IJBPAS, December, 2021, 10(12): 4614-4624

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

NOVEL RISK RATIO IN DIABETES AND ITS ASSOCIATED COMPLICATION

ELANGO K S[1*], BANU N[2] AND VIJAYALAKSHMI S[3]

1: Research Scholar, Vels Institute of Science, Technology & Advanced Studies (VISTAS), Pallavaram, Tamilnadu, Chennai, INDIA

Scientific Officer, HSEG/HISD/OHS, Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Chengalpat District, 603102

2: Assistant Professor, Department of Botany, Bharathi Women's College (Autonomous), Chennai, Tamilnadu, INDIA

3: Professor, Department of Biotechnology, VELS University, Pallavaram, Chennai, Tamilnadu, INDIA

***Corresponding Author: K.S. Elango: E Mail: kse@igcar.gov.in**

Received 23rd Feb. 2021; Revised 24th Mar. 2021; Accepted 20th April. 2021; Available online 1st Dec. 2021

https://doi.org/10.31032/IJBPAS/2021/10.12.5780

ABSTRACT

Objective: Diabetes blood sugar level fluctuates in chronic anti-diabetic medication. Furthermore, lipid assay value is higher, assessing the risk factor to prevent complications in diabetes and its associated diseases difficult. Previous studies confirm the association between lipid and insulin sensitivity. However, the compound effect of blood glucose, high-density lipoprotein ratio versus cholesterol, blood glucose ratio has not been assessed so far in insulin-resistant non-insulin-dependent diabetes. The present study aimed at calculating the novel ratio, Fasting blood sugar/High-density lipoprotein (FBS/HDL) ratio versus Cholesterol/Fasting blood sugar (CHO/FBS) ratio and investigating the correlation to Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), and Triglyceride-glucose (TyG) index in type 2 diabetes insulin-resistant patients.

Methods: The study includes 30 Type 2 Diabetic patients and 20 Non-diabetics (control). Fasting serum samples used to measure Insulin, Cholesterol, Triglyceride, HDL. A fasting plasma sample utilized to measure Plasma glucose.

Results: The Fasting blood sugar/ High-density lipoprotein ratio (FBS/HDL), HOMA-IR, TyG index and the Cholesterol/Fasting blood sugar ratio (CHOLESTEROL/FBS) between Non-diabetic and Diabetic at a significance of p-value < 0.001 level. Serum insulin level showed a significant difference between Non-diabetic and Diabetic at p-value < 0.05 level. The Receiver operating characteristic curve (ROC) explains the FBS/HDL ratio and CHOLESTEROL/FBS ratio with the good value of area under the curve by 0.907 and 0.935.

Conclusion: The FBS/HDL ratio versus CHOLESTEROL/FBS ratio suggests a significant correlation with each other, also with HOMA-IR, and TyG index value. Hence, we suggest beneficial risk assessment ratios in type 2 diabetes insulin resistance and its associated cardiovascular diseases complications.

Keywords: Type 2 diabetes; Novel lipid, sugar ratio; TyG index; Insulin resistant, Receiver operating characteristic curve

INTRODUCTION

Different type of lipid ratio analyses studied in type 2 diabetes. Dobiasova M *et al* derived log(TG/HDL-c) atherogenic index and examined the link with apoprotein B, small dense LDL. The study concludes that the TG/HDL-c ratio can also be an atherogenic marker and a high TG/HDL-c ratio noted in insulin resistance **[1, 2]**. Gaziano *et al*. reported that TG/HDL-c ratio strongly predicted the myocardial infarction risk **[3]**. Another study warranted that the high TG/HDL-c ratio in women as a risk of mortality even in the absence of coronary artery disease **[4]**. Low-density lipoprotein (LDL) is also one of the risk factors for atherosclerosis. The risk intensity of atherosclerosis further strengthened by studying LDL-c/HDL-c ratio **[5]**. High LDL-c/HDL-c ratio observed as an increased risk of sudden cardiac death **[6]**. Carotid intima-media thickness (CIMT) measured to study the proatherogenic development, LDL-c/HDL-c ratio significantly associated with CIMT as a strong predictor in human IMT progression **[7]**. In non-diabetes, euthyroid adults' high triglyceride/glucose index (TGI) proven the association with insulin resistance **[8]**. Recent study on TyG (Triglyceride-Glucose) index in stable coronary artery disease (CAD) patients suggest that TyG index was found to be positively correlated to cardiovascular risk factors and may be a useful predictive marker in patients with CAD **[9]**. In the present study, resistant to non-insulin-dependent diabetes and the risk factor for insulin resisted complication like coronary artery disease assessed through novel arrived ratios (FBS/HDL, CHOLESTEROL/FBS) compared with

HOMA-IR gold standard and clinically justified TyG index.

MATERIALS AND METHODS

Thirty non-insulin-dependent diabetes patients and twenty healthy control were studied. We followed Diabetes criteria according to the Indian Council of Medical Research (ICMR) guidelines. Individual informed consent received before the study. Age group between 40-60 males excluded with previous cardiovascular problem and parameters studied include plasma fasting blood sugar, serum insulin, serum triglyceride, cholesterol, HDL. Calculated indexes are HOMAR-IR, TyG index, and the novel ratios FBS/HDL, CHOLESTEROL/FBS. Blood samples collected after 10-12 hour fasting and two ml were transferred in sodium fluoride/Na2 EDTA anticoagulant tubes for fasting blood glucose estimation. The remaining 6 ml blood was transferred in plain tubes (without anticoagulant) to estimate serum fasting insulin and triglyceride, cholesterol and high-density lipoprotein. Beckman Coulter UniCel Dxi 800 biochemistry auto analyzer estimated the serum triglyceride, serum insulin. Serum insulin measured by a chemiluminescent method with access to an ultra-sensitive insulin assay kit. Serum insulin binds to the antibody on the solid phase while the conjugate reacts with a different antigenic site on the insulin molecule. After incubation in reaction vessels, materials attached to the solid phase held in a magnetic field, and unbound materials washed away. Chemiluminescent substrate Lumi-phos 530 measured the reaction with a luminometer. Measurement is directly proportional to the sample's insulin concentration **[10]**. Serum triglyceride analyzed through Enzymatic/GPO Trinder method. This Triglyceride procedure based on a series of coupled enzymatic reactions. The sample's triglycerides were hydrolyzed with a combination of microbial lipases to give glycerol and fatty acids. Adenosine triphosphate (ATP) phosphorylate the glycerol in the presence of glycerol kinase (GK) to produce glycerol-3-phosphate. The glycerol-3-phosphate oxidized by molecular oxygen in the presence of GPO (glycerol phosphate oxidase) to produce hydrogen peroxide (H_2O_2) and dihydroxyacetone phosphate. The formed hydrogen peroxide reacts with 4-aminophenazone and N,N-bis(4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB) in the presence of peroxidase (POD) to produce a chromophore and read at 660/800nm. The increase in absorbance at 660/800 nm is proportional to the sample's triglyceride content **[11, 12]**. Estimation of glucose done by GOD/POD method. Glucose oxidase (GOD) catalyzes the oxidation of glucose to gluconate and

hydrogen peroxide formation. Chromogenic oxygen acceptor, phenol, 4-Aminophenazone detect the hydrogen peroxide in the presence of peroxidase.

The colour complex's intensity is directly proportional to the glucose in the specimen and serum HDL measured by Cholesterol Esterase, Peroxidase endpoint method and serum cholesterol measured by endpoint method **[13]**. Previous studies confirm the accuracy and precision of the homeostasis model assessment (HOMA-IR) **[14]**. The HOMA-IR index formula is (Glucose x Insulin) / 405, HOMA-IR calculated according to the formula. Few studies described the TyG index in various conditions **[15, 16, 17]**. The formula for the TyG index is TyG = ln [Fasting triglyceride (mg/dl) x Fasting glucose (mg/dl)] / 2, TyG calculated as per the formula. The novel ratios calculated by dividing the two parameters value (i.e.) Fasting blood sugar value/High-density lipoprotein value. Cholesterol value/Fasting blood sugar value.

Statistical analysis

SPSS (version 20) statistical software used for analyses. Data categorized according to 40 to 60 years in age, patients and control, fasting serum insulin, triglyceride, fasting plasma glucose, HOMA-IR index, TyG index, FBS/HDL, CHOLESTEROL/FBS and evaluated using a Pearson's correlation coefficient analysis, Receiver operating characteristics curve (ROC). Patients and control parameters compared with a significance level of $p < 0.05$ **[18, 19]**.

RESULTS

Table 1 shows the significant difference in the Fasting blood sugar, HOMA-IR, TyG index, and the novel ratios FBS/HDL, CHOL/FBS between Non-diabetic and Diabetic at p-value < 0.001 level. Serum insulin level shows a significant difference between Non-diabetic and Diabetic at p-value < 0.05 level.

Analysis performed using Pearson's correlation coefficient to study the strength of a linear relationship between two quantitative variables. In this study novel ratios, FBS/HDL and CHOL/FBS correlation also with other variables Fasting blood sugar, HOMA-IR, Insulin, TyG index were studied **(Table 2)**.

The correlation between novel ratio FBS/HDL and Fasting blood sugar, TyG index shows highly significant positive correlation. FBS/HDL and HOMA-IR correlate significant moderate positive correlation. The correlation between another novel ratio CHO/FBS and Fasting blood sugar, TyG index shows highly significant negative correlation. CHO/FBS and HOMA-IR correlate significant moderate negative correlation. FBS/HDL versus CHO/FBS shows a highly

significant negative correlation with each other.

Figure 1 The ROC analysis of the FBS/HDL novel ratio shows good performance in distinguishing the positive and negative classes. The area under the curve value 0.91, shows the acceptable in using as a diagnostic ratio. From the curve tabulation coordinates, the cut off value for FBS/HDL ratio arrived as positive if greater than or equal to 3.373, screens positive 80% sensitivity in affected individuals with the specificity of 85%.

Figure 2 The ROC analysis of the CHO/FBS novel ratio shows good performance in distinguishing the positive and negative classes. The area under the curve value 0.935, shows the acceptable in using as a diagnostic ratio. From the curve tabulation coordinates, the cut off value for CHO/FBS ratio arrived as positive if less than or equal to 1.35, screens positive 86% sensitivity in affected individuals with the specificity of 90%.

Table 1: Fasting blood sugar, HOMA-IR, TyG index, and the novel ratios FBS/HDL, CHOL/FBS between Non-diabetic and Diabetic

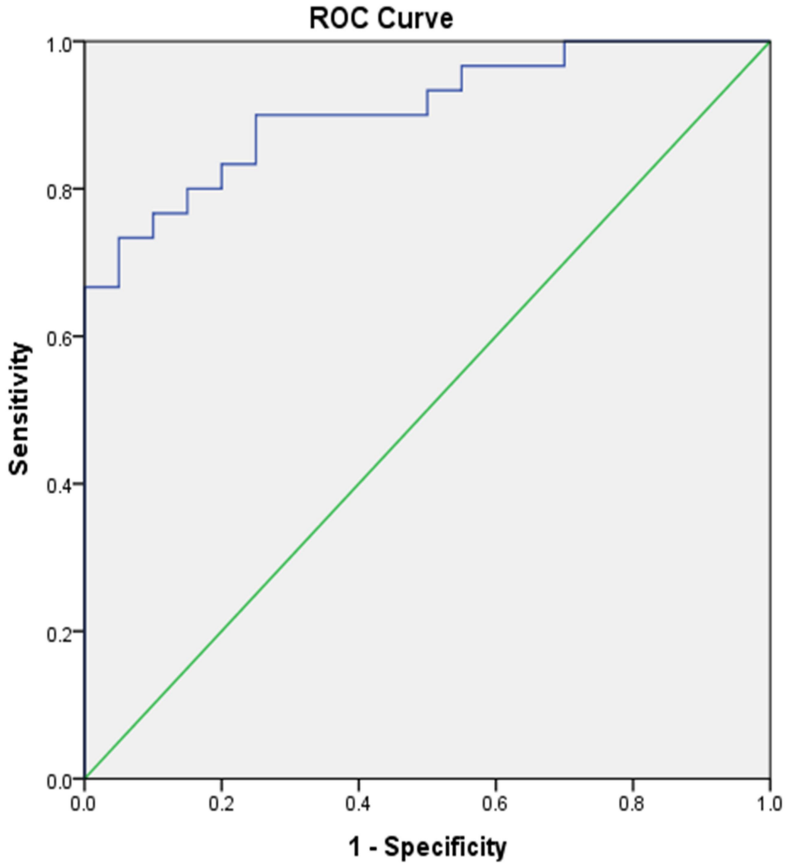
Biomarkers	Groups	Mean	Standard Deviation	*P*-value
Fasting blood sugar (mg/dl)	Non-Diabetic	103.35	12.57	< 0.001
	Diabetic	192.06	72.62	
HOMA-IR Index	Non-Diabetic	2.24	0.94	< 0.001
	Diabetic	6.10	4.09	
Insulin (µIU/mL)	Non-Diabetic	8.72	3.23	< 0.05
	Diabetic	13.86	9.66	
FBS/HDL ratio	Non-Diabetic	2.71	0.77	<0.001
	Diabetic	5.60	2.58	
CHOL/FBS ratio	Non-Diabetic	1.85	0.51	<0.001
	Diabetic	0.98	0.36	
Triglyceride-Glucose Index (TyG)	Non-Diabetic	4.85	0.22	< 0.001
	Diabetic	5.18	0.25	

The data are presented as mean, ± standard deviation, fasting blood glucose, TyG index, insulin, HOMA-IR index, FBS:HDL ratio, CHOL-FBS ratio between Non-Diabetic and Diabetic persons. The significance (p-value) estimated through the Independent sample ' t ' test.

Table 2

ANALYTE	FBS	HOMA-IR	Insulin	TyG Index	FBS/HDL ratio	CHO/FBS ratio
FBS	1	0.416**	-0.007	0.723**	0.912**	-0.782**
Significance		0.003	0.961	0.000	0.000	0.000
HOMA-IR	0.416**	1	0.859**	0.409**	0.409**	-0.457**
Significance	.003		0.000	0.003	0.003	0.001
Insulin	-0.007	0.859**	1	0.123	0.046	-0.152
Significance	0.961	0.000		0.394	0.751	0.293
TyG Index	0.723**	0.409**	0.123	1	0.767**	-0.636**
Significance	0.000	0.003	0.394		0.000	0.000
FBS/HDL ratio	0.912**	0.409**	0.046	0.767**	1	-0.765**
Significance	0.000	0.003	0.751	0.000		0.000
CHO/FBS ratio	-0.782**	-0.457**	-0.152	-0.636**	-0.765**	1
Significance	0.000	0.001	0.293	0.000	0.000	
No. Of Test	50	50	50	50	50	50

****. Correlation is significant at the 0.01 level (2-tailed).**



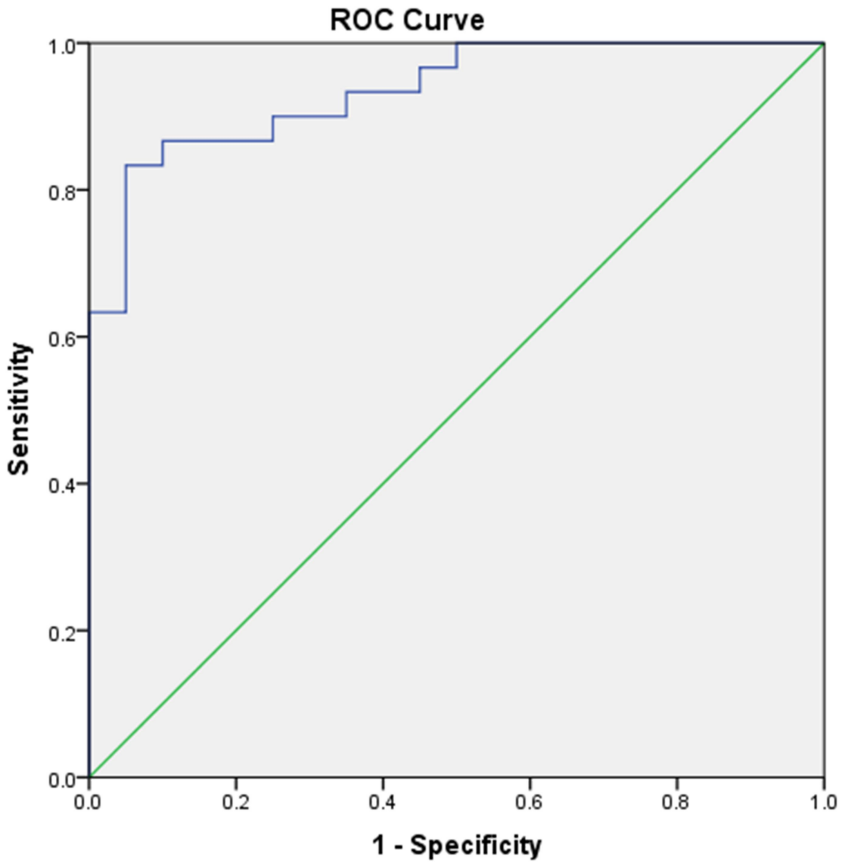
Area Under the Curve

Test Result Variable(s): FBS/HDL

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.907	0.040	0.000	0.827	0.986

a. Under the non parametric assumption
b. Null hypothesis : true area = 0.5

Figure 1: ROC curve analysis for performance of the Novel ratio Fasting blood sugar/High-density lipoprotein (FBS/HDL)



Area Under the Curve

Test Result Variable(s): CHO/FBS

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.935	0.032	0.000	0.871	0.999

a. Under the non-parametric assumption

b. Null hypothesis : true area = 0.5

Figure 2: ROC curve analysis for performance of the Novel ratio Cholesterol/Fasting blood sugar (CHOL/FBS)

DISCUSSION

Isabelle Lemieux et al. research on Total cholesterol/High-density lipoprotein ratio conclude the better risk assessment in insulin-resistant associated ischemic heart disease **[20]**. Mika Enomoto et al. study reports that Low-density lipoprotein/High-density lipoprotein ratio is a better prediction in the progression of Intima-media thickness, one of the risk factors atherosclerosis. Previous studies explain the TyG index associated with the prevalence of symptomatic coronary artery disease and correlate in ischemic stroke in risk assessment of diabetes **[21, 22, 23]**. However, non of the study reported on the FBS/HDL ratio and CHOL/FBS ratio. In our study, the novel ratio FBS/HDL differentiate significantly at <0.001 level between Diabetes and Non-diabetes. In diabetes, if HDL value in the protective level reduces the LDL by attaching and

transporting liver to remove from circulation. Also, the controlled fasting blood sugar reduces the triglyceride because the excess sugar converts into triglyceride. High triglyceride contributes to the hardening of artery walls and causative for coronary artery disease. Hence, in the FBS/HDL ratio, a higher HDL level reduces the ratio. The high FBS/HDL ratio indicates the risk factor for insulin-resistant associated cardiac disease as it significantly correlates with the TyG index. Another novel ratio CHOL/FBS shows a significant difference between Diabetes and Non-diabetes. The normal fasting blood sugar value incorporating with cholesterol give the higher ratio value indicates a normal ratio. In uncontrolled fasting blood sugar, the CHOL/FBS ratio shows the reduced ratio value, indicate risk ratio. Also, CHOL/FBS ratio significant negative correlation with TyG index. (i.e.) The TyG index raises in insulin-resistant risk factor in response to the reduced CHOL/FBS ratio indicating risk ratio. As we mentioned previously, the reduced CHOL/FBS ratio is a risk factor for insulin-resistant associated cardiac complications. In the same way, FBS/HDL versus CHOL/FBS shows a significant negative correlation. As the FBS/HDL ratio increase in risk factor the CHOL/FBS ratio low, but CHOL/FBS low ratio is a risk factor for cardiac and well explained in ROC cut off value as CHO/FBS ratio arrived as positive if less than or equal to 1.35.

CONCLUSION

In conclusion, the FBS/HDL versus CHOL/FBS correlated well with gold standard HOMA-IR. Also, the FBS/HDL ratio and CHOL/FBS ratio is well correlated with the TyG index, and in ROC curve shows good performance. Moreover, increased blood sugar value and reduced HDL value, mentioned in research studies as a risk factor for cardiovascular diseases. Hence, we suggest that the novel FBS/HDL ratio value positive if greater than or equal to 3.37, another novel ratio CHOL/FBS value positive if less than or equal to 1.35 in insulin-resistant non-insulin-dependent diabetes-associated coronary artery diseases and can be used for risk assessment in type 2 insulin resistant associated complication.

Conflicts of interest:

The authors declare no conflicts of interest

REFERENCES

[1] Dobiasova M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). Clinical Biochemistry 2001; 34: 583–588.

[2] McLaughlin T, Reaven T, Abbasi F. Is there a simple way to identify insulin-resistant individuals at increased risk of cardiovascular disease? American Journal of Cardiology 2005; 96: 399–404.

[3] Gaziano JM, Hennekens CH, O'Donnell CJ. Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. Circulation 1997; 96: 2520–2525.

[4] Vera Bittner, B. Delia Johnson, Issam Zineh, William J. Rogers, Diane Vido, Oscar C. Marroquin, C. Noel Bairey-Merz, and George Sopko. The TG/HDL Cholesterol Ratio Predicts All Cause Mortality in Women With Suspected Myocardial Ischemia A Report from the Women's Ischemia Syndrome Evaluation (WISE) American Heart Journal 2009; 157(3): 548–555.

[5] Kannel WB. Risk stratification of dyslipidemia: insights from the Framingham Study. Current Medicinal Chemistry - Cardiovascular & Hematological Agents 2005; 3: 187-193.

[6] Setor K Kunutsor, Francesco Zaccardi, Jouni Karppi, Sudhir Kurl, and Jari A Laukkanen. Is High Serum LDL/HDL Cholesterol Ratio an Emerging Risk Factor for Sudden Cardiac Death? Findings from the KIHD Study. Journal of atherosclerosis and thrombosis 2017; 1: 24(6): 600–608.

[7] Mika Enomoto, Hisashi Adachi, Yuji Hirai, Ako Fukami, Akira Satoh, Maki Otsuka, Shun-Ichi Kumagae, Yasuki Nanjo, Kuniko Yoshikawa, Eishi Esaki, Eita Kumagai, Kinuka Ogata LDL-C/HDL-C Ratio Predicts Carotid Intima1.-Media Thickness Progression Better Than HDL-C or LDL-C Alone. Journal of lipids 2011; Article ID 549137.

[8] Carlos J. Toro-Huamanchumo, Diego Urrunaga-Pastor, Mirella Guarnizo Poma, Herbert Lazaro-Alcantara, Socorro Paico-Palacios, Betzi Pantoja-Torres, Vitalia del Carmen Ranilla-Seguin, Vicente A. Benites-Zapata. Triglyceride and glucose index as an insulin resistance marker in a sample of healthy adults. Diabetes & Metabolic Syndrome: Clinical research and reviews 2019; 13: 1, 272-277.

[9] Jing-Lu Jin, Ye-Xuan Cao, Li-Guo Wu, Xiang-Dong You, Yuan-Lin Guo, Na-Qiong Wu, Cheng-Gang Zhu, Ying Gao, Qiu-Ting Dong, Hui-Wen Zhang, Di Sun, Geng Liu, Qian Dong, Jian-Jun Li.

Triglyceride glucose index for predicting cardiovascular outcomes in patients with coronary artery disease. Journal of thoracic disease 2018; 10(11): 6137-6146.

[10] Pack Insert for Beckman Access Ultrasensitive Insulin method. Beckman Coulter, Brea, USA. 2010.

[11] Trinder, P., Ann Clin Biochem, 1969; 6: 24.

[12] Bucolo, G. and David, H., Clincal Chemistry, 1973; 19: 476.

[13] Saunders W.B., Philadelphia, Tietz N.W. Clinical guide to laboratory tests, 1995. **3rd**edition.

[14] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment-insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28 (7): 412–9.

[15] Gyung-Min Park, Young-Rak Cho, Ki-Bum Won. Triglyceride glucose index is a useful marker for predicting subclinical coronary artery disease in the absence of traditional risk factors. Lipids in Health and Disease 2020; 19: 7.

[16] David Navarro-González, Laura Sánchez-Inigo, Juan Pastrana-Delgado , Alejandro Fernández-Montero, J Alfredo Martinez. Triglyceride-glucose index (TyG index) in comparison with fasting plasma glucose improved diabetes prediction in patients with normal fasting glucose: The Vascular-Metabolic CUN cohort. Preventive Medicine 2016; 99-105.

[17] Javad Alizargar, Chyi-Huey Bai, Nan-Chen Hsieh & Shu-Fang Vivienne Wu. Use of the triglyceride-glucose index (TyG) in cardio-vascular disease patients. Cardio-vascular Diabetology. 2020; 19: 8.

[18] Altman D.G., Machin D., Bryant T.N., Gardner M.J. Statistics with confidence, British medical journal books 2000 2nd edition.

[19] Campbell M.J., Machin D., Walters S.J. Medical statistics, A text book for the health sciences 2007 **4th** edition.

[20] Isabelle Lemieux, Benoît Lamarche, Charles Couillard, Agnès Pascot, Bernard Cantin, Jean Bergeron, Gilles R. Dagenais, Jean-Pierre Després. Total cholesterol/HDL cholesterol ratio vs LDL cholesterol/ HDL cholesterol ratio as indices of Ischemic Heart Disease Risk in men: The quebec cardiovascular

study. JAMA internal medicine 2001; 161(22): 2685-2692.

[21] da Silva A, silva caldas A.P., Miranda Hermsdorff H.H. Triglyceride-glucose index is associated with symptomatic coronary artery disease in patients in secondary care. Cardiovascular Diabetology 2019; 18(1): 89.

[22] Shi W, Xing L, Jing L. Value of triglyceride-glucose index for the estimation of ischemic stroke risk: insights from a general population. Nutrition Metabolism and Cardiovascular Diseases 2019; 30: 2.

[23] Chamroonkiadtikun P, Anan-chaisarp T, Wanichanon W. The triglyceride-glucose index, a predictor of type 2 diabetes development: a retrospective cohort study. Primary Care Diabetes 2019; 14: 2.