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Diagnosis of Thyroid Disorder for Women Using Some Biochemical Markers in Serum

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Abstract:
Background: thyroid disease are the most communal disorder that occur especially in women, and divided into hypo-and hyperthyroidism. Material and methods: seventy patients suffering from thyroid disorder are taken and divided into four study groups according to age and type of disorder. In present study was intended to asses serum levels of pcsk9,ORX-A in patients of hypothyroidism and hyperthyroidism using pcsk9 ELISA kit(A Catalog No: E-EL-H1579 and ORX-A ELISA kit(A Catalog No: E-EL-H1015).The results showed that pcsk9 increase in patients of hypothyroidism and decrease in patients of hyperthyroidism ,also ORX-A increase in patients of hypothyroidism and decrease in patients of hyperthyroidism.

Key words: PCSK9, ORX-A

INTRODUCTION

Thyroid gland one of the biggest endocrine glands in the human body, it lie in neck under Adams apple and anterior to trachea. It secret two types of hormones Triiodothyronine(T3) and Thyroxine(T4) which responsible on regulate metabolic processes in body, therefore any defect either hypo- or hyperthyroidism lead to many problems associated with metabolism [1].

Hyperthyroidism, which mean thyroid gland, is over active and produce additional hormone than normal, occur due to several causes such as immunologic state and thyroid tumor [2]. Hypothyroidism, which mean thyroid gland is under active, classified to two types primary which caused by either congenital or acquired such as destruction the gland by autoimmune diseases and secondary hypothyroidism which result from causes lie outside thyroid gland such as defect in secret Thyroid stimulating hormone (TSH) from pituitary gland or defect in hypothalamus [3,4].

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 used to diagnosis and prognosis thyroid disorders, such as, Proprotein convertase subtilisin kexin type nine and Orexin-a [5].

Proprotein converters subtilisin kexin kind 9 was one of nine serine proteases termed because bacterial subtlisin and families yeast kexin, it discovered in 2003 [6].In human, PCSK9 is rich expresses in liver, but it is also present in small amounts in intestine, central nervous system and kidneys [7]. This enzyme have role important in lipid homeostasis due to it regulate the action Low Density Lipoprotein Receptor (LDLR). It helps the degradation of the low-densities lipoproteins receptor (LDLR) in liver cell and rises LDL cholesterol (LDL-C) therefore; PCSK 9 can show a role in the progress of dyslipidemia linked with the metabolic disorder [8].

Orexin-A, also called as hypocretin-1 is one of neuropeptides which consist of 33 amino acid that produce in hypothalamus, at first detected in the brain of rats at 1998. Deficient in Orexin-A lead to narcolepsy (make the people sleep more than normal) [9,10].Orexin-A produced in the lateral hypothalamus are hypothalamic peptides involved in food intake, metabolic rate, growth hormone production, autonomic function, and the sleep/wake cycle [11,12].

MATERIALS AL METHODS

Healthy groups and Patient

Seventy women patient are separated in four group study: premenopausal hypothyroidism patients group 20 and postmenopausal hypothyroidism 15 and premenopausal hyperthyroidism patients groups 17 and postmenopausal hyperthyroidism 18, the control group was composed of 18 healthy women, also divided into premenopausal control and postmenopausal control. The samples were collected from the Center for diabetes and Endocrinology Unit in Teaching Hospital al-Sadder in al-Najaf /iraq, and AL FURAT AL AWSAT Hospital in al-Najaf /iraq and through a period from September until December 2017. The ages of patients and control ranged 18-77 years.

All persons women were expose to asker around age**,** menstrual cycle, disease history, family histories, take therapy**,** then blood samples and were send for testing.

Female have disease such as (disease heart, acute infection, rheumatoid disease, kidney disease, anemie (Hb<10% g/dl), and ages less than 15 years were excluded.

Inclusion criteria: The patients were diagnosed by serologic test using laboratory test such as (T3, T4, TSH) using new devices (minividus).patients show positive results when the test of (T4, T3 and TSH) out of normal value. Patient that show increase in T3, T4 and decrease in TSH are labeled as hyperthyroidism, and patient that show decrease in T3, T4 and increase in TSH are labeled as hypothyroidism and this depending on tests that conducted to the patient and symptoms.

RESULTS

The results of figure (1) revealed significant differences in orexin-a between hypo and hyperthyroidism compared with control, significantly increased ($p<0.05$) in orexin-a in hypothyroidism in contrast to control groups, while the level of orexin-a in patients with hyperthyroidism significant decrease ($p<0.05$) in contrast with control group.

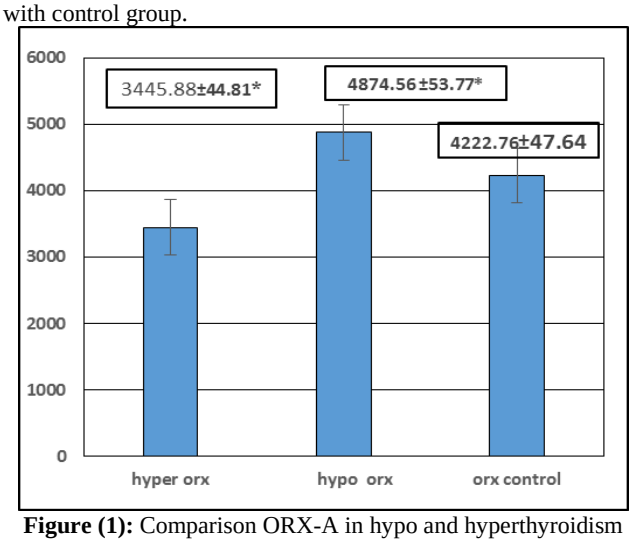

Figure (1): Comparison ORX-A in hypo and hyperthyroidism compared with control group.

Comparison of orexin-a in hyperthyroidism between pre and postmenopausal compared with control groups.

The results of figure (2) revealed significant differences in orexin-a in hyperthyroidism between pre and postmenopausal compared

with control, significant decrease (p< 0.05) In orexin-a in premenopausal in contrast with health groups, and significantly decrease (p< 0.05) In orexin-a In postmenopausal in contrast with health groups**.**

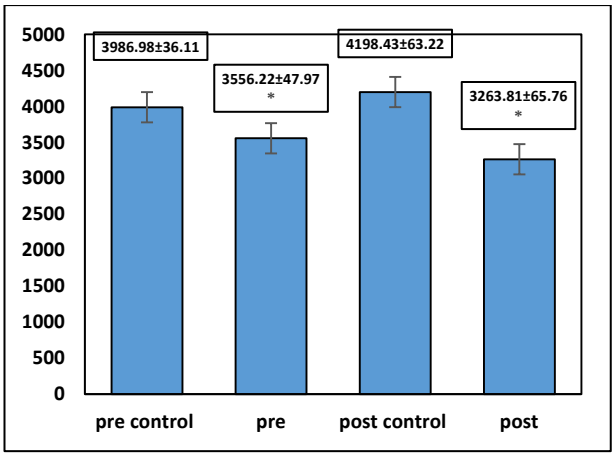

Figure (2): Comparison of ORX-a in hyperthyroidism between pre and postmenopausal compared with control group**.**

Comparison of orexin-a in hypothyroidism between the pre and postmenopausal matched with health groups.

The results of figure (3) revealed significant differences in orexin-a in hypothyroidism between the pre and the postmenopausal in contrast with the health, significantly increase (p<0.05) in orexin-a in premenopausal in contrast with the health groups, and significant increase (p< 0.05) In orexin-a in postmenopausal in contrast with the health groups in addition significantly increase In orexin-a in postmenopausal than premenopausal

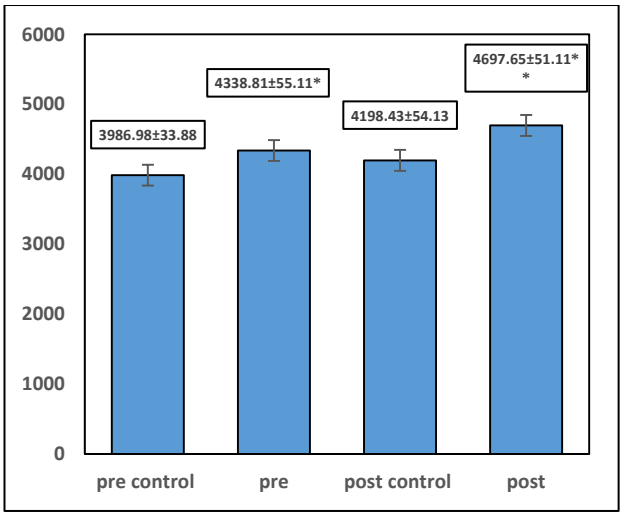

Figure (3): Comparison of ORX-a in hypothyroidism stuck between pre and postmenopausal compared with control group.

Comparison of pcsk9 in hypo and hyperthyroidism compared with control groups.

The results of figure (4) revealed significant differences in pcsk9 between hypo and hyperthyroidism compared with control, significantly increased (p<0.05) in pcsk9 in hypothyroidism matched with control groups, whereas an significantly decline (p< 0.05) In pcsk9 in hyperthyroidism in contrast with control group**.**

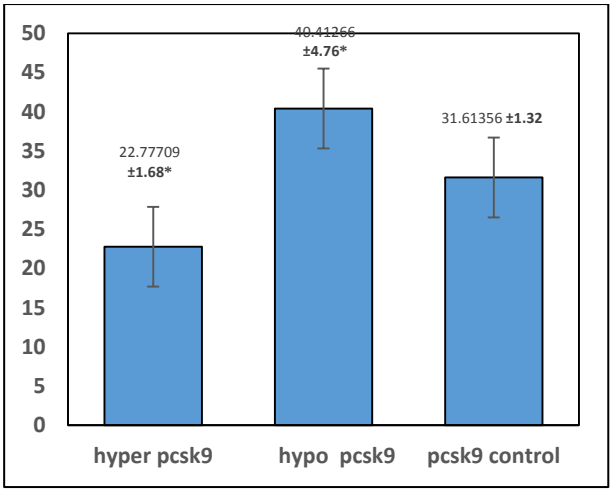

Figure (4): Comparison of pcsk9 in hypo and hyperthyroidism compared with control group.

Comparison of pcsk9 in hyperthyroidism between pre and postmenopausal compared with control groups.

The results of figure (5) revealed significant differences in pcsk9 in hyperthyroidism between pre and postmenopausal compared with control, significant reduction (p< 0.05) In pcsk9 in the premenopausal in contrast with the health groups, and the a significant reduction (p< 0.05) In pcsk9 In postmenopausal in contrast with the health groups**.**

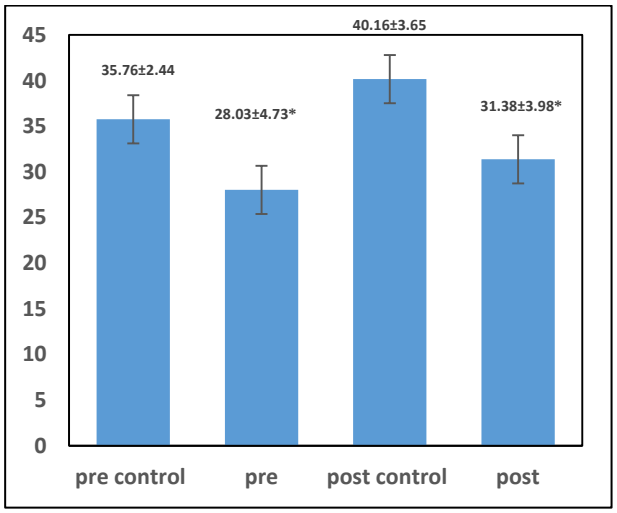

Figure (5): Comparison of pcsk9 in hyperthyroidism between the pre and postmenopausal compared with control group.

Comparison of pcsk9 in hypothyroidism between the pre and postmenopausal matched with the health groups.

The results of figure (6) revealed significant differences in pcsk9 in hypothyroidism between the premenopausal and the a postmenopausal related with a health, significantly increase (p<0.05) in pcsk9 in premenopausal related with a health groups, and the significantly rise (p< 0.05) In pcsk9 in postmenopausal in contrast with the control groups**,** also the significantly rise In pcsk9 in postmenopausal than premenopausal**.**

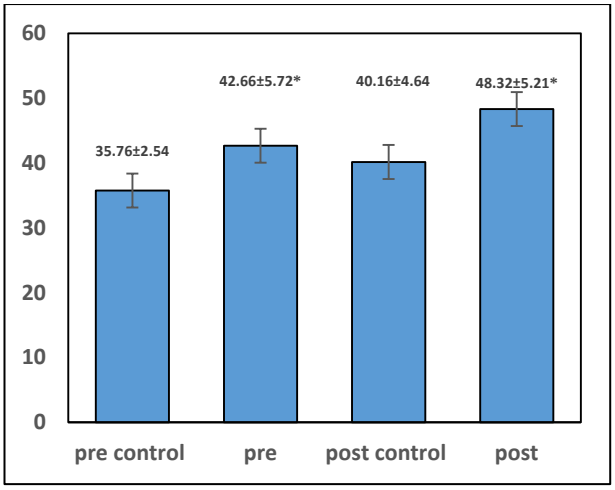

Figure (6): Comparison of pcsk9 in hypothyroidism between the pre and postmenopausal compared with control group.

Comparison of lipid profiles between the hypo and hyperthyroidism compared with control group.

The consequences of table (1) shown the significant dissimilarities in a lipid between the hypo and hyperthyroidism in contrast with health group, significantly improved ($p< 0.05$) In hypothyroidism in the serum cholesterols, triglyceride ,VLDL,LDL and HDL compare with the health groups whereas the significant decline ($p< 0.05$) In hyperthyroidism in cholesterol and triglyceride, VLDL, LDL and HDL compared with control groups.

Table (1): Comparison of the serum levels lipid profile between Hypo and Hyperthyroidism compared with control

Lipid profiles	Means ± S.E.		
	Control group	Hypo group	Hyper group
Cholesterol mg/dl	177±21.33	257±54.91 *	130±32.11*
TG mg/dl	121±14.2	358±47.6 *	110±28.53 *
VLDL-C mg/dl	26.2±1.24	71.6±4.33 *	24.2±2.51
HDL-C mg/dl	46±4.55	56±12 *	30±34 *
LDL-C mg/dl	55±1.83	188±8.92 *	50±5.66*

Correlation between biomarkers and lipid profile

Correlation between cholesterol and biomarker

Consequences of the association and a linear regression between the cholesterol and the biomarker concentrations revealed:

1- figure (9) showed, there are an significant positive relationship (r = 0.906) between orexin-a and the cholesterol concentration.

2- figure (10) indicated, there is an significantly positive relationship (r = 0.913) between the pcsk9 and the cholesterol level.

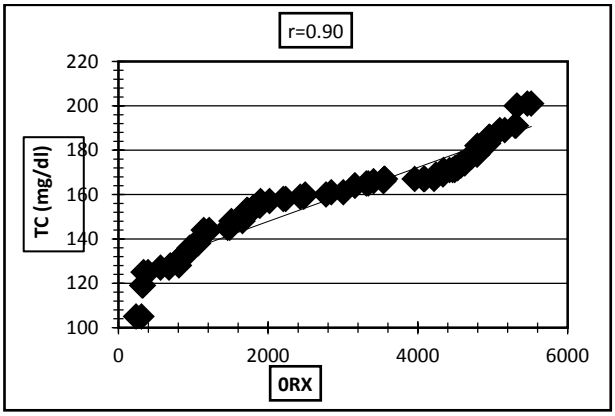

Figure (9): Correlation between serum cholesterols and the serum ORX-A

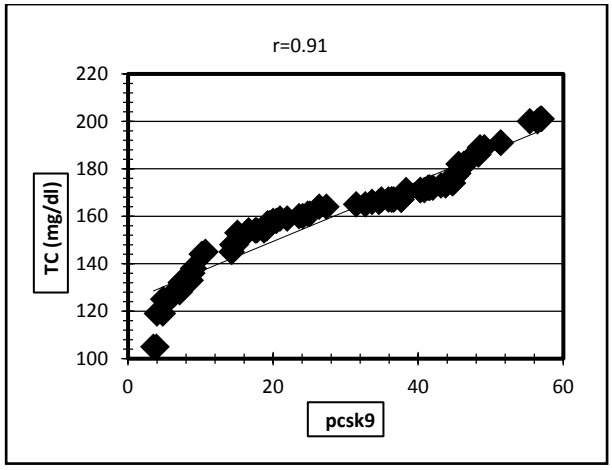

Figure (10): Correlation between serum cholesterols and the serum pcsk9

Correlation between the triglyceride and biomarkers

Consequences of the association and linear regression amongst the triglyceride and the biomarker concentrations revealed:

1- Figure (11) showed, there are an significantly positive relationship (r = 0.69) between the orexin-a and the TG concentrations.

2- Figure (12) showed, there are an significantly positive relationship (r = 0.74) between the pcsk9 and the TG level.

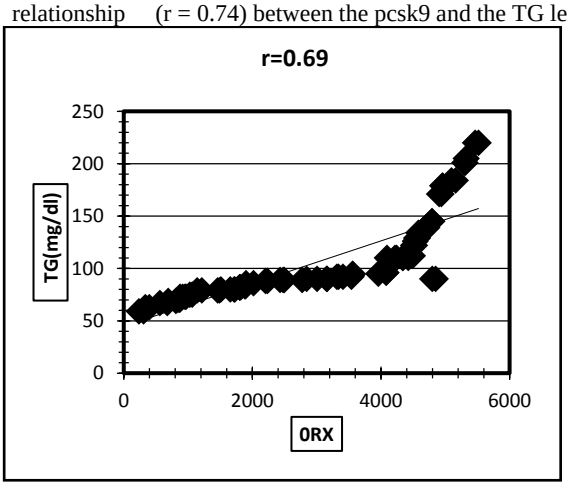

Figure (11): Correlation between serum triglyceride and serum ORX-A

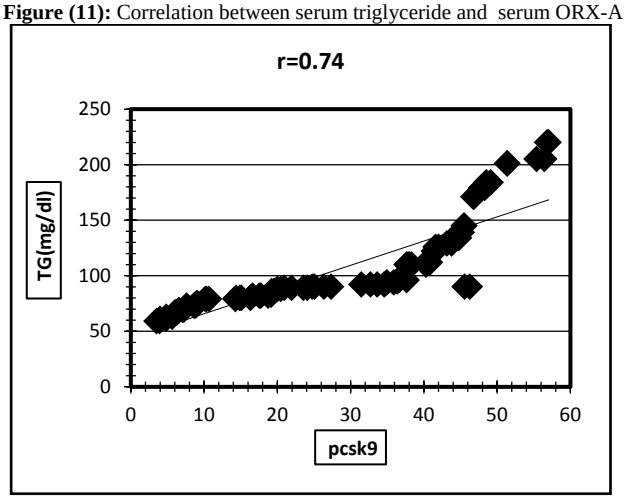

Figure (12): Correlation between serum triglyceride and serum pcsk9

Correlation between the VLDL and a biomarkers

Consequences of relationship and linear regresion amid very low-density lipoprotein and the biomarkers concentrations revealed

1-Figure (13) showed, there are an significantly positive relationship
(r = 0.74) between the orexin-a and the VLDL concentrations.
2-Figure (14) showed, there are an significantly relationship (r = 0.79) between the pcsk9 and the VLDL levels**.**

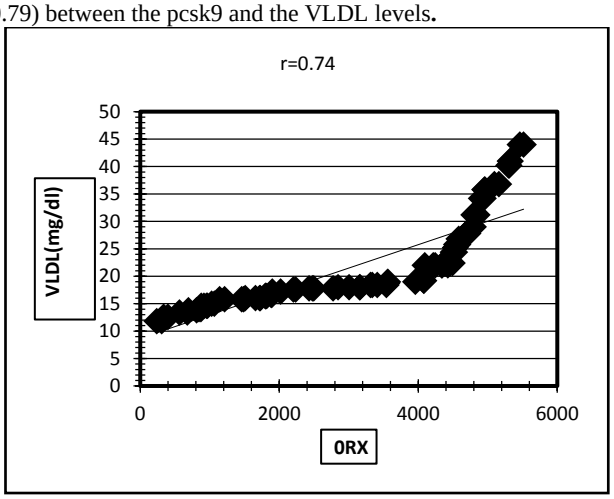

Figure (13): Correlation between serum VLDL and serum ORX-A

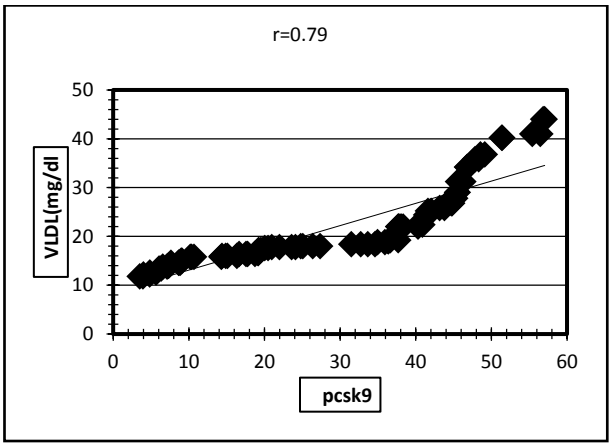

Figure (14): Correlation between serum VLDL and serum pcsk9

Correlation between the HDL and biomarkers

Consequences of relationship and linear regresion amongst the high-density lipoprotein and the biomarkers concentrations revealed:
1-Figure (15) showed, there are an significantly positive relationship (r = 0.97) between the orexin-a and the HDL concentrations.
2-Figure (16) showed, there are an significantly positive relationship (r = 0.98) between the pcsk9 and the HDL levels.

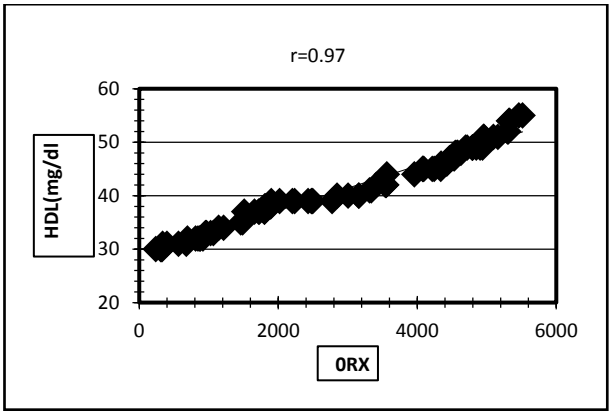

Figure (15): Correlation between serum HDL and serum orexin-a

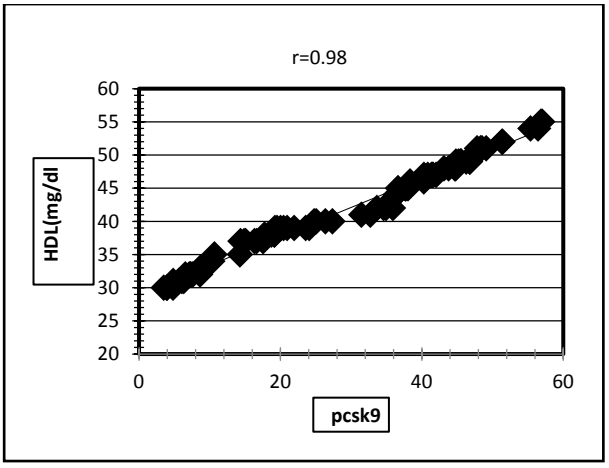

Figure (16): Correlation between serum HDL and serum PCSK9

Correlation between LDL and biomarkers

Consequences of relationship and linear regresion amongst the low-density lipoprotein and the biomarkers concentrations exposed**:**

1-Figure (17) showed, there are an significantly positive relationship (r = 0.91) among the orexin-a and the LDL concentrations.
Figure (18) showed, there are an significantly positive relationship (r = 0.90) among the pcsk9 and the LDL levels**.**

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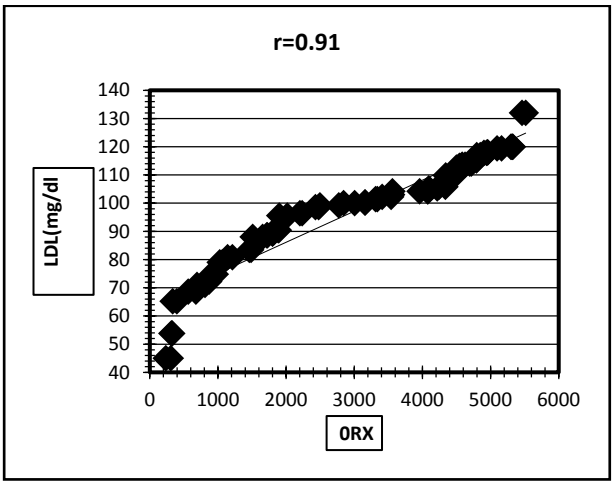

Figure (17): Correlation between serum LDL and serum orexin-a

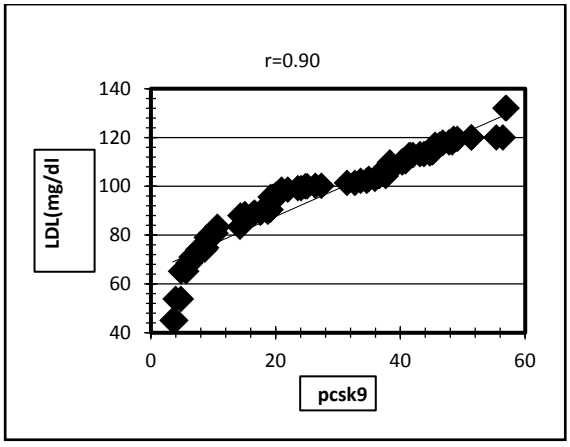

Figure (18): Correlation between serum LDL and serum pcsk9

DISCUSSION

The current study revealed significant differences in orexin-a between hypothyroidism and hyperthyroidism when compared with control group, significantly increased ($p<0.05$) in orexin-a in hypothyroidism compared with control group, while the level of orexin-a in patients with hyperthyroidism significant decrease ($p<0.05$) in contrast with control group.

The study was done by Tohma *et al*.,(2015) agreement with this current study which found that the ORX-A level was decrease in patients group with hyperthyroidism when contrast with control group. And also this study found ORX-A (an orexigenic peptide) in patients with hyperthyroidism has lower level and after euthyroidism the level of ORX-A was increase, and there level become similar to which found in health (control) group [13]. In addition, this study was found strong relationship between decrease level of ORX-A and predictive diagnosis for hyperthyroidism, and this agreement with current study.

There is no past study take the direct relationship between hypothyroidism and Level of ORX-A, but many of study(Messina *et al*., 2015c) take the relationship between the BMR(Basal metabolic rate) and hypothyroidism, and found BMR increase in patients with hyperthyroidism[14].

And decrease level of BMR in patients with hypothyroidism (Melmed *et al*., 2012) (163). Another study was done by Tohma *et al*., (2015) was found negative correlation between BMR and ORX-A. And more than this, this study found negative correlation between level of ORX-A and Ft3 and FT4 and in same time have positive correlation between ORX-A and TSH [15].

From all past studies can be conclude that the level of ORX-A increase in patients with hypothyroidism patients and this agreement with current study.

This current study agreement with study done by El-Sedeek *et al*., (2010) found negative relationship between ORX-A and level of estrogen, this meaning the level of ORX-A in postmenopausal patients higher than pre-menopausal women(estrogen Have inhibitory effect on ORX-A) [16].

Also there are study found, the women who take HRT(postmenopausal hormone-replacement therapy)or ERT (estrogen replacement therapy) have lower level of ORX-A in contrast with women who not receiving HRT or ERT.

Also there is another study found the postmenopausal women who don't receive ERT have higher plasma cholesterol and triglyceride more than postmenopausal women who receive ERT, and in general the post-menopausal women have higher plasma level of TG and cholesterol than pre-menopausal(Taylor and Samson, 2003) , and this study agreement with current study that found ORX-A higher in post-menopausal women than pre-menopausal women (estrogen decrease after postmenopausal) [17].

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

There are many studies found serum PCSK9 levels change in patients with thyroid disorder .The level of PSCK9 increase in patients with hypothyroidism by compared with control group[18].while decrease in patients with hyperthyroidism by compared with control group [19].and because PSCK9 have effect to increase the level of plasma lipid profile [20,21].,therefore found positively correlation between hypothyroidism patients with this marker while negatively correlation with hyperthyroidism [2]. 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 [18].

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[23].

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 [19]. The study of (Lakoski *et al*., 2009; Cui *et al* ., 2010; Chernogubova *et al*., 2012) have shown that PCSK9 levels correlate to age and gender, it is increase with age in a healthy population, and it is higher in females then in males. Plasma levels of pcsk9 are higher in postmenopausal women than pre-menopausal women, pcsk9 correlate with age only in women but not in men [24,25,26].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 (Bonde *et al*., 2014) and this is in accordance with current study[19].

The results of figure (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) indicate we found increased PCSK9 levels in patients with hypothyroidism in contrast with control. In current study, pcsk9 increase in both state premenopausal and postmenopausal but the study of (Moumita *et al*., 2015) show pcsk9 were 22% higher in postmenopausal than premenopausal [18,27].

Pcsk9 levels in healthy has variation, and age and gender can effect (influence) on pcsk9 [26].

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) show there are inversely correlation between pcsk9 and estrogen, as mentioned pcsk9 increase in postmenopausal than premenopausal and this is accordance with study [27,28,29].

The current study revealed a significant positive correlation between pcsk9 and, LDL-C, TG, VLDL, TC and HDL.

PCSK9 was synthesize in liver and secrete into the plasma. Pcsk9 act by combine to the LDL receptor (LDLR) on a cell surface and chaperones the LDLR in the direction of the lysosome compartment for degradation. Thus, this action of PCSK9 declines the number of LDLR, which is available to be recycled back to the cell surface to remove LDL from the plasma. Decreased LDLR results in increased a plasma LDL level [30].Deficiency of LDL-R accounts for the increased plasma concentrations of LDL and VLDL [31].also the study of (Chernogubova *et al*., 2012) show positive relationship between circulating pcsk9 levels and LDL concentration and provided further evidence for association between pcsk9 levels and plasma triglyceride[27].

The current study revealed significant positive association between ORX and TC, HDL, LDL-C, TG, and VLDL.

The study of (Skrzypski *et al*., 2011) show OXA can stimulates accumulation of lipid in an isolated adipocytes [32].An anabolic activity of the OXA are based on a phosphorinositide 3-kinase

(PI3K) - and peroxisome proliferator-activated receptor γ (PPARγ)- dependent that could inhibition of lipolysis and stimulation of lipogenesis. OXA PI3K-dependently elevated translocation membrane of Glucose transporter 4 (GLUT4) and an active uptake of the glucose, which are then converted to triacylglycerol. Activation of PI3K are know to enhancing accumulation of lipid in 3T3-L1 adipocytes. Agreement with this, we demonstrate that PI3K inhibition can prevent OXA-induced triacylglycerol accumulation. Earlier work demonstrated that OXA stimulates the proliferation of the 3T3-L1 pre-adipocytes [33].The differentiation of adipocytes critically depend upon PPARγ. Increased expression of PPARγ2 stimulates proliferation of a adipocytes and lipid accumulation. Isoform of PPARγ2 are restricted to and highly abundant in adipocytes. OXA increased PPARγ2 protein level in a adipocytes [34], possibly representing the mechanism of lipogenic activity of OXA. Evidence supporting the role of PPARγ in OXA stimulated triacylglycerol synthesis.

CONCLUSION

Conclude from the current study:

1. Orexin-A and PCSK9 play an important role inconfirming thyroid diseases diagnosis.
2. 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.
3. Orexin-A is closely associated with appetite for food as well as its relationship with the sleep/wake cycle, so this hormone is important in the diagnosis of disorders of metabolism and sleep disorder in the thyroid diseases.

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