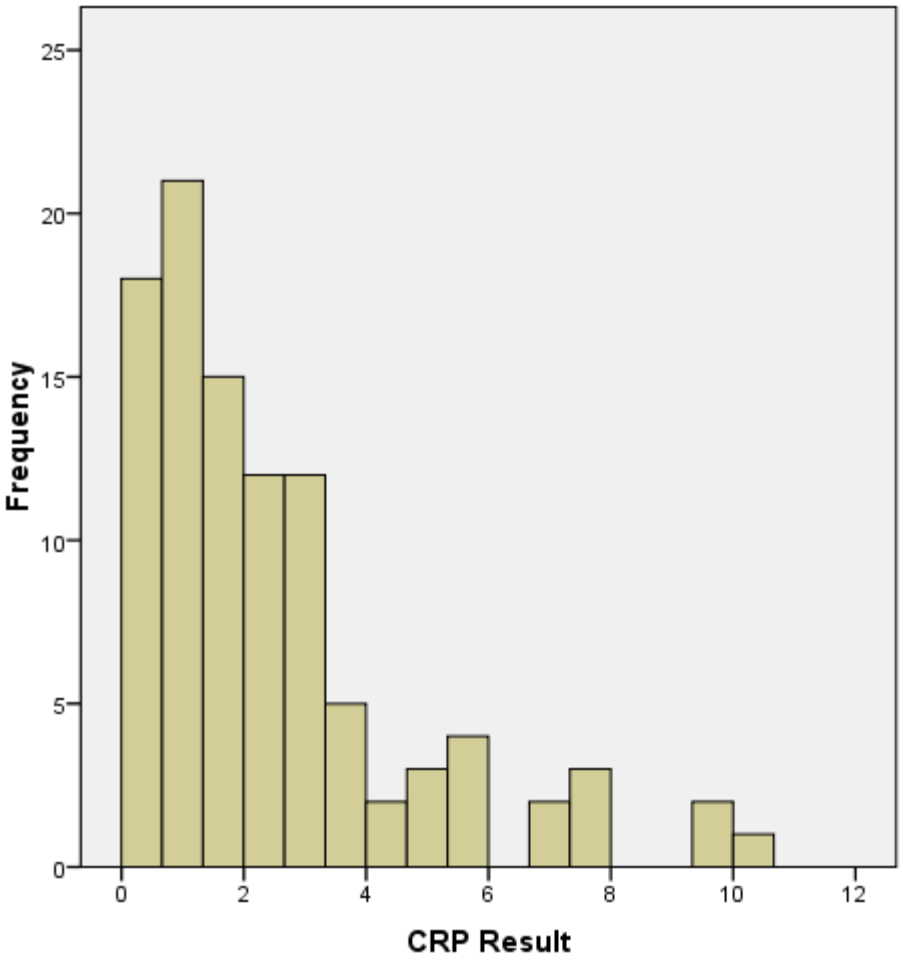

Fig 2: C- reactive protein result

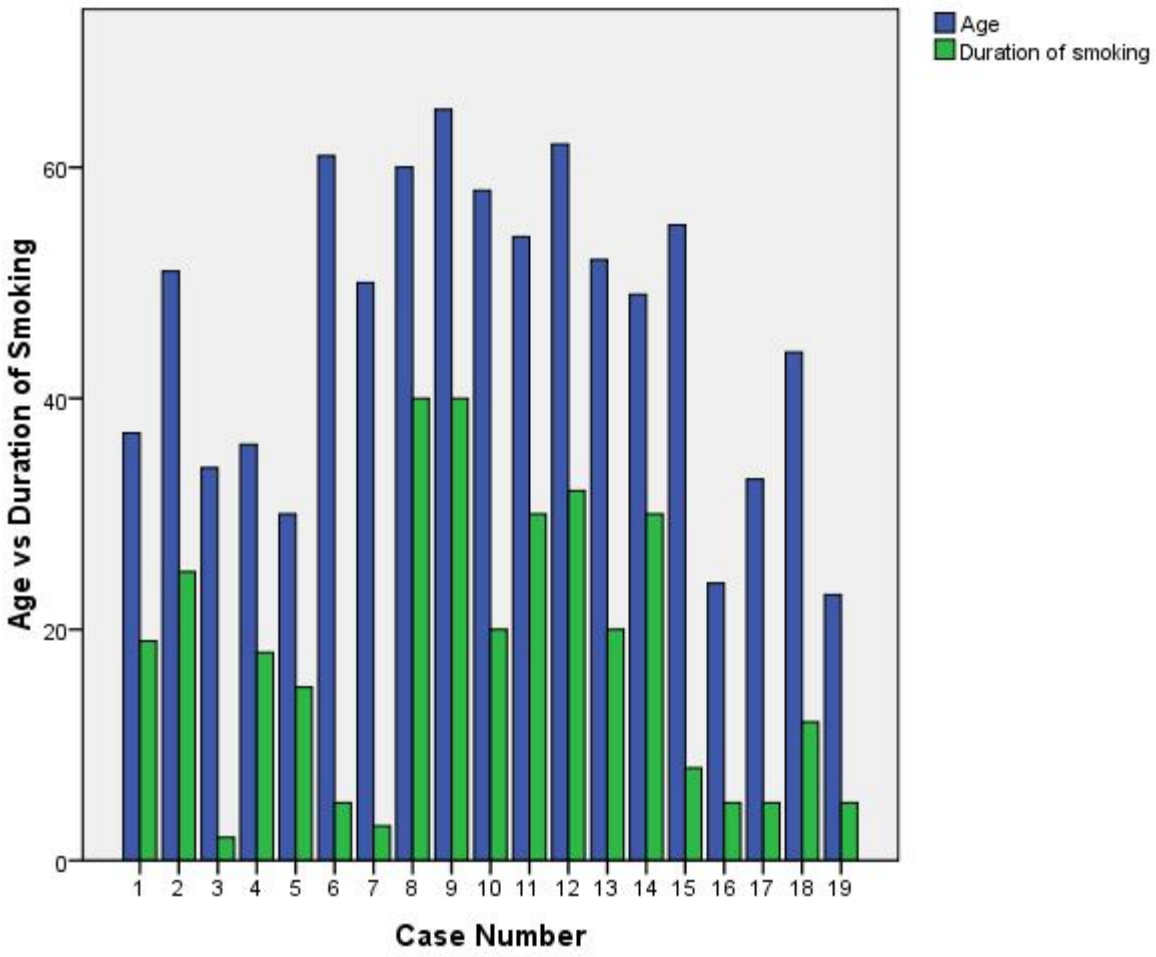

Fig 3: Age distribution and duration of smoking

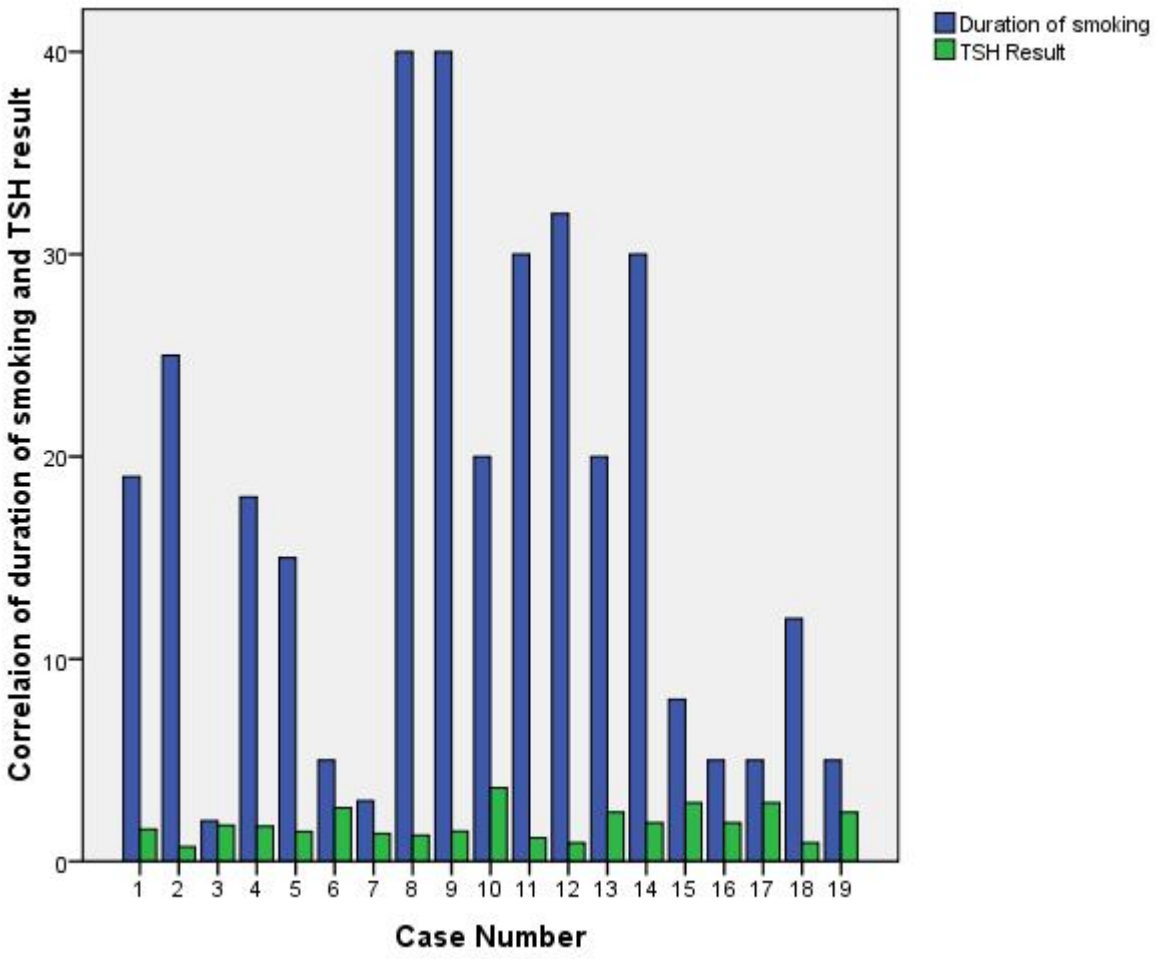

Fig 4: Duration of smoking and TSH result

Chapter Four

Discussion, Conclusions and Recommendations

4.1 Discussion:

Based on our findings from data analysis for 100 subjects included in this study these findings compared to other studies within this area our findings were significant for two reasons. Firstly there were so many studies that showed variable findings (Balhara , 2014; Karakaya, 1987).

Recent studies suggested that bias in reporting is inherent to all studies of smoking behavior; in fact, previous studies have concluded that self-reporting of smoking status is unreliable in many studies. A larger sample size is needed to confirm the current findings. Further, it would be of interest to determine the duration of tobacco-smoke-associated serum hormone concentrations related to active and passive smoking (Offie, 2009). We also rely on further studies involving fasting and ordinary subject's tobacco users. (Bjørn 2007; Fisher, 1997).

C-ReativeProtien (CRP) is an inflammatory marker in adults, little is known about the association between cigarette smoking and C-reactive protein (CRP) in adolescent smokers. Interestingly and based on our findings we found that CRPwas EXTEREMLY significant P= 0.0001 among tobacco chewers and both who were chewers and smokers (Tables 2 and 3). As opposed to other studies (Dietrich et al, 2007), (Ohsawa, 2005; O'Loughlin , 2008) and (Tonstad and Cowan, 2009), we found CRP to be reduced among chewers and chewers and smokers group, with its reduction may indicate that chewing may halt inflammatory response.

4.2. Conclusion:

Based on this study of thyroid hormones in Sudanese tobacco users in UAE, we may reach the following conclusions:

1- There was only a noticeable significance in those who smoke and use tobacco chewing.
2- Interestingly, CRP was significant among chewers and both chewers and smokers.

4.3. Recommendations

From the results of this study, it is recommended that:

1- Tobacco users is an important group and need further studies to monitor biochemical changes especially hormonal and immune-biochemical findings

2- The study highlighted significance in CRP that interestingly requires further studies and research in immune-biochemical findings.

3- Overall monitoring of thyroid hormones, and CRP with other supporting findings in tobacco users is crucial

4- This study requires further expansion with regard to duration, number of subjects and life style of tobacco users.

5- Further studies are required for more significant results.

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