Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/1554
DOI URL: http://dx.doi.org/10.21474/IJAR01/1554

RESEARCH ARTICLE

NON-INVASIVE PREDICTORS OF CORONARY ARTERY DISEASE SEVERITY.

Manar M. Al-Zaky[1] and Mai M .Hasan[2].

1. Cardiology department, Faculty of Medicine Zagazig University.
2. Physiology department, Faculty of Medicine Zagazig University.

Manuscript Info

Manuscript History

Received: 18 July 2016
Final Accepted: 16 August 2016
Published: September 2016

Key words:-
Coronary artery disease (CAD), Carotid intimal medial thickness (CMIT), two-dimensional strain speckle tracking, Gensini score and leptin.

Abstract

Background: -Coronary artery disease (CAD) is a worldwide health problem.Using non-invasiveevaluation of severity of CAD has many limitations and remains a challenging issue in current cardiology practice.Recently, carotid intimal medial thickness (CIMT), plasma leptin level and two-dimensional strain speckle tracking echocardiography have got importance in this regard.

Aim: -Investigatingwhether carotid intimal medial thickness, two-dimensional strain speckle tracking echocardiographyand plasma leptin level could be used as predictors of CAD severity denoted by Gensini score.

Methods:- one hundred -forty one(141) coronary artery patients underwent coronary angiography and echocardiography in our hospital after two weeks of acute attack, are examined for carotid intima media thickness (CIMT) testing via B-mode ultrasound, plasma leptin levelsmeasured by ELISA and strain imaging derived from 2D speckle tracking analysis. They are assessed according to Gensini score as mild (< 20 n = 105) or sever (≥20 n = 36). All patients have normal ejection fraction.

Results: -CIMTand leptin levelswere significantly higherin severe compared to mild CAD patients, and were positively correlated with the severity of CAD.GLS was significantly lowerin sever CAD patients and inversely correlatedwith the severity of CAD. CIMT,leptin levels and GLS weregood predictors of severity of CAD assessed by coronary angiography and scored by Gensini score with cut off points (>0.97mm,>13 ng/mLand > -17), sensitivity and specificity (91.7, 94.4, 77.8- 90.5, 93.3, 79) respectively.

Conclusion: -CIMT, leptin levelsand GLS could be useful non-invasive predictors of the severity of CAD.

Introduction:-

According to WHO 7.4 million deaths areattributed to coronary artery disease representing 13.2% of all global deathsin **2012 (WHO, 2014)**.Severe coronary disease is more common in patients with acute coronary syndrome or stable coronary disease than generally perceived, simple and low-cost tools may help in the selection of the most appropriate therapeutic approach**(D'Ascenzo et al, 2012)**.

Corresponding Author: -Manar M. Al-Zaky.
Address:- Cardiology department, Faculty of Medicine Zagazig University.

Carotid intima-media thickness (CIMT) is a simple and inexpensive tool to assess the cumulative effect of atherosclerotic risk factors. CIMT will help clinicians in effective identification of the vulnerable patient **(Limbu et al., 2015).**Increased intimal-media thickness is a non-invasive marker of arterial wall alteration, which can easily be assessed in the carotid arteries. In addition to CIMT measurement as a predictive value of future vascular events, the presence of plaques or stenosis in the carotid artery tree increases drastically the risk for cardiovascular events **(Ludwig et al, 2003**).

Speckle tracking echocardiography is a relatively new method for evaluating and measuring global and regional strain: longitudinal, circumferential, radial and transverse; the precise indices of ventricular function (**Liszka et al., 2014**). **Reant et al. (2008)** reported that, two-dimensional strain speckle tracking is a reliable method for detection of myocardial contraction abnormalities under both baseline and ischemic conditions. It is a non-invasive diagnostic tool to identify the high-risk coronary artery disease patients who are suspected to have coronary artery disease without regional wall motion abnormality at rest (**Rostamzadeh et al., 2015**).

Leptin, an adipose tissue-derived hormone, plays a central role in regulating human energy homeostasis. The role of leptin in regulating blood pressure, activating the sympathetic nervous system, platelet aggregation, arterial thrombosis, angiogenesis, and inflammatory vascular responses suggesting its close relationship with the development of coronary heart disease **(Chai et al., 2014).** Leptin receptors have been also identified in various peripheral tissues, including in the cardiovascular system and in human coronary arteries. It seems to have both vasodilatory and vasoconstrictory actions **(Hasan-Ali et al., 2011).** Serum leptin levels are reported to be associated with various cardiovascular risks, including stroke, chronic heart failure, acute myocardial infarction, coronary heart disease, and left cardiac hypertrophy **(Nalini et al., 2015).**However, some investigators emphasized a potential protective role of leptin in coronary artery disease **(Hasan-Ali et al., 2011).**

The aim of this study is to assess the relation ofcarotid intimal medial thickness, plasma leptin level and two-dimensional strain speckle tracking to coronary artery disease severity and whether they could be used as predictors of CAD severity.

Method:-

Study population:-

The study included one hundred -forty one(141) patients of acute coronary syndrome who underwent coronary angiography in Zagazig- University hospitals after two weeks of acute attack with no history or ECGchanges denoting old myocardial infarction. **Patients were excluded if** they had abnormal myocardial repolarization, bundle branch block,, ventricular pacing,prosthetic valve, myocarditis,infiltrative disorder of the left ventricle,poor echogenic window,valvular heart disease,cardiomyopathy,pericardial heart disease,congenital heart disease, acute myocardial infarction,percutaneous coronary intervention , coronary artery bypass graftor arrhythmias that alter the atrial depolarization and the late ventricular filling phase.

Echocardiographic evaluation:-

Echocardiographic evaluation was done using the commercially available equipment, the digital ultrasound system (GE healthcare) with a 2- to 3-MHz transducer. M-mode, 2- dimensional and Doppler echocardiographic assessment was performed for all patients. Examinations were done with the patient in left semi- lateral position. For the SI study, high frame rate 2-dimensional images were recorded from 3 standard LV apical views (2-, 3- and 4-chamber views of the LV). In each view, images from 3 consecutive cardiac cycles were recorded and stored on magneto-optical disks for offline analysis. The operator traced the LV endocardium manually, and then it was tracked by the soft- ware automatically. The LV wall was divided into 6 segments in each view. Both global and regional longitudinal strains were obtained: global strain values (GLS) were calculated by averaging all segmental peaks systolic strain values in a 17 segment model.

Carotid intimal-medial thicknessmeasurement:-

Measurement of carotid CIMT was done on USG B mode 7.5 MHZ probe (HP 5500 andover, Massachusetts).The left and right common carotid arteries were examined bythe same sonographer who was blinded to the clinical data of thepatients with a Vivid Logiq 7 (GE) device with a 7.5 MHz linear arraytransducer. Patients were examined in the supine position, withthe head turned 45◦from the side being scanned. For the commoncarotid artery measurement, 10 mm of the common carotid arterysegment after the bulbus was determined. On a longitudinal, two-dimensional ultrasound image of the carotid artery, the anterior(near) and posterior (far) walls of the carotid artery

are displayedas two bright white lines separated by a hypoechogenic space. Thedistance between the leading edge of the first bright line of the farwall and the leading edge of the second bright line indicates theintima–media thickness. CIMT measurements were made from four different points 1 cm distance from each other andtheir mean value was calculated. The mean and maximum CIMTvalues for both the right and left carotid arteries and the overallmaximum and mean values were determined. The intimal-medial thickness on both (right and left) sides was calculated and an average of the two values was also taken (CIMT).

Plasma leptin:-
The venous samples were collected into collection tubes containing EDTA; plasmawas obtained after centrifugation at 3000 rpm and was stored at-80°C for the subsequent analysis of the leptin. Plasma concentrations of leptin were measured by sandwich enzyme-linked immunosorbent assay (ELISA) kits.

Coronary angiography:-
All patients underwent coronary angiography from multiple projections and recorded on cine film.Severity of the lesion: the coronary artery narrowing was visually estimated and expressed as percentage of luminal diameter stenosis. Patients with ≥ 70% narrowing in LAD, circumflex artery or right coronary artery or their major branches and ≥50% in left main coronary were classified as having significant angiographic coronary artery disease**.** The severity of CAD was evaluated by Gensini score namely mild atherosclerosis (Gensini score <20) and severatherosclerosis (Gensini score ≥ 20)according to **Oishi et al. (2000).**

Other Measurements:-
Age, sex, smoking, family history and medical history were collected by self-report questionnaire. Smoking was defined as current tobacco use. Diabetes, hypertension and dyslipidemia were defined as self-report of, fasting glucose ≥ 126, Blood pressure > 140/90 mmHg, TC >200 mg/dL, HDL < 40 mg/dL, LDL > 150mg/dL, or taking medications for either disease.

Statistical Analysis:-
The data were coded, entered and checked to SPSS file using SPSS version 12-computer package.

Results:-

Table (1): shows the Sociodemographic data of the study population. They have mean age ±SD of 52.2 years ± 7.59 years with 37 years range. The higher percentage of them was males 78.7%, smokers 59.6%. The clinical data revealed higher percentage of being non- diabetic 66%, non- hypertensive 57.4 %, non- dyslipidemic 68.6 % with negative family history 97.9%and negative left ventricular hypertrophy(LVH)85.1%.

Table 1:-characteristics of the studied group:

Sociodemographic data of the patients		
Age Mean ±SD Range	**52.2 ± 7.59** **37**	
	Frequency (N=141)	**Percent**
Sex Male	**111**	**78.7**
Female	**30**	**21.3**
Smoker	**84**	**59.6**
Non	**57**	**40.4**
Clinical data		
Diabetic	**48**	**34**
Non	**93**	**66**
Hypertensive	**60**	**42.6**
Non	**81**	**57.4**
Positive family history	**3**	**2.1**
Negative family history	**138**	**97.9**
Positive dyslipidemia	**45**	**31.9**
Negative dyslipidemia	**96**	**68.1**
Positive LVH	**21**	**14.9**
Negative	**120**	**85.1**

Table 2:- Shows higher percentage of mild CAD 74.5% and sever CAD 25.5%.

Table 2:-comparison of the studied group regarding grades of Gensini score:

Mild <20	**105**	**74.5**
Sever ≥20	**36**	**25.5**

Table 3:- Shows comparison of mild CAD and sever CAD patients as regard patients characteristics and clinical data; mild group were significantly higher in age compared to sever group.Sever group were significantly higher in being male, smokers, diabetic, hypertensive, dyslipidemic, with negative family history. Both groups were insignificantly different as regard left ventricular hypertrophy.

Table 3:-Comparison between the grades of Gensini score regarding patient characteristics and clinical data:

	Mild (N=105)		**Sever (N=36)**		**t-test**	**p-value**
AGE (mean± SD)	**54±7. 2**		**48±7.3**		**3.91**	**<0.001****
	Mild (N=105)	**%**	**Sever (N=36)**	**%**	**X^2**	**p-value**
Male (n=111)	**78**	**74.3**	**33**	**91.7**	**4.84**	**0.03***
Female(n=30)	**27**	**25.7**	**3**	**8.3**		
Smoker(n=84)	**57**	**45.7**	**27**	**75**	**4.78**	**0.03***
Non(n=57)	**48**	**54.3**	**9**	**25**		
Diabetic(n=48)	**30**	**28.6**	**18**	**50**	**5.48**	**0.02***
Not (n=93)	**75**	**71.4**	**18**	**50**		
Hypertensive(n=60)	**36**	**34.3**	**24**	**66.7**	**11.5**	**0.001****
Not (n=81)	**69**	**65.7**	**12**	**33.3**		
Positive family history(n=3)	**3**	**2.9**	**0**	**0**	**1.05**	**0.31**
Negativefamily history (n=138)	**102**	**97.1**	**36**	**100**		
Positive dyslipidemia(n=45)	**21**	**20**	**24**	**66.7**	**26.87**	**< 0.001*****
Negative dyslipidemia(n=96)	**84**	**80**	**12**	**33.3**		
Positive LVH (n=21)	**13**	**12.4**	**8**	**22.2**	**2.05**	**0.15**
Negative LVH (n=120)	**92**	**87.6**	**28**	**77.8**		**NS**

***Significance <0.05 **Significance <0.01 ***significance<0.001 NS not significant**

Table 4:- Shows the comparison of mild CAD patients and sever CAD patients as regard the three diagnostic tools. It reveals significantly higher CIMT in sever group (1.5 mm) compared to mild group (1.05 mm), higher plasma leptin levels (14.4 ng/mL) in sever group compared to (9 ng/mL) in mild group and significantly lower global longitudinal shortening in sever group(-13.2) compared to mild group (-17.4)

Table 4:-Comparison between grades of Gensini score regarding common carotid medial intimal thickness (CCMIT), leptin and GLS:

	Mild (N=105) Median (Minimum-maximum)	**Sever (N=36) Median (Minimum-maximum)**	**Test**	**p-value**
CMIT	**1.05 mm (0.35-2.1)**	**1.5mm (0.65-1.9)**	**MW 3.01**	**0.003$^{\$\$}$**
Leptin	**9ng/mL (4-26 ng/mL)**	**14.5 ng/mL (7-27 ng/mL)**	**MW 4.31**	**<0.001$^{\$\$\$}$**
GLS	**-17.4 (-20.8 -- -5.3)**	**-13.2 (-18.1 -- -10.3)**	**t 8.19**	**<0.001*****

***significance for t- test$ significance for MW test**

Table 5:- Shows correlation of Gensini score to the diagnostic tools; it revealed positive correlation to CIMT, serum leptin levels and GLS.

Table 5:- Pearson's correlation between Gensini csore and the used diagnostic tools.

Variables	GENSINI SCORE	
	R –value	**p-value**
CIMT	**0.63**	**<0.001****
Leptin	**0.53**	**<0.001****
GLS	**0.60**	**<0.001****

****Significance <0.01** ****significance<0.001**

Figure 1:-Scatter plot showing the association between CIMT and Gensini score

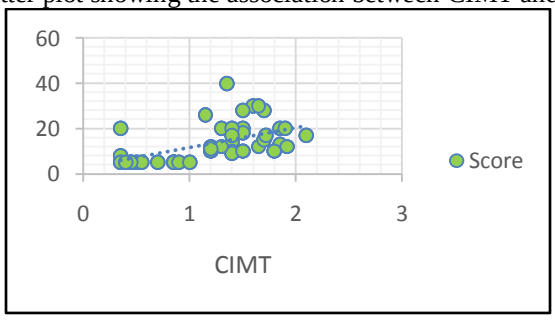

Figure2:- Scatter plot showing the association between leptin and Gensini score

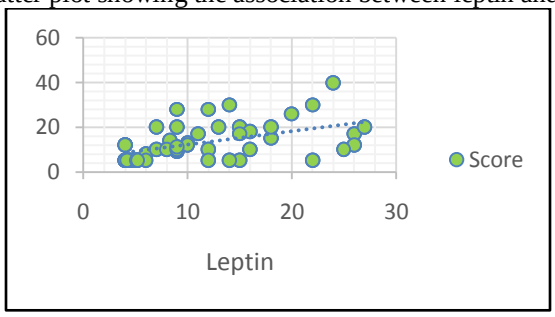

Figure 3:-Scatter plot showing the association between GLS and Gensini score

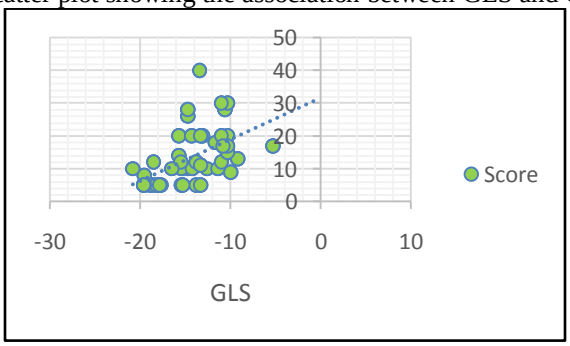

Figure 4:-ROC curve for CMIT, leptin and GLS

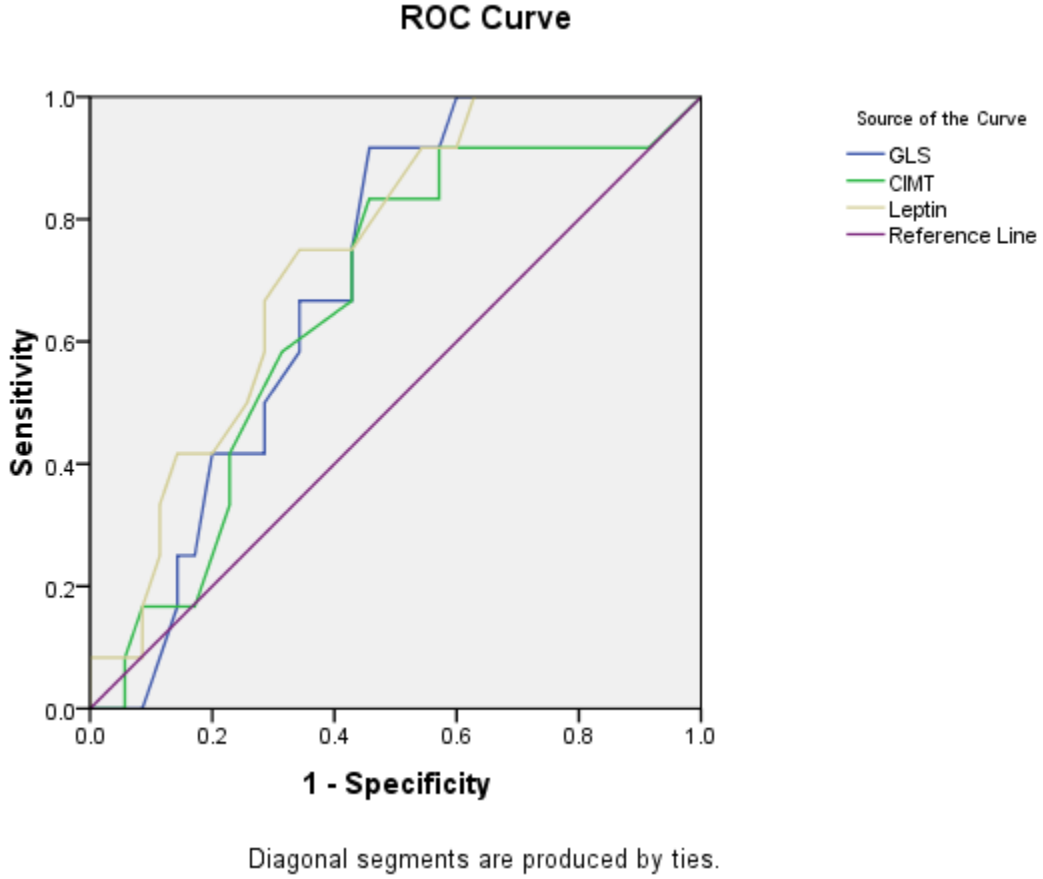

Table 7:-Reliability data of GLS, CIMT and leptin as a predictor for lesions diagnosed by Gensini score:

Variables	Cut off	AUC	PPV	NPV	P-value	95% CI	sensitivity	specificity	Accuracy
CIMT	**>0.97**	**0.701**	**76.7**	**96.9**	**<0.001*** **	**0.62 - 0.79**	**91.7**	**90.5**	**90.8**
Leptin	**> 13**	**0.740**	**82.9**	**98**	**<0.001*** **	**0.66 - 0.82**	**94.4**	**93.3**	**93.6**
GLS	**> -17**	**0.661**	**56**	**91.2**	**0.004****	**0.56 - 0.76**	**77.8**	**79**	**78.7**

Discussion:-

The present study showed significantly higher percentages of smoker, diabetic, dyslipidemic patients in severCAD patients than those of mild CAD according to Gensini score. These results are in line with **Tomizawa et al. (2015)**who reported that distribution and severity of CAD between patients with hypertension, diabetes mellitus and dyslipidemia is more significant than those without disease, diabetes and dyslipidemia were related to obstructive CAD.Moreover, serum lipoprotein (a) level was an independent predictor for the severity of CAD assessed by Gensini score in patients with diabetes (**Chen et al., 2015)**

In diabetic patients, advanced glycation end-products could promote mineralization of microvascular pericytes, which results in both intimal and medial calcification (**Madhavan et al., 2014**), lipoprotein (a) promotes atherosclerosis by increased lipoprotein (a)-associated cholesterol entrapment in the arterial intima, inflammation mediation, carrying of proinflammatory oxidized phospholipids, impairing fibrinolysis and enhancing coagulation**(Gouni-Berthold and Berthold 2011.)**

The results of the present study showed significant increased carotid intima-medial thickness in sever CAD compared to mild CAD patients and it was positively correlated to grade of Gensini score. These results are in agreement with**Tarzamni et al. (2006)**who found a significant correlation between CIMT and advancing CAD and **Timo´teoet al. (2013)** who showed that CIMT is significantlyhigher in patients with CAD than patients without

CAD. While **Sosnowski et al.(2007)** reported that femoral IMT enables better assessment of coronary artery atherosclerosis than CIMT.

However, increased CIMT is related to generalized atherosclerosis of all vascular beds especially the coronaries (**Bots et al., 1997**) and CIMT measurement is increasingly used as a surrogate marker for subclinical atherosclerosis because of its ability to predict future clinical cardiovascular endpoints (**Iwakiri et al., 2012**).This is in accordance to our results which showed high sensitivity and specificity of CIMT as predictors of severity of CAD.

The present study demonstrated significant increase in serum leptin levels in sever CAD than mild CAD patients with positive correlation between serum leptin levels and severity of CAD and serum leptin levels showed high sensitivity and specificity as predictors of severity of CAD.**Tsai et al. (2016)** found that serum leptin levelscorrelated positively with the number of stenotic coronary arteries (i.e. severity, extent). Leptin levels were higher in patients with coronary atherosclerosis than controls, and high serum leptin level was an independent factor for severity and pattern of coronary atherosclerosis. Also**, Khafaji et al,(2012)** demonstrated that serum leptin level may be a predictor of the degree ofcoronary atherosclerosis.

However, **Simiti et al.(2016)**suggested a protective role of leptin in CAD patients with overweight and mild obesity. While leptin deleterious effects to cardiovascularhealth maybe due toincrease oxidative stress leading to decreases in the bioavailability of nitric oxideor promotion of neo-intimalgrowth in mice (**Cooke and Oka 2002)**,stimulation of migration andproliferation of vascular smooth muscle cells **(Li et al., 2005)** orsynthesis and secretion of endothelin-1(**Quehenberger et al., 2002**). Moreover, leptin stimulateslipoprotein lipase secretion in macrophages and increasesaccumulation of cholesterol esters in foam cells, especiallyat high glucose concentrations (**Maingrette and Renier 2003**),promotes hepaticHDL and decreases plasma HDL level in mice (**Lundåsen et al., 2003**).

The present study revealed significant decrease in GLS in sever CAD compared to mild CAD patientsand inverse correlation between GLS and Gensini score grading of the patients, GLS was found to be a good sensitive and specific predictor of CAD severity.This is in accordance to **Biering-Sørensen et al (2014)** who reported that global longitudinal peak systolic strain assessed at rest is an independent predictor of significant CAD and the risk of multivessel disease increases with decreasing GLS. There is a reverse linear correlation between global longitudinal peak systolic strain (GLPSS) and the SYNTAX score (SS) in patients undergoing coronary angiography**(Vrettoset al., 2016).**

When CAD patients are at rest the myocardial systolic peak strainrate, the coronary blood-supply in segments of which the stenosis is more than at 70%, is much lower than that of other normal segments **(Liang et al., 2006).** Sever impairment of the longitudinal systolic function may be related to myocardial fiber arrangement and perfusion characteristics(**Xie et al., 2015**).As most branches of coronary blood vessels are deep in the myocardium, the heart exerts oppression on these vessels when it contracts, and deep coronary blood flow is much more influenced, causing significant increase in the resistance of blood flow to the subendocardial myocardium (**Palekar et al., 2015**).Left ventricular longitudinal motion is produced by the contraction of the longitudinal myocardial fibers under the endocardium. Thus, the endocardial myocardium is the most vulnerable part when coronary blood flow decreases (**Snider et al., 2014**).

Limitations:-

Asthe current study included a small number of patients, the result should be interpreted cautiously.

Conclusion:-

CIMT and serum leptin levels were significantly higher in sever CAD, positively correlated to the severity of CAD while GLS significantly decreased and inversely correlated with the severity of CAD. CIMT, serum leptin levels and GLS were good predictors of CAD severity.

References:-

1. **Biering-Sørensen T., Hoffmann S., Mogelvang R., Zeeberg Iversen A., Galatius S., Fritz-Hansen T., Bech J., Jensen J.S. (2014):** Myocardial strain analysis by 2-dimensional speckle tracking echocardiography improves diagnostics ofcoronary artery stenosis in stable angina pectoris. Circ. Cardiovasc. Imaging. 7(1):58-65.
2. **Bots M.L., Hoes A.W., Koudstaal P.J., Hofman A., Grobbee D.E.(1997):** Common carotid intima-media thickness and risk of stroke and myocardial infarction the Rotterdam Study. Circulation; 96(5):1432-37.
3. **Chai S.B., Sun F., NieX.L., Wang J.(2014):** Leptin and coronary heart disease: asystematic review and meta-analysis. Atherosclerosis;233(1):3-10.
4. **Chen J., Zhang Y., Liu J., Chen M.H., Guo Y.L., Zhu C.G., Xu R.X., Dong Q., Li J.J. (2015**):Role of lipoprotein(a)in predicting the severity of new on-set coronary artery disease in type 2 diabetes : A Gensini score evaluation. Diab. Vasc. Dis. Res. 12(4):258-64.
5. **Cooke J.P., Oka R.K. (2002):** Does leptin cause vascular disease? Circulation. 8; 106(15):1904-5.
6. **D'Ascenzo .F, Presutti D.G., Picardi E., Moretti C., Omedè P., Sciuto F., Novara M., Yan A.T., Goodman S., Mahajan N., Kosuge M., Palazzuoli A., Jong G.P., Isma'eel H., Budoff M.J., Rubinshtein R., Gewirtz H., Reed M.J., Theroux P., Biondi-Zoccai G., Modena M.G., Sheiban I., Gaita F.(2012) :** Prevalence and non-invasive predictors of left main or three-vessel coronary disease: evidence from a collaborative international meta-analysis including 22 740 patients. Heart. ; 98(12):914-9.
7. **Gouni-Berthold I., Berthold H.K. (2011):** Lipoprotein (a): current perspectives. Curr. Vasc. Pharmacol. 9(6):682-92.
8. **Hasan-Ali H., Abd El-Mottaleb N.A., Hamed H.B., Abd-Elsayed A. (2011):**Serum adiponectin and leptin as predictors of the presence and degree of coronary atherosclerosis. Coron. Artery Dis.; 22(4):264-9. (Cross Ref.)
9. **Hasan-Ali H., Abd El-Mottaleb N.A., Hamed H.B., Abd-Elsayed A.(2011) :** Serum adiponectin and leptin as predictors of the presence and degree of coronary atherosclerosis. Coron. Artery Dis. 22(4):264-9.
10. **Iwakiri T., Yano Y., Sato Y., Hatakeyama K., Marutsuka K., Fujimoto S., Kitamura K., Kario K., Asada Y.(2012):** Usefulness of carotid intima-media thickness measurement as an indicator of generalized atherosclerosis: findings from autopsy analysis. Atherosclerosis; 225(2):359- 62.
11. **Khafaji H.A., Bener A.B., Rizk N.M., Al Suwaidi J. (2012):**Elevated serum leptin levels in patients with acute myocardial infarction; correlation with coronaryangiographic and echocardiographic findings. BMC Res. Notes. 29; 5:262.
12. **Li L., Mamputu J.C., Wiernsperger N., Renier G. (2005):** Signaling pathways involved in human vascular smooth muscle cell proliferation and matrix metalloproteases-2 expression induced by leptin: inhibitory effect of metformin. Diabetes. 54(7):2227-34.
13. **Liang H.Y., Cauduro S., Pellkka P., Wang J., Urheim S., Yang E. H., Rihal C., Belohlavek M., Khandheria B., Miller F. A., Abraham T.P. (2006):**Usefulness of two-dimensional speckle strain for evaluation of lest ventricular diastolic deformation in patients with coronary disease. Am. J. Cardiol. 98: 1581-1586.
14. **Limbu Y.R., Rajbhandari R., Sharma R., Singh S., Limbu D., Adhikari C.M., Prajapati D.(2015):**Carotid intima-media thickness (CIMT) and carotid plaques in young Nepalesepatients with angiographically documented coronary artery disease. Cardiovasc.Diagn. Ther.;5(1):1-7.
15. **Liszka J., Haberka M., Tabor Z., Finik M., Gąsior Z. (2014):** Two- dimensional speckle tracking echocardiography assessment of left ventricular remodeling in patients after myocardial infarction and primary reperfusion. .Arch. Med. Sci.; 10(6):1091-100.
16. **Ludwig M., von Petzinger-Kruthoff A., von Buquoy M., Stumpe K.O.(2003):**Intima media thickness of the carotid arteries: early pointer to arteriosclerosis and therapeutic endpoint. Ultraschall. Med.; 24(3):162-74. (Abstract).
17. **Lundåsen T., Liao W., Angelin B., Rudling M. (2003):** Leptin induces high density lipoprotein receptor scavenger receptor B type I (SR-BI) but not cholesterol7alpha- hydroxylase (Cyp7a1)in Leptin-deficient (ob/ob)mice.J. Biol. Chem. 31; 278(44):43224-8.
18. **Madhavan M.V., Tarigopula M., Mintz G.S.., Maehara A., Stone G.W., Généreux P.(2014):** Coronary artery calcification: pathogenesis and prognostic implications. J. Am. Coll. Cardiol. 6;63(17):1703-14.
19. **Maingrette F., Renier G. (2003):** Leptin increases lipoprotein lipase secretion by macrophages; involvement of oxidative stress and protein Kinase C.Diabetes.52 (8):2121-8.
20. **Nalini D., Karthick R., Shirin V., Manohar G., Malathi R. (2015):** Role of the adipocyte hormone leptin in cardiovascular diseases – a study from Chennai based Population Thromb. J. 4;13:12.

21. **Oishi Y., Wakatsuki T., Nishikado A., Oki T., Ito S. (2000):**Circulating adhesion molecules and severity of coronary atherosclerosis. Coron. Artery Dis.; 11(1):77-81.
22. **Palekar R.U., Jallouk A.P., Lanza G.M., Pan H., Wickline S.A. (2015):** Molecular imaging of atherosclerosis with nanoparticle-based fluorinated MRI contrast agents. Nanomedicine (Lond). 10(11):1817-32.
23. **Quehenberger P., Exner M., Sunder-Plassmann R., Ruzicka K., Bieglmayer C., Endler G., Muellner C., Speiser W., Wagner O.(2002):** Leptin induces endothelin-1 in endothelial cells in vitro.Circ. Res. 5; 90(6):711-8.
24. Reant P**.,** Labrousse L.**,** Lafitte S.**, Bordachar P., Pillois X,. Tariosse L., Bonoron-Adele S., Padois P., Deville C., Roudaut R., Dos Santos P.(2008):** Experimental validation of circumferential, longitudinal, and radial 2-dimensional strain during dobutamine stress echocardiography in ischemic conditions. J. Am. Col. Cardiol. ; 51:149 –57.
25. **Rostamzadeh A., Shojaeifard M., Rezaei Y., Dehghan K.(2015):** Diagnostic accuracy of myocardial deformation indices for detecting high risk coronary artery disease in patients without regional wall motion abnormality.Int J. Clin. Exp. Med. 15;8(6):9412-20.
26. **Simiti L.A., Todor I., Stoia M.A., Goidescu C.M., Anton F.P., Farcas A.D. (2016):**Better prognosis in overweight/obese coronary heart disease patients with high plasma levels of leptin. Clujul. Med. 89(1):65-71.
27. **Snider P., Simmons O., Wang J., Hoang C.Q., Conway S.J. (2014):** Ectopic Noggin in a Population of Nfatc1 Lineage Endocardial Progenitors Induces Embryonic Lethality. J. Cardiovasc. Dev. Dis. 1(3):214-236.
28. **Sosnowski C., Pasierski T, Janeczko-Sosnowska E, Szulczyk A, Dabrowski R, Woźniak J, Sumiński A, Ruzytto W. (2007):** Femoral rather than carotid artery ultrasound imaging predicts extent and severity of coronary artery disease. Kardiol. Pol. 65(7):760-6; discussion 767-8.
29. **Tarzamni M.K, , Salehi R., Givian F., Farhang S (2006):**Association of carotid intima-media thickness with the presence and severity of coronary artery disease.Neurosciences (Riyadh). 11(4):308-11.
30. **Timóteo A.T., Carmo M.M., Ferreira R.C. (2013):** Carotid intima-media thickness and carotid plaques improves prediction of obstructive angiographic coronaryartery disease in women. Angiology.64 (1):57-63.
31. **Tomizawa N., Nojo T., Inoh S., Nakamura S. (2015):** Difference of coronary artery disease severity, extent and plaque characteristics between patients with hypertension, diabetes mellitus or dyslipidemia. Int. J. Cardiovasc. Imaging; 31:205–212.
32. **Tsai J.P., Wang J.H., Chen M.L., Yang C.F., Chen Y.C., Hsu B.G. (2016):** Association of serum leptin levels with central arterial stiffness in coronary artery disease patients. BMC Cardiovasc. Disord. 5; 16:80.
33. **Vrettos A., Dawson D., Grigoratos C., Nihoyannopoulos P. (2016):**Correlation between global longitudinal peak systolic strain and coronary artery disease severity as assessedby the angiographically derived SYNTAX score. Echo. Res. Pract. 3(2):29-34.
34. **World Health Organization (2014):**Department of Health Statistics and Information System, Global Health estimates Summery tables: Deaths by cause, age and sex, 2000-2012.Geneva. World Health Organization 2014.
35. **Xie M.Y., Yin J.B., Lv Q., Wang J.(2015):** Assessment of the left ventricular systolic function in multivessel coronary artery disease with normal wallmotion by two-dimensional speckle tracking echocardiography. Eur. Rev. Med. Pharmacol. Sci.19 (20):3928-34.