Al-Azhar Med. J. *Vol. 45(2), April 2016, 435-448*
DOI: 10.12816/0029141/ORG. No. 91235 ***Issues_title_English:*** 2016 Vol.45 Issue 2

SERUM LIPOCALIN-2 PREDICTS CARDIOVASCULAR MORBIDITY RISKS IN SUBCLINICAL HYPOTHYROID RAT MODEL

By

Nadine A. Raafat and Sama S. Khalil

Physiology Department, Faculty of Medicine, Zagazig University

ABSTRACT

Background: Subclinical hypothyroidism (SCH) and its associations with cardiovascular disease (CVD) remain controversial**.** Previous studies revealed that lipocalin -2 (LCN2) associated with increased cardiovascular mortality risk.

Objective: Evaluation of the serum level of LCN2 and its association with some cardiovascular risk factors in SCH rat model.

Material and methods: Twenty four adult male albino rats weighing 150-170 g were used and divided into two equal groups: control euthyroid group and SCH-induced group where SCH was induced by administration of methimazole (MMI) (10 mg/kg body weight) by gavage once daily for 3 months. Groups were compared for body mass index (BMI), serum thyroid-stimulating hormone (TSH), triiodothyronine (T3) thyroxin (T4), glucose, insulin, lipid profiles, C reactive protein (CRP), LCN2 , Homeostasis model assessment of insulin resistance (HOMA-IR), atherogenic index ,systolic (SBP) and diastolic blood pressure (DBP).

Results: SCH induced group showed significantly higher mean serum levels of TSH, LCN2, insulin, total cholesterol, low density lipoprotein - cholesterol (LDL- C), triglycerides (TG), CRP than that of control. Also, BMI, calculated HOMA, atherogenic index, SBP and DBP were significantly higher than that of control and all these parameters were positively correlated to LCN2. However, high density lipoproteins-cholesterol (HDL- c) levels were significantly lower than that of control group and negatively correlated with serum LCN2 levels.

Conclusion: Serum lipocalin-2 may be associated and can predict cardiovascular risk factors in SCH.

Key words: Lipocalin-2, subclinical hypothyroidism, cardiovascular disease, atherogenic index, calculated HOMA-IR, rats.

INTRODUCTION

Subclinical hypothyroidism (SCH) is precursor to overt hypothyroidism in which serum TSH levels is elevated while T3 and T4 levels are normal **(Cooper, 2001 and Karthick et al., 2013**). SCH has been associated with metabolic syndrome, hypertension, dyslipidemia **(Cinar et al., 2011)**.

Several studies reported that, SCH has been implicated in development of atherosclerosis **(Ochs et al., 2008; Valentina et al., 2011 and Billic-Komarica et al., 2012)** and coronary endothelial dysfunction **(Biondi et al., 2009 and Gao et al., 2015**), leading to an increased e4risk of CVD **(Meena et al., 2012).**Although other studies have denied that relation **(Cappola et al., 2006 and Biondi & Cooper, 2008).**

LCN2 cytokine primarily known as a protein of human neutrophils granules (NAGL), was reported to be closely associated with metabolic syndrome, impaired lipid profile, hyper-insulinemia, hyperglycemia, insulin resistance and obesity among humans and mice **(Wang et al., 2007)**.

NGAL up-regulation has been described in several conditions usually associated with hypertension, such as endothelial dysfunction, atherosclerosis and inflammatory vascular damage (**Paulsson et al., 2007 and Giaginis et al., 2010). Leoncini et al. (2011)** showed that increased NGAL is significantly associated with increase left ventricular mass in primary hypertensive patients. Elevated LCN2 levels were found in patients with coronary heart disease **(Choi et al., 2008)** and positively correlated with it and with metabolic syndrome in a Chinese cohort **(Ni et al., 2013)**.

Circulating NGAL is a significant predictor of CVD mortality in older adults **(Daniels et al., 2012)** and in chronic heart failure **(van Deursen et al., 2014).** Moreover, higher urinary (but not serum) NGAL concentrations are associated with increased cardiovascular mortality risk **(Helmersson-Karlqvist et al**., **2013**). Interestingly, NGAL (serum and urine) levels have been increased in acute **(Alvelos et al., 2013)** and chronic heart failure patients **(Damman et al., 2011)**. Recently, serum NGAL is associated with cardiovascular fatal and non-fatal events in patients with chronic kidney disease, independently of renal function and inflammation **(Solak et al., 2015)**.

This work aimed to measure serum levels of LCN2 in SCH rat model and its association with some cardiovascular risk factors.

MATERIAL AND METHODS

Animals: Twenty four adult male albino rats weighting 150-170 g obtained from the animal house of the faculty of veterinary medicine of Zagazig University for use in these experiments. All rats were housed individually in separate steel wire cages (4-6 rats/ cage) at comfortable temperature (20-24?C) and were maintained on a 12h light/dark cycle. Also, were provided free access to food and water and accommodated to animal house conditions for 2 weeks before the experiments took place. The experimental protocol was proved by local medical ethics committee in faculty of medicine of Zagazig University (Institutional Review Board, IRB).

The animals were randomly divided into two equal groups (n=12):

Group I: Control euothyroid rats: by administration of physiological saline for 3 months by gavage once daily.

Group II: **sub clinical hypothyroid-induced rats**: induction of SCH was done by administration of (10 mg/kg body weight) methimazole (MMI) by gavage once daily for 3 months (MMI obtained by sigma and dissolved in physiological saline) **(Gao et al., 2015).**

After 45 days, a rat model of SCH was established, but we continued the methim-azole administration and concurrently assessed the TSH, T3 and T4 levels (**Gao et al., 2015**) to evaluate the long term effects of SCH on cardiovascular risk factors.

At end of 3 months, all animals were weighted and their lengths were measured, BMI was estimated by the equation; body weight (g)/length^2 (cm^2) =BMI (g/cm^2) (**Novelli et al., 2007).**

Measurement of Blood Pressure (BP) according to **Zorniak et al.** (**2010) and Parasuraman & Raveendran (2012)**; an overnight fasted (8–10 h) each rat was anesthetized with urethane (1200 mg/kg), and placed on a suitable rodent none electrically conductive surgical table. The skin on the ventral side of the neck is shaved and disinfected. The skin was carefully cut open (1.5–2 cm), and a slit incision was made in the platysma muscles. The trachea was identified, small incision was made on the cartilage tissue, and the tracheostomy was performed using a small piece of tracheal intubation tube**.** One side of the carotid artery was separated from the adjacent connective tissue, and its cephalic end was tied and the cardiac end was clamped with a bulldog clamp and cannulated using a heparinized cannula (0.5 IU/ml in saline). The other end of the cannula connected to a three-way stopcock connected to the pressure transducer and a syringe filled with heparinized saline. Then the carotid artery cannulation site was tied with a thread without obstructing the blood flow in the carotid cannula. Then bulldog clamp was released slowly, ensuring that there was no bleeding at the cannulation site**.**

After cannulation, the animal is connected to the Power Lab **(AD Instruments Pty Ltd, Australia)** to record the BP. The pressure cuff of the sphygmomanometer was connected to the pressure transducer. Then, the pressure transducer is checked by inflating to a known pressure level. The calibration between the voltage (millivolts) and the pressure (mmHg), and the results are automatically calculated by power Lab software.

Blood sampling: Blood samples were taken from the cannula after measuring BP and were allowed to clot for 2 hours at room temperature before centrifuging for 20 minutes at approximately 500 rpm. The separated serum was stored at -20° C.

Serum analysis: the following serum levels were measured:

LCN2 levels according to **Goetz et al. (2002)** by using enzyme immunoassay test kit catalog: ELR-LCN2.

TSH levels according to **Engall (1980**) by using rat ELISA kits (Shanghai Sunred Biological Technology Co., Ltd).

T3 according to **Agharanya (1990)** by using rat ELISA kits (Shanghai Sunred Biological Technology Co., Ltd).

T4 according to **Schuurs and Van Weeman (1977)** by using rat ELISA kits (Shanghai Sunred Biological Technology Co., Ltd).

TC levels according to **Tietz (1995**) by using total cholesterol kits estimation (BioSource Europe S.A).

HDL-c levels according to **Nauk et al. (1997**) by using kits for HDL-cholesterol (BioSource Europe S.A).

LDL-c calculated according to **Friedewald et al. (1972),** LDL=TC-HDL-TG/5.

TG levels according to **Naito (1989)** using triglycerides ESPAS SL kits A (Elttech S.A., Lyon, France).

CRP levels according to **Ridker et al. (1998**) using CRP Kits (Monobind Inc Lake Forest, Ca 92630, USA).

Insulin levels according to **Temple et al. (1992)** using INS-EASIA, KAP1251 (BioSource Europe S.A).

Glucose levels according to **Tietz (1995)** using glucose enzymatic (GOD-PAP)-liquizyme rat Kits (Biotechnology, Egypt).

Calculation of HOMA-IR according to the equation of **Sun et al. (2007)** modification on **Matthews et al. (1985)** [HOMA-IR = insulin (?U/mL) x glucose (mg/dL) /405].

Calculation of atherogenic index (AI) according to the equation: Atherogenic index = (total cholesterol-HDL - cholesterol)/HDL-cholesterol **(Karthik and Ravikumar, 2011)**.

Statistical analysis: Data were presented as mean ± SDM. Statistical significance between groups was determined by unpaired t-test; Pearson correlations were performed to determine the correlations between LCN2 and all other parameters. P value of less than 0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS 20 software (SPSS Inc., Chicago, IL, USA).

RESULTS

As in **table (1)**: SCH rats showed significant increase in mean serum TSH and LCN2 levels ($p<0.001$, $p<0.01$ respectively) than that of control. However, the groups had no significant differences in terms of T3 and T4 ($p>0.05$). TSH levels were positively correlated to LCN2 in SCH rats ($r=0.72$, $p<0.01$).

BMI, insulin levels and HOMA-IR were significantly elevated in SCH rats when compared with control ($p<0.001$, $p<0.01$ and $p<0.01$ respectively). Moreover, a positive correlation was observed for LCN2 with those parameters {($r=0.69$, $p<0.05$); ($r=0.73$, $p<0.01$) and ($r=0.708$, $p<0.05$)} respectively, while glucose levels increased but insignificantly ($p>0.05$) in SCH compared with control and not correlated with LCN2 ($r=0.28$, $p>0.05$).

Regarding lipid profiles, CRP levels and AI in SCH rats, there were high significant levels of total cholesterol ($p<0.001$), LDL-C ($p<0.01$), TG ($p<0.001$), CRP ($p<0.001$) and AI ($p<0.001$) which were significantly positive correlated to LCN2 ($r=0.76$; $r=0.72$; $r=0.73$; $r=0.85$, and $r=0.72$, respectively ($p<0.01$ in all parameters). However, HDL-cholesterol levels were significantly lower than that of control group ($P<0.05$) and negatively correlated with serum LCN2 levels ($r=-0.071$, $p<0.01$).

Concerning blood pressure, as shown in **figures (1 and 2)** systolic BP and diastolic BP were significantly elevated in SCH group in comparison with control group ($P<0.01$) and both positively correlated with serum LCN2 levels ($r=0.68$; $r=0.65$, $P<0.05$ respectively).

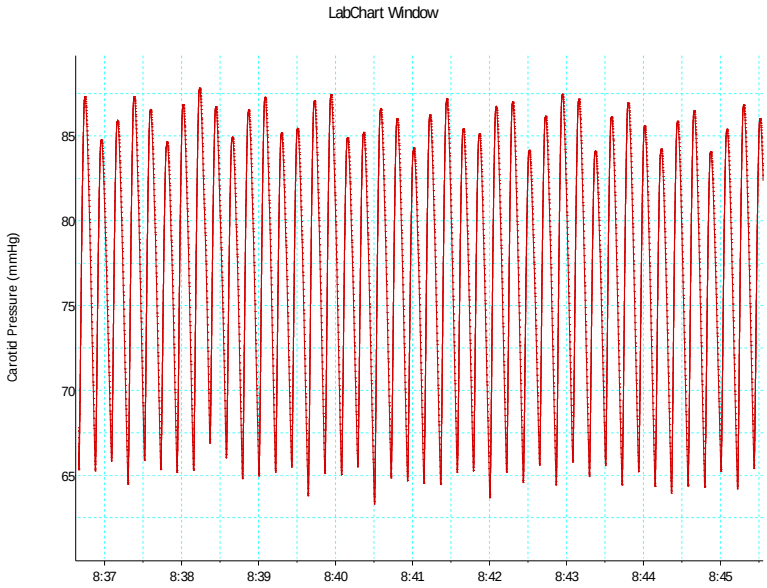

Figure (1): Blood pressure of rat from control group.

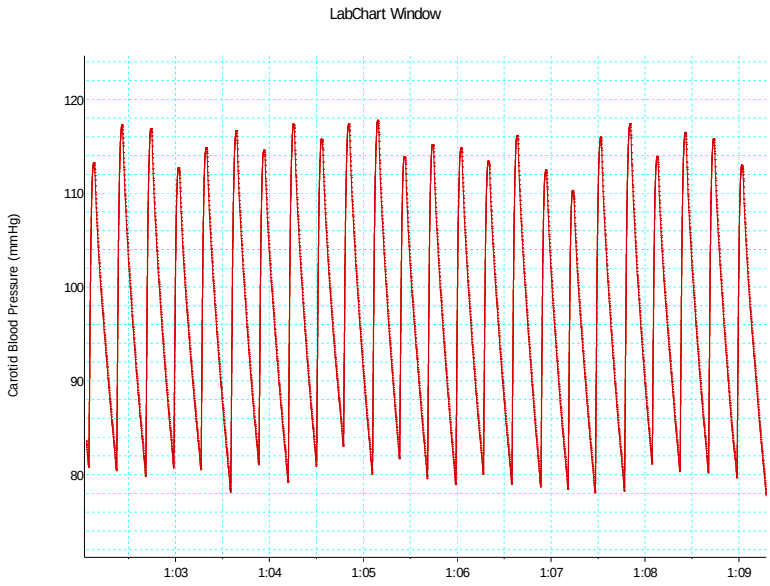

Figure (2): Blood pressure of rat from the SCH group.

Table (1): Serum TSH, T3, T4, LCN2, lipid profile, CRP, glucose, insulin, calculated HOMA-IR, AI, BMI, SBP and DBP in both groups.

Groups / Parameters	Control		SCH		
	mean ±SD	r	mean ± SD	P value	r
Lcn2 (ng/ml)	56.62±10.35		79.9±17.12	<0.01	
T3 (ng / mL)	1.45±0.28	0.34	1.38±0.42	0.63	-0.041
T4 (ug / dL)	6.41±1.7	0.37	5.8 ± 1.65	0.38	-0.087
TSH (uIU/ mL)	0.0044± 0.0007	0.18	0.0081± 0.0001	<0.001	0.72* <0.01
Glucose (mg/dL)	81.2±6.34	-0.29	84.69±7.76	0.24	-0.28
Insulin (MIU/mL)	16.75±2.34	-0.07	21.08±3.93	<0.01	0.73* <0.01
HOMA-IR	3.35±0.54	-0.24	4.4±0.94	<0.01	0.708* <0.05
TG (mg/dl)	86.57±12.6	0.35	113.93 ±16.13	<0.001	0.73* <0.01
Total cholesterol (mg/dL)	105.3±5.54	0.27	173.78±17.02	<0.001	0.72* <0.01
LDL-C (mg/dl)	62.9±7.4	0.017	79.12±12.6	<0.01	0.76* <0.01
HDL-C (mg/dL)	51.9±6.5	0.39	45.22±7.59	<0.05	- 0.71* <0.01
Atherogenic index (AI)	1.05±0.27	-0.28	2.92±0.65	<0.001	0.72* <0.01
CRP (Ug/Ml)	0.87±0.23	0.034	2.05±0.49	<0.001	0.85* <0.01
SBP (mmHg)	92.83±5.52	-0.26	106.7±12.5	<0.01	0.68* <0.05
DBP (mmHg)	64.5±9.8	-0.36	79±9.12	<0.01	0.65* <0.05
BMI (g/cm^2)	0.65±0.08	-0.08	0.81±0.07	<0.001	0.69* <0.05

r = correlation with L

DISCUSSION

SCH rat model in current study was confirmed by the significant increase in serum TSH levels, while there was no significant difference in serum levels of both T3 and T4.

Our results had revealed a significant increase in serum LCN2 levels in SCH rats as compared with those of euothyroid rats. Moreover, LCN2 levels were positively correlated with TSH levels in same group.

Our findings of insulin resistance (as measured by HOMA-IR) in SCH rats can be attributed to impaired translocation of GLUT-4 transporters on cell surface of adipose tissue and muscle **(Maratou et al., 2009)**. Similarly, **Tuzcu et al. (2005)** and **Vyakaranam et al. (2014)** found insulin resistance and a positive correlation between TSH and insulin in SCH.

Interestingly, LCN2 levels were positively correlated with BMI, insulin levels and HOMA index. This is in accordance with **Wang et al. (2007)** who suggested that this adipokine might be an independent risk factor for hyperglycemia and insulin resistance in humans. Serum lipocalin-2 levels showed significant positive correlations with BMI in SCH patients **(Zorlu et al., 2014).**

Concerning lipid profile, our findings came in agreement with **Canaris et al. (2000)** who showed that total cholesterol levels were higher in subjects with subclinical hypothyroidism Also, **Danese et al. (2000)** found that lipid profile was improved with levothyroxine treatment. Furthermore, **Owen et al. (2006)** and **Gao et al. (2015**) reported high total cholesterol and LDL-c levels in SCH female patients and SCH rats model, respectively.

Interestingly, even with a mild elevation of serum TSH, SCH is associated with atherogenic lipid profiles in postmenopausal women independent of thyroid hormones **(Geng et al., 2015)**.

Recently**, Brenta at al. (2016**) showed that women with SCH have higher remnant-like lipoproteins, small dense LDL levels and reduced hepatic lipase activity, which was improved significantly following levothyroxine therapy.

Also, in accordance with our results, **Choi et al. (2008)** showed that serum LCN2 levels have been positively correlated with serum TG and negatively correlated with serum HDL-c indicating that the related pathogenic mechanism of atherosclerosis may involve disruption of lipid metabolism.

On the other hand, the LCN2 levels were negatively associated with HDL cholesterol **(Ni et al., 2013)**.

Accordingly; we found that atherogenic index (AI) was significantly higher in SCH rats than that of control group, where there was also a positive correlation between serum LCN2 levels and AI in SCH group which was not detected in control group.

SCH represents an independent risk factor for coronary heart disease **(Walsh et al., 2005)** and may be associated with increases in carotid intima-media thickness and carotid plaque formation **(Valentina et al ., 2011 and Gao et al., 2013).** Moreover**, Gao et al. (2015**) demonstrated that SCH rats displayed an increased serum endothelin level and a decreased vasodilator nitric oxide level in the SCH rats.

Conversely, **Delitala et al., (2015)** showed no association between SCH and intima-media thickness or carotid atherosclerotic plaque. Also, **Schultz et al. (2011)** failed to find any association between SCH and CVD.

Galis and Khatri (2002) elucidated the pathogenic mechanism of LCN2 in atherosclerosis and CAD as it destabilizes the artery plaque and promote atheroscle-

rosis by degrading the vascular basement membrane, leads to increasing endothelial permeability , allowing more white blood cells and inflammatory cytokines to infiltrate the intima. And induced the conversion of macrophages to foam cells **(Oberoi et al., 2015).**

Interestingly, NGAL is predominantly expressed in atherosclerotic plaques of mice with myocardial infarction **(Hemdahl et al., 2006)** .This indicated that LCN2 could be involved in creating the local and systemic pro-inflammatory environment characteristic for atherosclerosis. Moreover, LCN2 may be used as a prognostic marker to determine the status of CAD progression **(Oberoi et al., 2015).**

Concerning serum levels of CRP, our results came in contact with results of many reports **(Christ-Crain et al., 2003; Guldiken et al., 2008 and Belen, 2015)** who showed that CRP levels were elevated in SCH when compared with the control group.

Interestingly, significant correlations between serum levels of LCN2 and serum CRP were showed in patients with metabolic syndrome **(Wang et al., 2007)** and acute coronary syndrome (**Singh et al., 2009 and Ni et al., 2013)**.

Results regarding blood pressure alterations in SCH are still controversial; in agree with us, **Cai et al. (2011)** has indicated that SCH is associated with increases in SBP and DBP, moreover a population-based study observed positive linear associations between TSH levels and both systolic and diastolic BP (**Asvold et al.,2007)**. Similarly, SCH is associated with higher systolic BP **(Belen, 2015 and Ye et al., 2014),** subclinical hypothyroidism and high normal TSH are independent risk factors for hypertension in middle-aged and elderly Chinese women **(Jian et al., 2013).**

However, a number of studies showed only increased diastolic blood pressure in SCH patients **(Saltiki et al., 2008; Anand et al., 2012 and Pesic et al., 2015),** others showed no association between SCH and increased BP **(Walsh et al., 2006 ; Iqbal et al,. 2007; Duan et al., 2009 and Amouzegar et al., 2016).**

In contrast to our results, **Gao et al. (2015)** showed decreased SBP and DBP in SCH rats. Or decreased only systolic BP (**Chen et al., 2013)**.

Correlation of LCN2 with systolic and diastolic BP in our study was also confirmed by **Sachan et al (2014)** in patients of hypertensive disorders of pregnancy and in children with nephrotic syndrome (**Gheissari et al., 2013).** In addition, **Aksan et al.(2015**) suggested that circulating NGAL concentration might be a useful marker in identifying hypertensive patients with higher risk for cardiovascular mortality .also**,** predicts major adverse cardiovascular events after cardiac care unit discharge **(Ito et al., 2016).**

Furthermore, NGAL level was positively correlated with severity of coronary artery disease in patients with non-ST elevation acute coronary syndrome **(Soylu et al., 2015)**.

On the other hand, our results differ from those of **Zorlu et al. (2014)** who showed no significant difference in lipocalin-2 levels in patients with subclinical hypothyroidism.

We attributed these controversial findings to spices difference and to heterogeneity of study groups as etiology of hypothyroidism in this study was due to drug induced, but others varied from autoimmune thyroiditis, postpartum thyroiditis to thyroid function insufficiency after radioiodine treatment or after thyroidectomy.

CONCLUSION

Serum lipocalin-2 may be associated and can predict cardiovascular risk factors in subclinical hypothyroid induced rats.

REFERENCES

1. **Agharanya JC (1990):** Clinical usefulness of ELISA technique in the assessment of thyroid function West Afr. J. Med., 9(4):258-63.
2. **Aksan G, Inci S, Nar G, Sigirci S, Gedikli O, Soylu K, Nar R, Yuksel S and Şahin M (2015)**: Serum neutroph?l gelat?nase-assoc?ated l?pocal?n levels in patients with non-dipper hypertension. Clin Invest Med., 8; 38(2):E53-62.
3. **Alvelos M, Lourenço P, Dias C, Amorim M, Rema J and Leite AB (2013):** Prognostic value of neutrophil gelatinase-associated lipocalin in acute heart failure. Int J Cardiol., 165(1):51–55.
4. **Amouzegar A, Heidari M, Gharibzadeh S, Mehran L, Tohidi M and Azizi F (2016):** The Association Between Blood Pressure and Normal Range Thyroid Function Tests in a Population Based Tehran Thyroid Study.Horm Metab Res., 48(3):151-6.
5. **Anand P, Sudharani D and Nagaraj DD (2012):** Assessment of the cardiovascular risk in subclinical hypothyroidism. Int J Pharm Biol Sci., 2(21):128-134.
6. **Asvold BO, Bjoro T, Nilsen TI and Vatten LJ (2007):** Association between blood pressure and serum thyroid-stimulating hormone concentration within the reference range: a population based study. J Clin Endocrinol Metab., 92(3):841-5.
7. **Belen E (2015):** Evaluation of arterial elastic parameters in patients with subclinical hypothyroidism. Turk Kardiyol Dern Ars., 43(8):678-83.
8. **Billic-Komarica E, Beciragic A and Junuzovic D (2012):** The Importance of HbA1c Control in Patients with Subclinical Hypothyroidism. Materia Socio-Medica, 24(4):212–9.
9. **Biondi B and Cooper DS (2008):** The clinical significance of subclinical thyroid dysfunction. Endocrine reviews, 29(1):76–131.
10. **Biondi B, Galderisi M, Pagano L, Sidiropulos M, Pulcrano M and D'Errico A (2009):** Endothelial-mediated coronary flow reserve in patients with mild thyroid hormone deficiency. European Journal of Endocrinology / European Federation of Endocrine Societies , 161(2):323–9.
11. **Brenta G, Berg G, Miksztowicz V, Lopez G, Lucero D, Faingold C, Murakami M, Machima T, Nakajima K and Schreier L (2016)**: Atherogenic Lipoproteins in Subclinical Hypothyroidism and Their Relationship with Hepatic Lipase Activity: Response to Replacement Treatment with Levothyroxine .Thyroid , 26(3):365-72.
12. **Cai Y, Ren Y and Shi J (2011)**: Blood pressure levels in patients with subclinical thyroid dysfunction: a meta-analysis of cross-sectional data. Hypertension research: official journal of the Japanese Society of Hypertension., 34(10):1098–105.
13. **Canaris G, Manowitz NR, Mayor G and Ridgway EC (2000):** The Colorado thyroid disease prevalence study. Arch. Int. Med., 160(4): 526–534.
14. **Cappola AR, Fried LP, Arnold AM, Danese MD, Kuller LH and Burke GL (2006):** Thyroid status, cardiovascular risk, and mortality in older adults. JAMA: The journal of the American Medical Association, 295(9): 1033–41.
15. **Chen X, Zhang N, Cai Y and Shi J (2013):** Evaluation of left ventricular diastolic function using tissue Doppler echocardiography and conventional doppler echocardiography in patients with subclinical hypothyroidism aged

<60 years: a meta-analysis. Journal of Cardiology, 61(1):8–15.

16. Choi KM, Lee JS, Kim EJ, Baik SH, Seo HS, Choi DS, Oh DJ and Park CG (2008): Implication of lipocalin-2 and visfatin levels in patients with coronary heart disease. Eur J Endocrinol , 158(2):203–207.

17. Christ-Crain M, Meier C, Guglielmetti M, Huber PR, Riesen W, Staub JJ and Müller B (2003): Elevated C-reactive protein and homocysteine values: cardiovascular risk factors in hypothyroidism? A cross-sectional and a double-blind, placebo-controlled trial. Atherosclerosis, 166(2):379 –386.

18. Cinar N, Ersʔz N, Aydƀn K, Akƀn S, Usman A and Gürlek A. (2011): Serum vaspin levels in hypothyroid patients. Eur J Endocrinol., 165(4):563–9.

19. Cooper DS (2001): Clinical practice. Subclinical hypothyroidism. N Engl J Med., 345 (4):260–265.

20. Damman K, Masson S, Hillege HL, Maggioni AP, Voors AA and Opasich C (2011): Clinical outcome of renal tubular damage in chronic heart failure. Eur Heart J., 32(21):2705–2712.

21. Danese MD, Landerson PW, Meinert CL and Powe NR (2000): Effect of thyroxine therapy on serum lipoproteins in patients with mild thyroid failure: a quantitative review of the literature. J. Clin. Endocrinol. Metab., 85(9): 2993–3001.

22. Daniels LB, Barrett-Connor E, Clopton P, Laughlin GA, Ix JH and Maisel AS (2012): Plasma neutrophil gelatinase-associated lipocalin is independently associated with cardiovascular disease and mortality in community-dwelling older adults: the Rancho Bernardo Study. J Am Coll Cardiol., 59(12):1101–1109.

23. Delitala AP, Filigheddu F, Orrù M, AlGhatrif M, Steri M, Pilia MG, Scuteri A, Lobina M, Piras MG, Delitala G, Lakatta EG, Schlessinger D and Cucca F (2015): No evidence of association between subclinical thyroid disorders and common carotid intima medial thickness or atherosclerotic plaque. Nutr Metab Cardiovasc Dis., 25(12):1104-10.

24. Duan Y, Peng W, Wang X, Tang W, Liu X, Xu S, Mao X, Feng S, Feng Y, Qin Y, Xu K and Liu C (2009): Community-based study of the association of subclinical thyroid dysfunction with blood pressure. Endocrine, 35(2): 136–142.

25. Engall, E (1980): Methods in Enzymology, Volume 70, Van Vunakis, H. and Langone, J. J. (eds.), Pbl. Academic Press, New York, PP. 419-492.

26. Friedwald WT, Levy RI, and Fredrickson DS (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

27. Galis ZS and Khatri JJ (2002): Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res., 90:251–262.

28. Gao C, Li T, Liu J, Guo Q and Tian L (2015): Endothelial Functioning and Hemodynamic Parameters in Rats with Subclinical Hypothyroid and the Effects of Thyroxine Replacement. PLoS One., 10(7): e0131776.

29. Gao N, Zhang W, Zhang YZ, Yang Q and Chen SH (2013): Carotid intima-media thickness in patients with subclinical hypothyroidism: a meta-analysis. Atherosclerosis, 227(1):18-25.

30. Geng H, Zhang X, Wang C, Zhao M, Yu C, Zhang B, Wang Y, Ban B and Zhao J (2015): Even mildly elevated TSH is associated with an atherogenic lipid profile in postmenopausal women with subclinical hypothyroidism. Endocr Res., 40(1):1-7.

31. Gheissari A, Rezaii Z, Merrikhi A, Madihi Y and Kelishadi R (2013): Association of neutrophil gelatinase associated lipocalin and cystatin-c with kidney function in children with nephrotic syndrome. Int J Prev Med.; 4(8):956-63.

32. Giaginis C, Zira A, Katsargyris A, Klonaris C and Theocharis S. (2010): Clinical implication of plasma neutrophil gelatinase-associated lipocalin (NGAL) concentrations in patients with advanced carotid atherosclerosis. Clin Chem Lab Med Parameters, 48(7):1035–41.

33. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN and Strong RK (2002): The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition, Mol Cell, 10(5): 1033-1043.

34. Guldiken S, Demir M, Arikan E, Azcan S, and Tugrul A (2008): Levels of High-Sensitivity C - reactive protein, Leptin, and Resistin in Patients With Overt Hypothyroidism and Subclinical Hypothyroidism. The Endocrinologist, 18(1): 30–33.

35. Helmersson-Karlqvist J, Larsson A, Carlsson AC, Venge P, Sundstr m J and Ingelsson E (2013): Urinary neutrophil gelatinase- associated lipocalin (NGAL) is associated with mortality in a community-based cohort of older Swedish men. Atherosclerosis, 227(2):408–413.

36. Hemdahl AL, Gabrielsen A, Zhu C, Eriksson P, Hedin U, Kastrup J, Thoren P and Hanssor GK (2006): Expression of neutrophil gelatinase associated lipocalin in atherosclerosis and myocardial infarction. Arteriosclerosis, Thrombosis, and Vascular Biology, 26(1):136–142.

37. Iqbal A, Schirmer H, Lunde P, Figenschau Y, Rasmussen K and Jorde R (2007): Thyroid stimulating hormone and left ventricular function. The Journal of Clinical Endocrinology and Metabolism, 92(9):3504–3510.

38. Ito M, Doi K, Takahashi M, Koyama K, Myojo M, Hosoya Y, Kiyosue A, Ando J, Noiri E, Yahagi N, Hirata Y and Komuro I (2016): Plasma neutrophil gelatinase-associated lipocalin predicts major adverse cardiovascular events after cardiac care unit discharge. J Cardiol., 67(2):184-91.

39. Jian WX, Jin J, Qin L, Fang WJ, Chen XR, Chen HB, Su Q and Xing HL (2013): Relationship between thyroid-stimulating hormone and blood pressure in the middle aged and elderly population. Singapore Med J., 54 (7):401-5.

40. Karthik D and Ravikumar S (2011): A Study on the Protective Effect of Cynodondactylon Leaves Extract in Diabetic Rats. Biomed Environ Sci., 24(2): 190-199.

41. Karthick N, Dillara K, Poornima KN and Subhasini AS (2013): Dyslipidaemic changes in women with subclinical hypothyroidism. Journal of clinical and diagnostic research: JCDR., 7(10):2122–2125.

42. Leoncini G, Mussap M, Viazzi F, Fravega M, Degrandi R, Bezante GP, Deferrari G and Pontremoli R (2011): Combined use of urinary neutrophil gelatinase-associated lipocalin (uNGAL) and albumin as markers of early cardiac damage in primary hypertension .Clin Chim Acta., 412(21-22):1951-6.

43. Maratou E, Hadjidakis D , Kollias A, Tsegka K, Peppa M, Alevizaki M, Mitrou P, Lambadiari V, Boutati E, Nikzas D, Tountas N, Economopoulos T, Raptis SA and Dimitriadis G. (2009): Studies of insulin resistance in patients with clinical and sub clinical hypothyroidism. Eur J Endocrinol., 160(5):785–790.

44. Matthews DR, Hosker JP, Rudenski AS, Naylor BA and Turner RC (1985): Homeo-stasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 28(7):412-9.

45. Meena CL, Meena RD, Nawal R, Meena VK, Bharti A and Meena LP (2012): Assessment of left ventricular diastolic dysfunction in sub-clinical hypothyroidism. Acta Informatica Medica, 20(4):218–20.

46. Naito HK (1989). Triglycerides in clinical chemistry: theory, analysis and correlation. Pbl. KaplanL A and Pesce A.J., U.S.A., PP. 997.

47. Nauk M, Marz W and Jarausch J (1997): Multicenter evaluation of homogenous assay for HDL-Cholesterol without sample pretreatment. Clin Chem., 43(9):1622-1629.

48. Ni J, Ma X, Zhou M, Pan X, Tang J, Hao Y, Lu Z, Gao M, Bao Y and Jia W (2013): Serum lipocalin-2 levels positively correlate with coronary artery disease and metabolic syndrome. Cardiovascular Diabetology, 12:176.

49. Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F, Fernandes AA, Cicogna AC and NovelliFilho JL (2007): Anthropometrical parameters and markers of obesity in rats., Lab Anim., 41(1):111-9.

50. Oberoi R, Bogalle EP, Matthes LA, Schuett H, Koch AK, Grote K, Schieffer B, Schuett J and Luchtefeld M (2015): Lipocalin (LCN) 2 Mediates Pro-Atherosclerotic Processes and Is Elevated in Patients with Coronary Artery Disease. PLoS One. 10(9):e0137924.

51. Ochs N, Auer R, Bauer DC, Nanchen D, Gussekloo J and Cornuz J (2008).: Meta-analysis: subclinical thyroid dysfunction and the risk for coronary heart disease and mortality. Annals of Internal Medicine, 148(11):832–45.

52. Owen PJ, Rajiv C, Vinereanu D, Mathew T, Fraser AG and Lazarus JH (2006): Subclinical, arterial stiffness, and myocardial reserve. The Journal of Clinical Endocrinology and Metabolism, 91 (6):2126–32.

53. Parasuraman S and Raveendran R (2012): Measurement of invasive blood pressure in rats. Journal of Pharmacololgy & Pharmacotherapeutics, 3(2):172-177.

54. Paulsson J, Dadfar E and Held C (2007): Activation of peripheral and in vivo transmigrated neutrophils in patients with stable coronary artery disease. Atherosclerosis, 192:328–34.

55. Pesic MM, Radojkovic D, Antic S, Kocic R and Stankovic-Djordjevic D (2015): Subclinical hypothyroidism: association with cardiovascular risk factors and components of metabolic syndrome. Biotechnology & Biotechnological Equipment, 29 (1) 157-163.

56. Ridker PM, Rifai N, Pfeffer MA, Sacks F M, Moye LA., Goldman S, Flaker GC and Braunwald E (1998): Inflammation, Pravastatin and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Circulation, 98(9): 839- 844.

57. Sachan R, Patel M, Gaurav A, Gangwar R and Sachan P (2014): Correlation of serum neutrophil gelatinase associated lipocalin with disease severity in hypertensive disorders of pregnancy. Adv Biomed Res., 3:223.

58. Saltiki K, Voidonikola P, Stamatelopoulos K, Mantzou E, Papamichael C and Alevizaki M. (2008): Association of thyroid function with arterial pressure in normotensive and hypertensive euthyroid individuals: a cross-sectional study. Thyroid Res., 1(1):1-3.

59. Schultz M, Kistorp C, Raymond I, Dimsits J, Tuxen C and Hildebrandt P (2011): Cardiovascular events in thyroid disease: a population based prospective study. Hormone and Metabolic Research, 43(9):653–9.

60. Schuurs AM and Van Weeman BK (1977): Review, Enzyme- Immunoassay. Clin Chem Acta, 81:1-20.

61. Singh U, Devaraj S and Jialal I (2009): C-reactive protein stimulates myeloperoxidase release from polymorphonuclear cells and monocytes: implications for acute coronary syndromes. Clin Chem ., 55(2) :361–364.

62. Solak Y, Yilmaz MI, Siriopol D, Saglam M, Unal HU, Yaman H, Gok M, Cetinkaya H, Gaipov A, Eyileten T, Sari S, Yildirim AO, Tonbul HZ, Turk S, Covic A and Kanbay M (2015): Serum neutrophil gelatinase-associated lipocalin is associated with cardiovascular events in patients with chronic kidney disease. Int Urol Nephrol., 47(12):1993–2001.

63. Soylu K, Aksan G, Nar G, Ozdemir M, Gulel O, Inci S, Aksakal A, Soylu AI and Yılmaz O (2015): Serum neutrophil gelatinase-associated lipocalin levels are correlated with the complexity and the severity of atherosclerosis in acute coronary syndrome. Anatol J Cardiol., 15(6):450-5.

64. Sun G, Bishop J, Khallili S, Vasdev S, Gill V and Pace D (2007): Serum visfatin concentrations are positively correlated with serum triacylglycerols and down-regulated by overfeeding in healthy young men. Am J Clin Nutr., 85(2): 399- 404.

65. Temple RC, Clark PM and Hales CN (1992): Measurement of insulin secretion in type II diabetes: problems and pitfalls. Diabetic Medicine, 9(6): 503-512.

66. Tietz NW (1995): Clinical guide to laboratory tests. Pbl. W.B. Saunders, Co., Philadelphia, PP. 509-512.

67. Tuzcu A, Bahceci M, Gokalp D, **Tuzun Y and Gunes K. (2005):** Subclinical hypothyroidism may be associated with elevated high sensitive C-reactive protein (low grade inflammation) and fasting hyperinsulinemia. Endocr J., 52(1):89–94.

68. Valentina VN, Marijan B, Chedo D and Branka K (2011): Subclinical hypothyroidism and risk to carotid atherosclerosis. Arq Bras Endocrinol Metab., 55(7):475–80.

69. Van Deursen VM, Damman K, Voors AA, van der Wal MH, Jaarsma T and van Veldhuisen DJ (2014): Prognostic value of plasma neutrophil gelatinase-associated lipocalin for mortality in patients with heart failure. Circ Heart Fail., 7(1):35–42.

70. Vyakaranam S, Vanaparthy S, Nori S, Palarapu S and Bhongir AV (2014): Study of Insulin Resistance in Subclinical Hypothyroidism. Int J Health Sci Res., 4(9):147-153.

71. Walsh JP, Bremner AP and Bulsara MK (2005): Subclinical thyroid dysfunction as a risk factor for cardiovascular disease. Arch. Int. Med., 165(21): 2467–2472.

72. Walsh JP, Bremner AP, Bulsara MK, O'Leary P, Leedman PJ, Feddema P and Michelangeli V(2006): Subclinical thyroid dysfunction and blood pressure: a community-based study. Clin Endocrinol (Oxf.), 65(4): 486–491.

73. Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J and Tso AWK (2007): Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clin Chem., 53(1):34–41.

74. Ye Y, Xie H, Zeng Y, Zhao X, Tian Z and Zhang S (2014): Association between subclinical hypothyroidism and blood pressure - a meta-analysis of observational studies.Endocr Pract. , 20 (2):150-158.

75. Zorlu M, Kiskac M, Karatoprak C, Kesgin S, Cakirca M, Yildiz K, Ardic C, Cikrikcioglu MA and Erkoc R (2014): Assessment of serum apelin and lipocalin-2 levels in patients with subclinical hypothyroidism. Ann Endocrinol. (Paris), 75(1):10-4.

76. Zorniak M, Mitrega K, Bialka S, Porc M and Krzeminski TF (2010): Comparison of thiopental, urethane, and pentobarbital in the study of experimental cardiology in rats in vivo. J Cardiovasc Pharmacol, 56 (1):38-44.

الليبوكالين 2 فى مصل الدم يتنبأ بمخاطر إعتلال القلب و الأوعية الدموية في نموذج الجرذان المصابة بقصور تحت السريري فى الغدة الدرقية

نادين أحمد رأفت - سما صلاح خليل

قسم الفسيولوجيا - كلية الطب - جامعة الزقازيق

خلفية البحث : القصور تحت السريري فى الغدة الدرقية وعلاقته بأمراض القلب والأوعية الدموية لا تزال مثيرة للجدل، وكشفت دراسات سابقة أن مستويات الليبوكالين 2 فى الدم مرتبطة بزيادة خطر وفيات القلب والأوعية الدموية.

الهدف من البحث :قياس مستوى الليبوكالين2 فى مصل دم الجرذان المحدث لها قصور تحت السريري فى الغدة الدرقية و بحث مدى تأثيره على بعض عوامل الخطورة على القلب والأوعية الدموية.

مواد وطرق البحث :تم إستخدام 24 من ذكور الجرذان البيضاء البالغة وزنها حوالى 150-170 جرام. وقسمت إلى مجموعتين متساويتين: مجموعة جرذان ضابطة و مجموعة جرذان محدث لها قصور تحت السريري فى الغدة الدرقية –و ذلك بواسطة إعطائها ميثيمازول10 (ملغم / كغم من وزن الجسم) من خلال أنبوب تغذية مرة واحدة يوميا لمدة 3 أشهر. و قد تم قياس كل من هرمونات الغدة الدرقية و الهرمون المحفز لها، وكذلك مستويات الليبوكالين وسكر الدم و الإنسولين ، و كل أنواع دهون الدم ، وبروتين سي المتفاعل، ومؤشر كتلة الجسم ، ومعادلة مقاومة الإنسولين ، و مؤشر تصلب الشرايين ، و ضغط الدم الإنقباضي والإنبساطي فى كل من المجموعتين.

النتائج :وجد أن في مجموعة الجرذان المحدث لها قصور تحت السريري فى الغدة الدرقية مستويات كل من الهرمون المحفز للغدة الدرقية، والليبوكالين، والإنسولين ، والكولسترول الكلي، والبروتين الدهنى المنخفض الكثافة، والدهون الثلاثية، وبروتين سي المتفاعل فى الدم عالية و ذات دلالة إحصائية عن مثيلاتها فى مجموعة الجرذان الضابطة. أيضا، كان مؤشر كتلة الجسم ومعادلة مقاومة الإنسولين و مؤشر تصلب الشرايين و ضغط الدم الإنقباضي والإنبساطي فى نفس ذات المجموعة أعلى و ذات دلالة إحصائية عنه فى مجموعة الجرذان الضابطة ، وارتبطت كل هذه المعايير بشكل إيجابي ذو دلالة إحصائية مع الليبوكالين. وعلى العكس، كانت مستويات البروتين الدهنى العالى الكثافة في مجموعة الجرذان المحدث لها قصور تحت السريري فى الغدة الدرقية قليلة و ذات دلالة إحصائية عن مثيلاتها فى المجموعة الضابطة ، ووجد أن لها إرتباط سلبي بدلالة إحصائية مع مستويات الليبوكالين.

الإستنتاج :مستويات الليبوكالين 2 فى مصل الفئران المحدث لها قصور تحت السريري فى الغدة الدرقية عالية و لها إرتباط و تتنبأ بمخاطر إعتلال القلب والأوعية الدموية.