Physiological and Molecular Study for Some Cases of Coronary Artery Disease

Zahraa Abdali Mohammad[1], Ali MHussein[2], Hassan R Mobarek[3]

[1]Research Scholar, Corresponding Author, Department of Biology, College of Education for Pure Sciences, Thi-Qar University, Iraq, [2]Professor, Department of Biology, Sumer University , Iraq, [3]Professor, Department of Biology, College of Education for Pure Sciences, Thi-Qar University, Iraq

Abstract

Coronary artery diseases (CAD) known as the disorders of the heart and the blood vessels, which claimed 17.1 million lives a year worldwide. to identify the correlation between the coronary artery disease and some physiological and molecular parameters in men. one hundred blood samples (5 ml) collected from coronary artery diseases patients, the Complete blood count parameters (CBC), Serum concentrations of Creatinine, Glucose, Cholesterol, Triglycerides, HDLLDL, T3 , T4 and TSH were measured in current study. Multiplex polymerase chain reaction used to determine the presence or absence of GSTM1 and GSTT1 genes in the genomic DNA and CYP1A1 as an internal control**.** The results of current study showed significant increase in the total WBC, Neutrophil, Lymphocyte, MCH, MCHC,PLT, MPV, PCT, creatinine, glucose, cholesterol, Triglyceride, LDL andTSH in patients group when compared with control.The percentage of coronary artery diseases were increase 47% when the GSTM1 gene absence in patients group compared with control,while no significant differences were recorded when GSTT1 gene absence and the two genes absence in patient group compared with control.The study concluded that physiological indicators may increase in patients with Coronary artery diseases .

Keywords*: Coronary artery disease, Physiological, GSTM1, GSTT1*

Introduction

Coronary artery diseases (CAD) are known as the disorders of the heart and the blood vessels, which claimed 17.1 million lives a year worldwide [(1)]. Cardiovascular diseases (CVD) is the main causes of death globally[(2)]. Research and epidemiological studies have concluded that 17.3 million people died from CVD in 2008. Of the entire death takes place due to CVD, 80% of the deaths are from low-middle income countries. It has been projected that by the year 2030, >23.3 million people will die annually from Cardiovascular diseases [(3)].

The Coronary artery diseases (CAD) can play a crucial role to decide the cause of sudden death whether natural or unnatural [(4)]. Getting at the root cause of Coronary artery diseases requires a different approach. Coronary artery diseases begins with progressive endothelial injury, inflammatory oxidative stress, diminution of nitric oxide production, foam cell formation, and development of plaques that may rupture to cause a myocardial infarction (MI) or stroke[(5)] Conventional risk factors for the development of CAD are hypertension, hypercholesterolemia, diabetes, sedentary lifestyle, obesity, smoking, and family history have an adverse influence on prognosis in those with established disease[(6)].Atherosclerosis is the main cause of coronary artery disease. Atherosclerosis is often seen as a chronic low-grade inflammatory condition

Corresponding Author:
Zahraa Abdali Mohammad
Department of Biology,
College of Education for Pure Sciences, Thi-Qar University, Iraq,
E-Mail : hawra.mohmad33@yahoo.com

with a complex pathogenesis involving endothelial dysfunction, lipoprotein build up and oxidation, pro-inflammatory cytokines, and various other factors [7,8].

Glutathione S-transferases (GSTs) detoxify environmental agents which influence the onset and progression of disease, Dysfunctional detoxification enzymes are responsible for prolonged exposure to reactive molecules and can contribute to endothelial damage, an underlying factor in coronary artery disease [9].

Methods

The current study included 100 blood samples (5 ml) collected from male patients infected with coronary artery diseases with age ranged between (20-79)years and sleeping in Heart center of Nassiryia city during the period from 1/9/2019 to 1/3/2020 and 50 blood samples collected from males who were not infected with coronary artery diseases as control group. The serum separated and stored at –20C°.

Physiological study

Complete blood count parameters (CBC)were measured for patients and control groups by using coulter (Genex, Germeny), Serum concentrations of Creatinine and Urea were determined by using (Biolabo-France kit) , Glucose, Cholestrol (Plasmatec kit), Triglycerides, HDL and LDL (Biolabo-France kit) , T3 (Bioactive – USA kit), T4 and TSH (Monobind –UAS kit).

Molecular study

DNA Extraction: genomic DNA was extracted from blood samples of patients and control groups by using gSYAN DNA Kit provided from Geneaid American company.

DNA Amplification: Multiplex polymerase chain reaction was used to determine the presence or absence of GSTM1 and GSTT1 genes in the genomic DNA and CYP1A1 as an internal control. The primers of GSTM1 gene were: (forward) 5/- GAACTCCCTGAAAAGCTAAAGC -3/ and (reverse) 5/-GTTGGGCTCAAATATACGGTGG-3/. The primers of GSTT1 gene were: (forward) 5/-TTCCTTACTGGTCCTCACATCTC -3/ and (reverse) 5/- TCACGGGATCATGGCCAGCA -3/. The primers of CYP1A1 gene were: (forward) 5/-GAACTGCCACTTCAGCTGTCT -3/ and (reverse) 5/-CAGCTGCATTTGGAAGTGCTC -3/.

Results

The results of current study showed significant increased in the total white blood cells count, Neutrophil and Lymphocyte (P≤0.05) of patients group when compared with control, while no significant differences were recorded in the Lymphocyte, Eosinophil and Basophil counts between the patients and control group (Table 1).Significant decrease was record in HGB and HCT in patients group when compared with control (P≤0.001), the results showed significant increase in MCH and MCHC and no significant increase in MCV of patients group when compared with control (Table. 2).The results of current study refer to significant increase in PLT, MPV and PCT (P ≤0.001) in patient group compared with control (Table. 3).Significant increase was record in concentration of creatinine and glucose in patients group when compared with control (P≤0.001), while no significant differences in concentration of urea between patients group and control (Table. 4).The results of current study showed significant increase in cholesterol, Triglycerid and LDL concentration in patients group when compared with control (P≤0.01) and significant decrease in HDL concentration in patients group compared with control (P≤0.01) (Table. 4).Significant decrease recorded in T4 concentration of patients group compared with control (P≤0.05), while no significant increase in T3 concentration in patients group compared with control and significant increase in TSH concentration in patients group compared with control (P≤0.01) (Table. 4). The two genes GSTM1 and GSTT1 were amplified with CYP1A1 gene as internal control in current study by using Multiplex PCR, these genes were detect by electrophoresis in Agarose gel, the GSTM1 gene was amplified at 215 bp, GSTT1 gene at 480 bp and CYP1A1 at 312bp (Figure. 1).

Table 1: Comparison between patients and control in WBC and differential of WBC.

Group	Total ofWBC(10^3/µL) Mean ±SD	Neut. (10^3/µL) Mean ±SD	Lym. (10^3/µL) Mean ±SD	Mono. (10^3/µL) Mean ±SD	Eos. (10^3/µL) Mean ±SD	Bas. (10^3/µL) Mean ±SD
Patients	9.55 ± 0.82	5.61 ± 0.09	2.13 ± 0.07	0.580 ± 0.03	0.271 ± 0.05	0.090 ± 0.01
Control	5.01 ± 0.27	2.87 ± 0.26	1.6 5 ± 0.14	0.344 ± 0.04	0.162 ± 0.03	0.041 ± 0.01
P-value	0.040	0.0001	0.061	0.0110	0.524	0.294

Table 2: Comparison between patients and control in RBC and blood parameters

Groups	RBC (106 /µL) Mean ±SD	Hb (g/dL) Mean ±SD	HCT (%) Mean ±SD	MCV (fL) Mean ±SD	MCH (Pg) Mean ±SD	MCHC (g/L) Mean ±SD
Patients	5.01 ± 0.06	13.90 ± 0.11	40.01 ± 0.39	78.68 ±0.68	28.53 ±0.42	351.03 ± 1.85
Control	5.97 ± 0.14	15.03 ±0.23	44.43 ± 0.61	74.55 ±1.17	25.26 ± 0.52	308.27 ± 30.61
P-value	0.0001	0.0024	0.0008	0.0644	0.011	0.0002

Table 3: Comparison between patients and control in PLT, MPV, PCT

Group	PLT (10^3/ µL) Mean ±SD	MPV (fL) Mean ±SD	PCT mL/L Mean ±SD	T3 (nmol/L)	T4 (nmol/L)	TSH (µLU/L)
Patients	232.72 ± 1.37	9.54 ± 0.09	1.83 ± 0.08	1.54 ± 0.09	87.41 ± 0.75	2.85 ± 0.09
Control	163.00 ± 13.43	6.89 ± 0.25	0.110 ± 0.01	1.40 ± 0.13	94.81 ± 4.46	1.81 ± 0.42
P-value	0.0001	0.0001	0.0001	0.388	0.0503	0.0052

Table 4: Comparison between patients and control inCreatinine, Urea , glucose and lipid profile

Group	Creatinine (mg/dl) Mean ±SD	Urea (mg/dl) Mean ±SD	Glucose (mg/dl) Mean ±SD	Cholesterol (mg/dl) Mean ±SD	Triglyceride (mg/dl) Mean ±SD	HDL (mg/dl) Mean ±SD	LDL (mg/dl) Mean ±SD
Patients	1.136 ± 0.05	34.86 ± 1.36	134.79 ± 2.47	237.55 ± 6.01	175.89 ± 9.11	1.89 ±28.30	136.91 ±4.63
Control	0.833 ± 0.06	25.83 ± 1.42	91.00 ± 3.22	126.00 ± 3.98	100.50 ± 4.60	1.077 ±38.67	69.18 ± 3.69
P-value	0.0086	0.109	0.0001	0.0001	0.0001	0.0001	0.0001

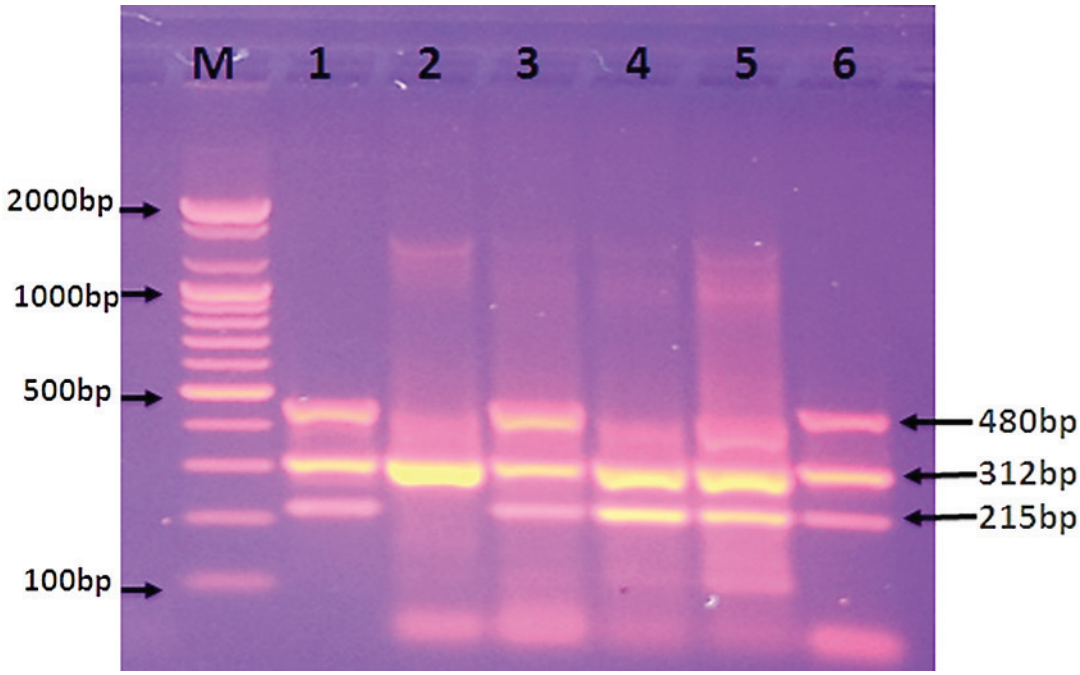

Figure (1): Agarose gel electrophoresis show the Multiplex PCR product analysis forGSTM1 and GSTT1 gene polymorphisms with CYP1A1 gene as internal control. Where M: marker (2000-100bp). The lane (1, 3, 5, and 6) were showed positive amplification of GSTM1 and GSTT1 gene polymorphisms with CYP1A1 gene as internal control at 215bp, 480bp, and 312bp respectively. The lane (2) was showed no amplification of GSTM1 and GSTT1 gene polymorphisms and only internal control at 312bp. The lane (4) were showed positive amplification of GSTM1 gene polymorphisms with CYP1A1 gene as internal control at 215bp and312bp respectively.

Discussion

WBCs participate in the chronic inflammatory process and affect the development of CVD through multiple mechanisms that mediate inflammation, cause proteolytic and oxidative damage to the endothelial cells, block the microvasculature, induce hypercoagulability, and promote infarct expansion [10]. Endothelial cell damage in vasculature is caused by changes in haemodynamics and oxidative stress. The continuous cycle of inflammation from atherosclerotic plaques and arterial wall lesions contributes to elevated levels of C-reactive protein in CAD. The low-grade inflammation, recruitment and activation of leucocytes to the atherosclerotic lesion plays an important role in

ROS generation in an attempt to circumvent the spread of foreign material, while simultaneously exposing biomolecules to oxidative stress[11]. There is evidence from multiple studies in patients with chronic stable heart failure on standard therapy that anaemia in this condition is at least in part due to dilution by increased plasma volume. These studies have shown increased extracellular fluid volume,increased plasma volume (normalized for body mass) in the absence of differences in red blood cell volume and increased plasma volume6 in patients with chronic stable heart failure[12].Anemia increases cardiac output, may lead to eccentric left ventricular hypertrophy, activation of the sympathetic nervous system, and stimulation of the renin angiotensin aldosterone system, and is closely associated with chronic inflammation and increased oxidative stress[13]. Increase MPV index has also been found to independently predict the risk for cardiovascular disease[14]. High PCT, MCHC, and MPV levels in acute coronary syndrome may be beneficial predictive values in terms of complications that may develop, TVCAD, and mortality [15]. MPV is an exact method to measure platelet size which reflects platelet function. It has been shown that larger platelets have higher hemostatical activity than their normal-size counterparts. This in turn escalates the risk for platelets aggregation (due to increased ADP secretion), thrombosis and TE [16].

Several mechanisms are involved in the relationship of serum creatinine with the increased risk for CAD[17]. Serum creatinine was strongly correlated to other indicators of kidney function and to the indices of body water status[18]. Hyperglycemia results in multiple biochemical changes, a few of which we will list: an increase in the reduction of nicotinamide adenine dinucleotide to NADH is thought but not proven yet to be a cellular oxidative stressor; an increase in the production of uridinediphosphate N-acetyl glucosamine is thought to alter cellular enzymatic function. Very importantly, the glycosylation of proteins in the arterial wall is thought to contribute to diabetic atherosclerosis. The nonenzymatic reaction between glucose and arterial wall proteins results in the formation of advanced glycation end products process that is enhanced in hyperglycemia. AGEs are thought to directly interfere with endothelial cell function and accelerate atherosclerosis. Additionally, hyperglycemia increases potent vasodilator and regulator of platelet activation [19]. Endothelial dysfunction is a very early step in atherosclerosis, which is one of the most common pathological manifestations of vascular disease, The interaction between lipoproteins and endothelial cells plays a crucial role in the generation and development of atherosclerosis, Ox-LDL promotes the pathogenesis and development of atherosclerosis and the proliferation, migration and phenotype alteration of vascular smooth muscle cells into foam cells are critical changes in atherosclerosis, It might play a novel role in the pathology [20,21]. In dyslipidemias, hepatic LDL receptors become incompetent; LDL circulates more through the body, its degree of oxidation increases and contact with the vascular endothelium is prolonged Thus, the atherogenic effect is increased [22]. Thyroid hormone mediates the expression of both structural and regulatory genes in the cardiac myocyte[23]. Thyroid hormones exert chronotropic and inotropic effects on the heart, and their main effects are an increase in heart rate, shortening of contraction time and an increase in stroke volume with increased end diastolic volume and cardiac output, resulting in increased left ventricular ejection fraction (LVEF)[24].Furthermore, peripheral resistance is reduced, probably due to both a direct relaxing effect of thyroid hormones on the vasculature and normal or reduced sympathetic tone[25].CAD is a multifactorial disease affected by both acquired and inherited factors [26]. Oxidative stress is the result of overproduction of reactive oxygen species and/or deficiency of antioxidant mechanisms and depends on the balance between generation of ROS and enzymatic or non-enzymatic systems of antioxidative protection [25]. GST is a family of enzymes that detoxify reactive electrophiles, particularly present in tobacco smoke, reactive oxygen species, and known or suspected carcinogenic compounds [27].

Conclusion

The study concluded that physiological indicators may increase in patients with Coronary artery diseases

Ethical Clearance : Taken from University of Thi-Qar ethical committee

Source of Funding : Self

Conflict of Interest : Nil

References

1- Sharma K, Gulati M. Coronary artery disease in women: A 2013 update. Glob Heart 2013;8:105-12.

2- Dyck GJB, Raj P, Zieroth S, Dyck JRB, Ezekowitz JA. The Effects of Resveratrol in Patients with Cardiovascular Disease and Heart Failure: A Narrative Review. Int J Mol Sci. 2019;20(4):904.

3- Capewell, S, Ford, E., Croft, J., Critchley, J., Greenlund, K., Labarthe, D. Cardiovascular risk factor trends and potential for reducing coronary heart disease mortality in the United States of America. Bulletin of the World Health Organization 2010; 88: 120-130.

4- Verma AK, Kumar S, Kumar N, Verma RK, Singh M. Study of atherosclerosis in sudden deaths and its medico-legal relevance. J Indian Acad Forensic Med. 2012;34:12.

5- Esselstyn CB Jr., Gendy G, Doyle J, Golubic M, Roizen MF. A way to reverse CAD? J FamPract 2014;63:356-364b.

6- Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, Budaj A, Bugiardini R, Crea F, Cuisset T, Di Mario C, Ferreira JR, Gersh BJ, Gitt AK, Hulot JS, Marx N, Opie LH, Pfisterer M, Prescott E, Ruschitzka F, Sabaté M, Senior R, Taggart DP, van der Wall EE, and Vrints CJ. 2013 ESC guideline in the management of stable coronary artery disease: The task force on the management of stable coronary artery disease of the European society of cardiology Eur. Heart J. 34 2949-3003.

7- Golia, E.; Limongelli, G.; Natale, F.; Fimiani, F.; Maddaloni, V.; Pariggiano, I.; Bianchi, R.; Crisci, M.; D'Acierno, L.; Giordano, R.; et al. Inflammation and cardiovascular disease: From pathogenesis to therapeutic target. Curr. Atheroscler. Rep. 2014 ; 16 : 435.

8- Libby, P.; Ridker, P.M.; Maseri, A. Inflammation and atherosclerosis. Circulation. 2002 ; 105 : 1135–1143.

9- Phulukdaree, Alisa & Khan, Sajidah&Moodley, Devapregasan&Chuturgoon, Anil. GST polymorphisms and early-onset coronary artery disease in young South African Indians. South African Medical Journal. 2012 ; 102 : 627.

10- Madjid M, Awan I, Willerson JT, Casscells SW. Leukocyte count and coronary heart disease: implications for risk assessment. J Am CollCardiol. 2004; 44(10):1945–56.

11- Hulsmans M, Holvoet P. The vicious circle between oxidative stress and inflammation in atherosclerosis. J Cell Mol Med 2009;14:70-78.

12- Westenbrink, B. D., Visser, F. W., Voors, A. A., Smilde, T. D., Lipsic, E., Navis, G., ... & van Veldhuisen, D. J.Anaemia in chronic heart failure is not only related to impaired renal perfusion and blunted erythropoietin production, but to fluid retention as well. European heart journal. 2007 ; 28(2) : 166-171.

13- Aronow, W. S. Cardiac Arrhythmias-Mechanisms, Pathophysiology, and Treatment, InTech, Rijeka, Croatia, 2014.

14- Yavuz B, Ertugrul DT, Yalcin AA, Kucukazman M, Ata N, Dal K. Increased mean platelet volume in rheumatic mitral stenosis: A possible factor for thromboembolic events. J Cardiol. 2009; 53: 204-7.

15- Karaman, S. and Coskum, A. Do MCHC, MPV, and Procalcitonin Levels Determine Prognosis in Acute Coronary Syndrome? Emergency Medicine International, 2019; 2019:6721279 .

16- Thompson CB, Jakubowski JA, Quinn PG, Deykin D, Valeri CR. Platelet size and age determine platelet function independently. Blood 1984; 63: 1372-5.

17- Hall WD. Abnormalities of kidney function as a cause and a consequence of cardiovascular disease. Am J Med Sci. 1999; 317(3): 176-182.

18- Bagheri B, Radmard N, Faghani-Makrani A, Rasouli M. Serum Creatinine and Occurrence and Severity of Coronary Artery Disease. Med Arch. 2019 Jun;73(3):154-156. doi: 10.5455/medarh.2019.73.154-156.

19- A. D'Souza, M. Hussain, F. C. Howarth, N. M. Woods, K. Bidasee, and J. Singh, "Pathogenesis and pathophysiology of accelerated atherosclerosis in the diabetic heart," Molecular and Cellular Biochemistry, 2009 ;331 (1-2) : 89–116 .

20- Sumino H, Nakajima K, Murakami M. Possibility of New Circulating Atherosclerosis-Related Lipid Markers Measurement in Medical and Complete Medical Checkups: Small Dense Low-Density Lipoprotein Cholesterol and Lipoprotein Lipase. RinshoByori 2016;64(3):298-307.

21- Johnston N, Jemberg T, Lagerrvist B, Siegbehn A, et al. Improved identification of patients with coronary artery disease by the use of new lipid and lipoprotein biomarkers. Am J Cardiol

2006;97(5):640-5.

22- Kopin, L.; Lowenstein, C.J. Dyslipidemia. Ann. Intern. Med. 2017, 167, ITC81–ITC9.

23- Danzi S, Klein I. Thyroid hormone and the cardiovascular system. Minerva Endocrinologica. 2004;29:139 –150.

24- Klein I, Ojamaa K: Thyroid hormone and the cardiovascular system. N Engl J Med 2001;344:501–509.

25- Al-fahham, AA. . Correlation between oxidative stress and thyroid hormone levels in infertile women .inter. j. Sci. Res. Pub. 2015 ; 5(12) :128-131 .

26- Zhao Q, Wei H, Liu D, et al. PHACTR1 and SLC22A3 gene polymorphisms are associated with reduced coronary artery disease risk in the male Chinese Han population. Oncotarget. 2017;8(1):658.

27- Li R, Boerwinkle E, Olshan AF, Chambless LE, Pankow JS, Tyroler HA, Bray M, Pittman GS, Bell DA, Heiss G. Glutathione S-transferase genotype as a susceptibility factor in smoking-related coronary heart disease. Atherosclerosis. 2000; 149: 451-62.