Role of Pcsk9 in the Diagnosis Thyroid Disorder in Patient Women

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ABSTRACT

This study for the diagnosis of thyroid using pcsk9. The current study was conducted on seventy women patients suffering from thyroid disease attending in Center for diabetes and Endocrinology Unit in each of Al-Sadder Teaching Hospital and AL FURAT AL AWSAT Hospital in Al-Najaf province/ Iraq, and during the period from September until December 2017. The sample patients were divided into four study groups: premenopausal hypothyroidism patients group (20), postmenopausal hypothyroidism (15), premenopausal hyperthyroidism patients groups (17), and postmenopausal hyperthyroidism (18). The control group composed of 18 healthy women, also divided into premenopausal control and postmenopausal control. The results indicated a significant increase ($p < 0.05$) in pcsk9 in hypothyroidism patients compared with the control group, while a significant decrease ($p < 0.05$) in pcsk9 in hyperthyroidism patients compared with the control group. The results indicated a significant decrease ($p < 0.05$) in pcsk9 in premenopausal hyperthyroidism patients compared with the control group, and a significant decrease ($p < 0.05$) in pcsk9 in postmenopausal hyperthyroidism patients compared with the control group. The results indicated a significant increase ($p < 0.05$) in pcsk9 in premenopausal hypothyroidism patients compared with the control group, and a significant increase ($p < 0.05$) in pcsk9 in the postmenopausal hypothyroidism patients compared with the control group, also a significant increase in pcsk9 in postmenopausal than premenopausal.

Keywords: Pcsk9, Thyroid, Menopause

Article Information

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INTRODUCTION

Thyroid gland is one of the biggest endocrine glands in the human body, it lies in neck under larynx and anterior to trachea. It secretes two types of hormones Triiodothyronine (T3) and Thyroxine (T4) which are responsible of regulating metabolic processes in body, therefore any defect either in hypo- or hyperthyroidism leads to many problems associated with metabolism (Guyton and Hall, 2016). Hyperthyroidism, which means thyroid gland, is over active and produces additional hormone than normal, this occurs due to several causes such as immunologic state and thyroid tumor (Barrett *et al.*, 2016).

Hypothyroidism, which means thyroid gland is under active, is classified to two types primary which is caused by either congenital or acquired such as destruction the gland by autoimmune diseases and secondary hypothyroidism which results from causes lie outside thyroid gland such as defect in secrete Thyroid stimulating hormone

(TSH) from pituitary gland or defect in hypothalamus (Almandoz and Gharib., 2012 ; Caturegli *et al.*, 2014). There are many testes achieved in laboratory to investigate any disorder associated with thyroid gland such as quantitate measure of TSH, T3 and T4. In addition, many markers are used to diagnosis and prognosis thyroid disorders, such as, proprotein convertase subtilisin/kexin type 9 (PCSK9) (Kwakernaak *et al.*, 2013b; Walker and Lawrence, 2017).

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is one of nine mammalian serine proteases aptly named due to its relation to the bacterial subtilisin and yeast kexin family, it was discovered in 2003 (Seidah *et al.*, 2003). In human, PCSK9 is abundantly expresses in liver, but it is also present to a lesser extent in the small intestine, kidney, and central nervous system (Cohen *et al.*, 2005). This enzyme has an important role in lipid homeostasis due to it regulates the action Low Density

Lipoprotein Receptor (LDLR).Pcsk9 promotes the degradation of the low-density lipoprotein receptor (LDLR) in hepatocytes and increases plasma LDL cholesterol (LDL-C) therefore PCSK9 could play a role in the development of dyslipidemia associated with the metabolic syndrome(Weinreich and Frishman ,2014).

Aim of study:

The present study aims at to determining the levels of some rare proteins in the serum for future use for the purpose of diagnosis of thyroid diseases.

Review of literature

Thyroid gland

Anatomy and histology

The thyroid gland is one of the largest of the endocrine glands, which lies in neck under Adams apple on each side and anterior to the trachea, normally weighing 15 to 20 grams in adults (Guyton and Hall, 2016). The thyroid gland contains two main cell type follicular cell that secretes two major hormones, thyroxine (T4) and triiodothyronine (T3) and parafollicular cell or C cells that secrete calcitonin (Kratzsch and Pulzer , 2008). The normal thyroid is made up of 2 lobes joined by a thin band of tissue, the isthmus, which is approximately 0.5 cm thick, 2 cm wide, and 1 to 2 cm high. The individual lobes normally have a pointed superior pole and a poorly defined blunt inferior pole that merges medially with the isthmus, each lobe is approximately 2.0 to 2.5 cm in thickness and width at its largest diameter and is approximately 4.0 cm in length, (Roher and Schulte, 2007). The thyroid gland is composed of great numbers of closed follicles (100 to 300 micrometers in diameter) (fig 2-1) that are filled in a secretory substance called colloid and lining with cuboidal epithelial cells that secrete into the interior of the follicles (Nicholson *et al.*, 2006). Fig(1)

Figure (1): anatomy and histology of thyroid gland adapted by (Mathur *et al.* ,2007).

Thyroid hormones

Thyroxine (T4)

About 93 % of the metabolically active hormones secreted by the thyroid gland is T4; though; almost totally, the T4 is eventually convert to T3 in the tissues.T4 formed from condensation 2 of DIT by coupling reaction. On entering blood most T4 is combined with several of plasma protein such as thyroid binding globulin(TBG) and less albumins and small amount remain free ,so half-life are long(6_7 days),because high affinity of plasma binding protein for T4. T4 is released to tissue slowly (Bogazzi *et al.*, 2012).

Triiodothyronine (T3)

About 7 % of the metabolically active hormones secreted by the thyroid gland is T3. It is present in the blood in much smaller quantities and persists for a much shorter time compared with T4 (Gardner and shoback., 2011).

The actions of T3 occur about four times as rapidly as those of T4, with a latent period as short 6 - 12 hours and maximal cellular activity occurring within 2 to 3 days. Half of T3 because of its lower affinity to plasma binding protein is released to the cells in about 1 day (Melmed *et al.*, 2016).

Calcitonin

An additional hormone produced by the thyroid gland contributes to the regulation of blood calcium levels. Parafollicular cells produce calcitonin in response to hypercalcemia; Calcitonin stimulates movement of calcium into bone, in opposition to the effects of parathyroid hormone (PTH) (Pritzer *et al.*, 2015).

Thyroid disorders

Dysfunction in thyroid can result from three factors: (1) alterations in the circulating levels of thyroid Dysfunction hormones, (2) impaired metabolism of TH in the periphery, and (3) resistance to TH actions at the tissue level. The clinical state resulting from a change in thyroid function is classified as either **hypothyroidism** (few thyroid function) or **hyperthyroidism** (much thyroid function) (Demers and Spencer., 2003).

Hypothyroidism

Little production of thyroid hormone is the main feature of the medical state called hypothyroidism (Vaidya and Pearce., 2008; Almandoz and Gharib., 2012). The hypothyroidism classified into primary and secondary hypothyroidism, if permanent loss or damage of the thyroid, through processes such as autoimmune damage or irradiation injury, was described as primary hypothyroidism (Caturegli *et al.*, 2014), whereas if deficient stimulation of the normal gland, as a result hypothalamic or pituitary

disease or defects in the TSH molecule or cause outside gland itself, it is called central or secondary hypothyroidism (Persani, 2012).

2.3.1.2: Secondary hypothyroidism

Central hypothyroidism is due to TSH deficiency caused by either acquired or congenitally hypothalamic or pituitary gland disorders. The causes of TSH deficiency may be classified as those of pituitary (secondary hypothyroidism) and hypothalamic (tertiary hypothyroidism) origins, but this distinction is not necessary in the initial separation of primary from central hypothyroidism (Melmed *et al.*, 2016).

Hypothyroidism due to central causes is less severe than primary hypothyroidism. The causes of central hypothyroidism are both acquired and congenital. Congenital weaknesses in either the stimulation or the synthesis of TSH or in its structure have been identified as rare causes of congenital hypothyroidism (Gruters and Krude, 2012).

Biochemical markers

Proprotein convertase subtilisin kexin type 9

Proprotein convertase subtilisin/kexin type 9 (pcsk9) and prior (alternative name) known as neural apoptosis-regulated convertase, PCSK9 was discovered in 2003 (Abifadel *et al.*, 2003). PCSK9 is one of the nine mammalian serine proteases and named because of their relation to the bacterial subtilisin and yeast kexin family (Seidah *et al.*, 2003).

In human, PCSK9 gene is present on chromosome 1, it is abundantly expressed in liver, but it is also present to a lesser amount in the small intestine, kidney, and central nervous system (Cohen *et al.*, 2005).

Structure and function of PCSK9

PCSK9 is a 692 amino acid (aa) protein with a molecular weight of 72 kDa that contains a signaling peptide (aa 1–30), prodomain (aa 31–152), catalytic domain (aa 153–404), hinge region (aa 405–454), and the C-terminal domain rich in cysteine and histidine (CHRD) (aa 452–692) (Denis *et al.*, 2012) (fig 2).

Figure (2): structure of pcsk9 adapted by (Horton *et al.*, 2007).

A mature form of PCSK9 consisting of 60 kDa and a 14 kDa N-terminal prodomain is created in the endoplasmic reticulum; this process requires autocatalytic cleavage at position FAQ152 (Seidah and Prat, 2007). The autocatalytic cleavage of PCSK9 is necessary for its maturation and secretion from the ER to the Golgi (Benjannet *et al.*, 2004). Even with the division of the original form of PCSK9, they are still connected by non-covalent binding between the prodomain and catalytic domain and a C-terminal (Hampton *et al.*, 2007). In this way, the prodomain allosterically blocks the action of the other two domains; experimental elimination of the prodomain results in a ten times higher affinity of PCSK9 for LDLR and a four times higher rate of degradation of the LDLR (Lagace *et al.*, 2006; Kwon *et al.*, 2008).

The prosegment-PCSK9 complex then leaves the ER and enters the secretory pathway for release of mature PCSK9, even with its inactive state, PCSK9 binds to LDL-Rs and promotes their lysosomal degradation, thereby preventing receptor from recycling back to the cell surface (Park *et al.*, 2004; McNutt *et al.*, 2007).

Although not enzymatically active, the catalytic domain of secreted PCSK9 links with LDL-R at the hepatocyte plasma membrane by interacting with the epidermal growth factor precursor homology domain-A (EGF-A) of LDL-R (Zhang *et al.*, 2007).

The PCSK9/LDL-R complex then enters the endosomal pathway and, in contrast to the LDL-R/LDL complex, does not separate at low pH in the endosome, likely because of the enhanced affinity of PCSK9 for EGF-A resulting from conformational changes in the LDL-R (Kwon *et al.*, 2008; Yamamoto *et al.*, 2011).

So, the LDL-R does not recycle to the cell surface; rather, the PCSK9/LDL-R complex is directed to the lysosomal compartment for degradation of both PCSK9 and LDL-R (Benjannet *et al.*, 2004) (fig 3).

The interaction of PCSK9 with LDL-R can also happen within the hepatocyte, in which case the prosegment-PCSK9/LDL-R is directed from the Golgi to the lysosome for degradation before LDL-R transport to the cell surface (Maxwell *et al.*, 2005).

Even though the molecular mechanism mediating PCSK9-induced LDL-R degradation is not totally understood, new studies suggest that PCSK9 may mediate transport of LDL-R to the lysosome via interactions

with amyloid precursor-like protein 2,47,48 a protein previously implicated in the transport of transmembrane proteins to lysosomes (DeVay *et al.*, 2013, DeVay *et al.*, 2015).

Figure (3): A-Degradation of LDLR by PCSK9, B-LDL-C clearance and recycling of LDLR, adapted by (Ghosh *et al.*, 2015).

Plasma concentration of PCSK9

The plasma concentration of PCSK9 follows a diurnal rhythm similar to cholesterol synthesis with an increased plasma concentration in the morning and a minor concentration in the afternoon (Persson *et al.*, 2010, Cariou *et al.*, 2013).

The plasma PCSK9 concentration is higher in female compared to male and the PCSK9 concentrations decrease with age in males, but increase in females (Baass *et al.*, 2009, Lakoski *et al.*, 2009).

DISCUSSION

Pcsk9

The current study revealed significant differences in pcsk9 between hypo and hyperthyroidism compared with control, significantly increased ($p < 0.05$) in pcsk9 in hypothyroidism compared with control group, while a significant decrease ($p < 0.05$) in pcsk9 in hyperthyroidism compared with control group.

Many studies found serum PCSK9 levels change in patients with thyroid disorder. The level of PCSK9 increases in patients with hypothyroidism by in contrast with control group (Ozkan *et al.*, 2015) while decrease in patients with hyperthyroidism by in contrast with control group (Bonde *et al.*, 2014), and because PCSK9 have effect to increase the level of plasma lipid profile (Dedecjus *et al.*, 2003, Pearce *et al.*, 2008), therefore found positively correlation between hypothyroidism patients with this marker while negatively correlation with hyperthyroidism (Duntas and Brenta, 2012) and this agreement with current study.

In addition, the study of (Ozkan *et al.*, 2015) indicate PCSK9 levels were positively correlate with TSH and negatively with FT3 and FT4. The correlations we observed between PCSK9 and TSH, FT3 and FT4 support the idea that thyroid hormones affect PCSK9 levels. This study in accordance with current study.

Current study also agree with study done by (Kwakernaak *et al.*, 2013) which show determined decline in thyroid function, as indicated by high-normal TSH levels, may confer increase PCSK9 plasma level.

Comparison of pcsk9 in hyperthyroidism

The current study revealed significant differences in pcsk9 in hyperthyroidism between pre and postmenopausal compared with control, significantly reduction ($p < 0.05$) in pcsk9 in premenopausal in contrast with control groups, while the level of pcsk9 in postmenopausal patients with hyperthyroidism significant decrease ($p < 0.05$) in contrast with control group.

The study was done (Bonde *et al.*, 2014) agreement with this current study which found that serum PCSK9 levels were reduce in hyperthyroidism.

PCSK9 regulates hepatic LDLR numbers by disrupting their intracellular recycling, and high plasma PCSK9 levels are thus linked to high LDL-cholesterol and vice versa (Lambert *et al.*, 2012; Tavori *et al.*, 2013). In hyperthyroidism, serum PCSK9 levels were 22% reduced. There were clear positive correlations between PCSK9 and plasma total cholesterol and LDL-cholesterol in hyperthyroidism, as predicted from previous data

(Lakoski et al., 2009; Chernogubova et al., 2012), the change in PCSK9 levels in response to TH is compatible with a substantial reduction of LDL cholesterol. Thus, in addition to transcriptional stimulation of the LDLR gene, the reduced PCSK9 level should contribute substantially to increase the number of hepatic LDLRs in hyperthyroidism. The study of (Cui et al., 2010; Chernogubova et al., 2012) has shown that PCSK9 levels correlate to age and gender, it is increase with age in a healthy population, and it is higher in females than in males. Levels of pcsk9 are greater in postmenopausal women than pre-menopausal women, pcsk9 correlate with age only in women but not in men. As a mentioned above, there are found difference in pcsk9 in female between pre- and postmenopausal when compared with control groups in hyperthyroidism, in both states it is decrease in pcsk9.

Comparison of pcsk9 in hypothyroidism

The results of figure (4.6) revealed significant differences in pcsk9 in hypothyroidism between pre and postmenopausal compared with control, significantly increase ($p < 0.05$) in pcsk9 in premenopausal in contrast with control groups, while the level of pcsk9 in postmenopausal patients with hypothyroidism significant increase ($p < 0.05$) in contrast with control group Also significant increase in pcsk9 in postmenopausal than premenopausal.

The study of (Ozkan et al., 2015) indicates increasing PCSK9 levels in patients with hypothyroidism in contrast with control group. In current study, pcsk9 increases in both states premenopausal and postmenopausal but the study of (Moumita et al., 2015) shows pcsk9 is 22% higher in postmenopausal than premenopausal.

Pcsk9 levels in healthy have variation, and age and gender has an effect (influence) on pcsk9 (Chernogubova et al., 2012).

The study of (Moumita et al., 2015) indicate that PCSK9 level rise in female after menopause, and the Variation in the endogenous estrogen levels during menstrual cycle can contributes to the interindividual difference in LDL-C and pcsk9 in the normal females. In postmenopausal there were lower estrogen levels compared with premenopausal women. The study of (Persson et al., 2009; Person et al., 2012) shows there are inversely correlation between pcsk9 and estrogen, as mentioned pcsk9 increases in postmenopausal than premenopausal and this is accordance with the present study.

CONCLUSION

Conclude from the current study:

PCSK9 is closely related to lipid homeostasis, so with the determination of the levels of fat in the body

can be confirmed diagnosis lipid Disruption of the thyroid diseases.

Recommendations

1. PCSK9 as routine test in standard laboratories for diagnosis of thyroid disorder rather than classical tests.
2. Use of these proteins in another study involving laboratory animals for the purpose of confirming the results of this study.
3. Other tests include other types of proteins and another study in addition to the current proteins for the purpose of raising the level of diagnosis.

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