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Association of Acid Ceramidase Enzyme Polymorphisms in Patients with Ischemic Heart Disease- A filed study in Karbala Province of Iraq

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Abstract

Myocardial infarction (MI) is a common occurrence in all communities worldwide and is one of the leading causes of hospitalization. Heart cell death occurs after MI because blood flow is cut off to that area of the heart after a rupture or erosion of an atheromatous plaque in a coronary artery. The current study aimed to detect the principal gene of myocardial infarction. A total of 120 participants were included in this study, 60 of whom were patients suffering from acute coronary syndrome, and 60 were healthy individuals who served as control. The patients were admitted to Imam Al-Hussein Medical City, Karbala, Iraq, for cardiac diseases and surgery. Blood sample was collected from each patient (2 ml), placed in EDTA-tube, and used for genetic study. Considering the distribution of genotype groups rs7844023 among patients with ischemic heart disease (IHD) and control, it was found that six patients with IHD had TT genotype in their chromosome, 14 patients had TC, and 40 patients had wild CC genotype frequency. Regarding the distribution of the genotype groups rs2427746, the result indicated that 23 IHD patients had GG genotype in their chromosome, 17 patients had AG, and 20 patients had wild AA genotype frequency.

Keywords: Cardiac disease, Ischemic heart disease, rs7844023, rs2427746

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1. Introduction

The heart muscle can die due to coronary artery disease, which is called a myocardial infarction. Ischemic heart disease was the leading cause of death in high- and middle-income nations in 2022, second only to lower respiratory infections in low-income [1]. Ischemic heart disease (IHD) mortality rates have decreased in most high-income countries although cardiovascular disease remains the leading cause of death worldwide, especially in the United States [2]. Moreover, the IHD prevalence is rising as a leading killer in South Asia [3]. Patients with unstable angina or other forms of acute coronary syndrome need to have their symptoms and indicators evaluated carefully [4]. To properly diagnose angina, a complete medical history must be compiled first. A diagnosis based purely on the patient's history can be relatively accurate; however, a physical examination and objective tests are often necessary to confirm the diagnosis and evaluate the severity of the underlying condition [5]. Incidences of chronic heart disease (CHD) are more likely in those who have already experienced some risk factors, such as cardiac events, peripheral arterial disease, abdominal aortic aneurysm, and carotid artery problems. Some people do not have common risk factors, but elevated low-density Lipoprotein (LDL) cholesterol is the primary goal of cholesterol-lowering therapy [6].

Those with a strong family history of certain cardiovascular disease (CVD) forms, such as IHD, are more likely to develop heart diseases [7]. Multiple studies have shown that having a family history of IHD is a significant risk factor for the disease [8-10]. Ischemic heart disease might suddenly strike down individuals with no prior warning. Risk factors may represent a unique underlying disease process, but only if genetic testing is used to differentiate between cardiovascular risk factors and chromosomal events. The identification of complex monogenic disorders, such as cardiac ailments, requires the addition of a detailed, targeted investigation [11].

2. Materials and methods

A total number of 120 patients were included in this study. The study was carried out on 60 patients suffering from acute coronary syndrome admitted to Imam Al-Hussein Medical City, Karbala, Iraq, for cardiac diseases and surgery, from March 2021 until the end of December 2021. The diagnosis of symptomatic acute coronary syndrome was made by the cardiologists depending on the presence of typical symptoms, electrocardiogram (ECG), and biomarkers. The control group in the current study included 60 healthy individuals of both sexes. Blood sample was collected from each participant (2 ml), placed in EDTA-tube, and used for genetic study.

2.1. DNA extraction

DNA Genome was extracted from blood sample according to protocol G-spin™ Total DNA Extraction Kit.

2.2. Polymerase chain reaction

Polymerase chain reaction (PCR) was performed using specific primer pairs designed for the acid ceramidase (AC) gene. Based on NCBI database, all gene information and single nucleotide polymorphisms (SNPs) detail were collected using Genius software designed. The primer sequences used for PCR amplification of AC gene rs7844023 and rs2427746 SNPs are presented in Tables 1 and 2, respectively. One PCR reaction was performed, one with outer forward primer rs7844023 (OF) and outer reverse primer rs7844023 (OR) and inner forward rs7844023 (IF) and inner reverse rs7844023 (IR) for detection of AC rs7844023 SNPs. The same thing happened with rs2427746 SNP; meaning that one PCR reaction was performed, one with outer forward primer rs2427746 (OF) and outer reverse primer rs2427746 (OR) and inner forward rs2427746 (IF) and inner reverse rs2427746 (IR) for detection of AC rs2427746 SNPs, and run side by side on an agarose gel.

3. Results and discussions

Patients involved in this study had an age of 56.61 ± 13.32 years, about 28% of the patients aged <50 years, and 72% were ≥ 50 years old (Figure 1). Age groups could significantly affect the prevalence of IHD ($P< 0.05$). However, MI may occur at any age, but it occurs mainly in individuals within the age range of 55-85 years [12].

The MI frequency rises sharply at the age of 40, partly because risk factors started to accumulate around the age of 44 years [13]. Few studies have focused on ischemic stroke in young adults, and the etiology of such a stroke is complex and rarely studied in this age group. Some of them identify the various risk factors for stroke in young and elderly people and predictors of in-hospital mortality in patients [14].

Considering the distribution of genotype groups rs7844023 among IHD patients and control, 6 patients with IHD had TT genotype in their chromosome, 14 had TC, and 40 had wild CC genotype frequency. There was no significant association between mutation factor TT and heterozygote patient TC (OR = 3.21, 95% CI: 0.78-13.24). Moreover, no significant difference was found between mutation factor TT and patients with wild CC (OR = 0.975, 95% CI: 0.25-3.79, $P> 0.05$). This might result from the sample size used in the study, and the fact that two-thirds of patients carried the dominant gene CC genotype frequency. This result disagrees with the findings of Lewis et al.[15], reporting that STRRIDE I and STRRIDE II individuals have T allelic frequency of 0.503 and 0.489, respectively.

The distribution of genotype groups rs2427746 among IHD patients and control indicated that 23 patients with IHD had GG genotype in their chromosome, 17 had AG, and 20 had wild AA genotype frequency. The result showed no significant association between mutation factor GG and heterozygote patient AG odd ratio (OR = 1.57, 95% CI: 0.81-6.91). On the other hand, there was a significant difference between mutation factor GG and patients with wild AA (OR = 5.4, 95% CI: 2.07-14.41, $P< 0.05$). The results revealed a significant association between alleleic frequency of the control and that of the patients (OR = 3.2, 95% CI: 1.48-6.94) Researchers increasingly seek the SNPs linked to disease phenotypes. The obtained results of the current study can shed light on how diseases work in the body, which will help the development of more effective preventative and treatment methods. Discovering disease susceptibility loci in the human genome has never been easier, thanks to genome-wide association studies (GWAS). It is a non-prescriptive method that uses many markers to identify genetic variations associated with a disease phenotype in a large number of samples [16].

Table 1. Specific primers of rs7844023 SNPs

Primer	Sequence (5´–3´)	Allele	Size (bp)	Company/ Country
Outer Forward	GAAACTGTCATTTGCAGCTCACA	355		BIONEER/ Korea
Outer reverse	GTGACCAGCGACACATGACTTAA			
Inner forward	TCCAACCACACCTGGGTTTT	T	172	
Inner reverse	CAGGGAATCCAGTAGCAGACG	C	244	

Table 2. Specific primers of rs2427746 SNPs

Primer	Sequence (5´ –3´)	Allele	Size (bp)	Company/ Country
Outer Forward	CTCTGGATGTCCCTGAGACACTT	379		BIONEER/ Korea
Outer reverse	CCAAGTGGAAAAGTTATGCAGGT			
Inner forward	CCTCTGAAGTTCTGACAGTGAGATG	G	118	
Inner reverse	GGGCAAATCTGCATGAAGCT	A	243	

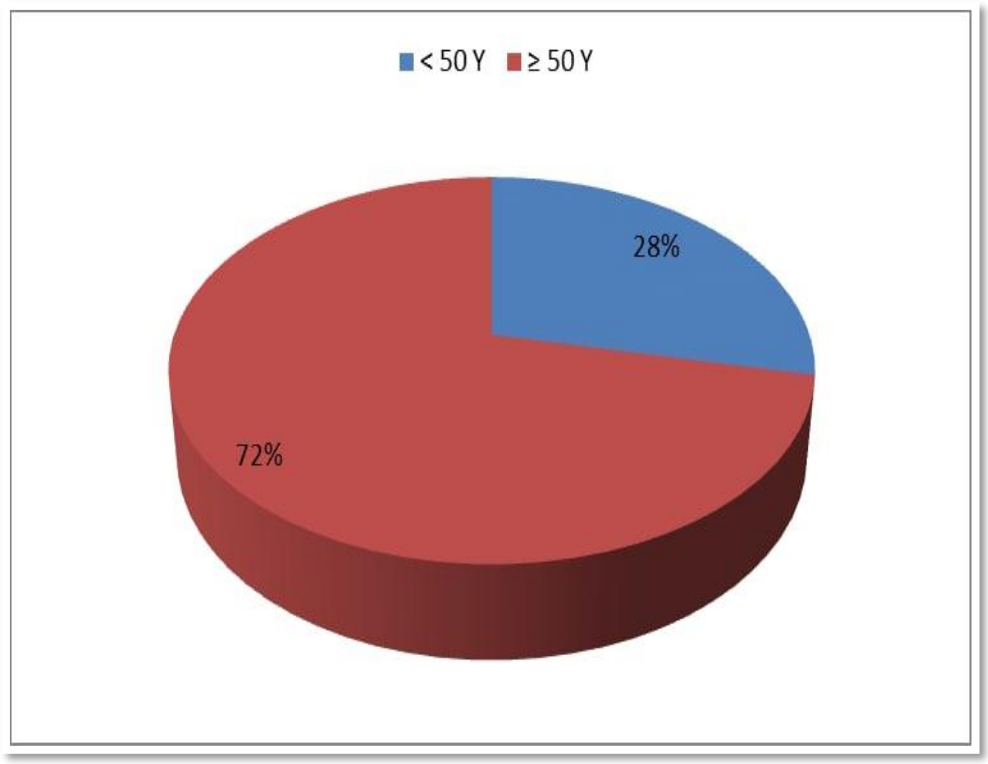

Figure 1. Age distribution in patient group

Table 3. Distribution of genotype groups “rs7844023” among patients with ischemic heart disease and control

Study of genotypes "rs7844023"	Patient with IHD N(%)	Control	Odd ratio	95% CI	P value
TT	6(10%)	2(3.3%)	Reference	_	_
TC	14(23.3)	30(50%)	3.21	0.78-13.24	0.105
CC	40(66.7)	28(46.7%)	0.975	0.25-3.79	0.97
T	13	17	Reference	_	_
C	47	43	0.59	0.26-1.35	0.217

Table 4. Distribution of genotype groups "rs2427746" among patients with ischemic heart disease and control

Study of genotypes " rs2427746"	Patient with IHD	Control	Odd ratio	95% CI	P value
GG	23(38.3%)	8(13.3%)	Reference	_	_
AG	17(28.3%)	14(23.3%)	1.57	0.81-6.91	0.114
AA	20(33.4%)	38(63.4%)	5.4	2.07-14.41	0.0054
G	31	15	Reference	_	_
A	29	45	3.2	1.48-6.94	0.003

4. Conclusions

Regarding the distribution of the genotype groups rs2427746, the result indicated that 23 IHD patients had GG genotype in their chromosome, 17 patients had AG, and 20 patients had wild AA genotype frequency. This shows association between acid ceramidase enzyme polymorphisms in patients with ischemic heart disease in Karbala city of Iraq.

References

[1] World Health Organization (2022). The global burden of disease. Geneva: World health organization.

[2] V. L. Roger, A. S. Go, D. M. Lloyd-Jones, E. J. Benjamin, J. D. Berry, W. B. Borden, D. M. Bravata, S. Dai, E. S., Ford, C. S. Fox. (2012). Heart disease and stroke statistics–2012 Update: A report from the American Heart Association. Circulation, 125(1): e2-e220. DOI: 10.1161/cir.0b013e31823ac046

[3] R. Gupta, P. Joshi, V. Mohan, K. S.Reddy, S. Yusuf. (2008). Epidemiology and causation of coronary heart disease and stroke in India. Heart, 94(1): 16-26. DOI: 10.1136/hrt.2007.132951

[4] K. Kimura, T. Kimura, M. Ishihara, Y. Nakagawa, K. Nakao, K. Miyauchi, K. Tsujita, , Hagiwara, N. Miyazaki, S.J. Ako. (2019). JCS 2018 guideline on diagnosis and treatment of acute coronary syndrome. Circulation Journal, 83(51085-1196. DOI: 10.1253/circj.cj-19-0133

[5] C .Albus, J. Barkhausen, E. Fleck, J. Haasenritter, O. Lindner, S. Silber. (2017). The diagnosis of chronic coronary heart disease. Deutsches Ärzteblatt International, 114(42): 712-719. DOI: 10.3238\\arztebl.2017.0712

[6] G. Matfin, C. M. Porth. (2009). Disorders of blood flow in the systemic circulation. Pathophysiology, concepts of altered health states. 8th ed. Porth CM, G. Matfin (Eds). Wolters Kluwer Health, Lippincott Williams & Wilkins, p. 477-503.

[7] The Texas heart institute, Heart Information Center, 2012, update: February 2012 available at: http//texasheart.org/HIC/Topics/HSmart /riskfact.cfm ladt

[8] R. Patel, Sh. Ye. (2011). Genetic determinants of coronary heart disease: New discoveries and insight from genome wide association studies, BMJ Journal 97(18):1463-1473. DOI: 10.1136/hrt.2010.219675

[9] S. Sivapalaratnam, S. M. Boekholdt, M. D. Trip, M. S. Sandhu, R. Luben, J. J. Kastelein, N. J. Wareham, K. T. Khaw. (2010). Family history of premature coronary heart disease and risk prediction in the EPIC-Norfolk prospective population study. Heart, 96(24): 1985-1989. DOI: 10.1136/hrt.2010.210740

[10] G. Fischer, F. N. Tubiello, H. Van Velthuizen, D. A. Wiberg. (2007). Climate change impacts on irrigation water requirements: Effects of mitigation, 1990–2080. Technological Forecasting and Social Change, 74(7): 1083-1107. DOI: 10.1016/j.techfore.2006.05.021

[11] S. Huma, R. Tariq, F. Amin, K. T. Mahmood. (2012). Modifiable and non-modifiable predisposing risk factors of myocardial infarction-A review. Journal of Pharmaceutical Sciences and Research; Cuddalore, 4(1): 1649-1653. Available at: https://www.proquest.com/docview/1009276246

[12] A. Groenewegen, F. H. Rutten, A. Mosterd, A. W. Hoes. (2020). Epidemiology of heart failure. European Journal of Heart Failure, 22(8): 1342-1356. DOI: 10.1002/ejhf.1858

[13] J. Putaala, A. J. Metso, T. M. Metso, N. Konkola, Y. Kraemer, E. Haapaniemi, M. Kaste, T. Tatlisumak. (2009). Analysis of 1008 consecutive patients aged 15 to 49 with first-ever ischemic stroke. Stroke, 40: 1195-1203. DOI: 10.1161/STROKEAHA.108.529883

[14] H. K. Ibrahem. (2020). Stroke in young versus elderly clinical characteristic and in hospital mortality a retrospective cross-sectional study. Sohag Medical Journal, 24(3): 94-99. DOI: 10.21608/smj.2020.35598.1175

[15] L. S. Lewis, K. M. Huffman, I. J. Smith, M. P. Donahue, C. A. Slentz, J. A. Houmard, M. J. Hubal, E. P. Hoffman, E. R. Hauser, I. C. Siegle. (2018). Genetic variation in acid ceramidase predicts non-completion of an exercise intervention. Frontiers in Physiology, 9, 781. DOI: 10.3389/fphys.2018.00781

[16] T. H. Pers, P. Timshel, J. N. Hirschhorn. (2015). SNPsnap: A web-based tool for identification and annotation of matched SNPs. Bioinformatics, 31(3): 418-420. DOI: 10.1093/bioinformatics/btu655