Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1561-1574
Received 25 April 2021; Accepted 08 May 2021.

An investigation of *SORT1* gene polymorphism with lipid profile biomarkers in Iraqi patients having Myocardial infarction.

Batool Luay*1, Mufeed Jalil Ewadh2 Shokry Faaz Alsaad3

1College;of Health and Medical Technology, Al-Zahraa University for Women, Kerbala, Iraq.
2,3 College of medicine, University of Babylon, Hilla, Iraq.
batoolluay1992@gmail.com

Abstract:

Introduction:

Myocardial infarction (MI) is one of the greatest frequent encounter causes for hospital admittance besides is common see in all populaces worldwide. The limit researches are carry out in Kerbala Province populace, and we object to examine the molecular relationship of single nucleotide polymorphism (SNPs) recognized done GWAS investigates in Myocardial infarction patient groups in Karbala Province populace.

Method: The case-control study design. This study involved (140) samples collected from patients with MI and control group**.** The blood five ml samples determination by collect in two changed tubes, besides 3mL of coagulate blood determination to use for biochemical analysis (lipid tests) besides 2mL determinate to use for molecular (DNA) analysis. Genomic DNA determinate extract by collect blood sample, besides specific primer can be design for chose SNP **(*SORT1*-rs464218 polymorphisms)** beside use Genotype technique is Allele specific-PCR.

Results: The ***rs464218*** polymorphisms was significant associate with genotype in Odd Ratio (O.R.) analysis ($p < 0.05$) in Heterozygous genotype (GA) of *SORT1* gene. The correlate in the middle of lipid profile, BMI and genotype have shown non association of rs464218 variant by combine parameters, the ANOVA analyse is carry out by research effect of (rs464218) variant on variance parameter. The analyse distribute of the variable around various genotype in select polymorphism. BMI was non-association with any of genotype in (rs464218) variant. Also the non-association in result with TG, TC, HDL-C, LDL-C, VLDL-C in (rs464218) *SORT1* gene (p=0.255), (p=0.31), (p=0.187) (p=0.416) and (0.213) were non-significantly associated variants respectively.

Conclusion: The outcomes of this research confirm the (rs464218) polymorphism in *SORT1* gene SNP is associated in Kerbala Province populaces.

Keywords: Genome-wide association studies, Myocardial infarction, SORT1, genetics.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1561-1574
Received 25 April 2021; Accepted 08 May 2021.

Introduction

Coronary artery diseases (CAD) has become record widespread serious global burden of morbidity and mortality in industrialized countries. Accord to Third research via World Health Organization, 12 million individuals death every year of CVD worldwide, and it is estimated that via 2025, cardiovascular death on worldwide measure determination probable exceed the all main diseases groups, containing infection, trauma, and cancer (World Health Organization; Nascimento *et al.*, 2019). Atherosclerosis is considered main cause of acute myocardial infarction (AMI), in which 70% of fatal events among patients with AMI are caused by occlusion from atherosclerotic plaques (Bhatt *et al.*, 2006).Atherosclerosis is considered via vascular inflammation, endothelial dysfunction, besides formation of atherosclerotic plaque. This buildup of atherosclerotic plaque causes an inadequate supply of oxygen to myocardial tissue, leading to myocardial hypoxia. Consequently, the plaque rupture and atherothrombosis cause further narrowing of coronary arteries and almost occluding the blood flow, leading to fatal acute coronary syndromes. The most evident manifestation of CAD is the AMI (Scheen, 2018).

In Iraq, the epidemiological statistics on prevalence besides incidence of CAD as evidence of awareness are limited due to the unavailability of indication based national guideline for organization of cardiovascular disease (Traina *et al.*, 2017). The prior study in 2014, cardiovascular disease mortality was estimated to account for 33% in Iraq (GBD 2015 Eastern Mediterranean Region Cardiovascular Disease Collaborators, 2018).

Sortilin (*SORT1* gene) was be recognized done GWAS researches, it seems arranged chromosome 1p13.3 region (Hubáček, 2016). *SORT1* gene is encode for sorting protein that performance an central role in uptake of lipid (Arvind *et al.*, 2014). *SORT1* SNPs are among first that are associate by together LDL -C level besides CHD in GWASs (Samani *et al.*, 2007), Risk of MI is decreased via (40%) in homozygous carrier compare to non-carriers (Kathiresan *et al.*, 2008).

The *SORT1* gene encode sortiline, its was identified to be there a multi-ligand cells surfaces receptors besides intra-cellular traffick, its theorized the sortiline may possibly involve in traffick besides pre secretory degradate of VLDL-C in hepatocyte to decrease VLDL-C secrete and eventual circulate LDL-C level. The over expression of *SORT1* gene is show to decrease VLDL-C, TG besides apo-lipoprotein B secrete in mouse, shown in (Figure 1a) (Musunuru *et al.*, 2010).

The difference to expectation since human genetic finding, the *SORT1* gene hit mice by expression of abnormal sortiline protein go across by LDL-R lacking contextual exhibit decrease LDL-C besides decrease VLDL-C secrete (Kjolby *et al.*, 2010). Additional

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1561-1574
Received 25 April 2021; Accepted 08 May 2021.

supported for the result move toward from one more hit typical with whole absence the *SORT1* gene (Zeng, Racicott and Morales, 2009), which likewise confirmed decrease VLDL-C secret (Strong *et al.*, 2012). The results suggested that, paradoxical, together hepatic over expression besides whole insufficiency of *SORT1* gene decrease VLDL-C secreted in mouse. The recent be present confirmed the sortiline bind LDL-C direct besides is a physiological related cells surface LDL-C receptor in liver (Strong *et al.*, 2012). The recent research prove the sortiline is involve in LDL-C uptake via macrophage, shown in (Figure 1b) (Patel *et al.*, 2015). It is notable the sortiline was chief recognized through GWASs as taking a role in LDL-C metabolism (Orho-Melander, 2015).

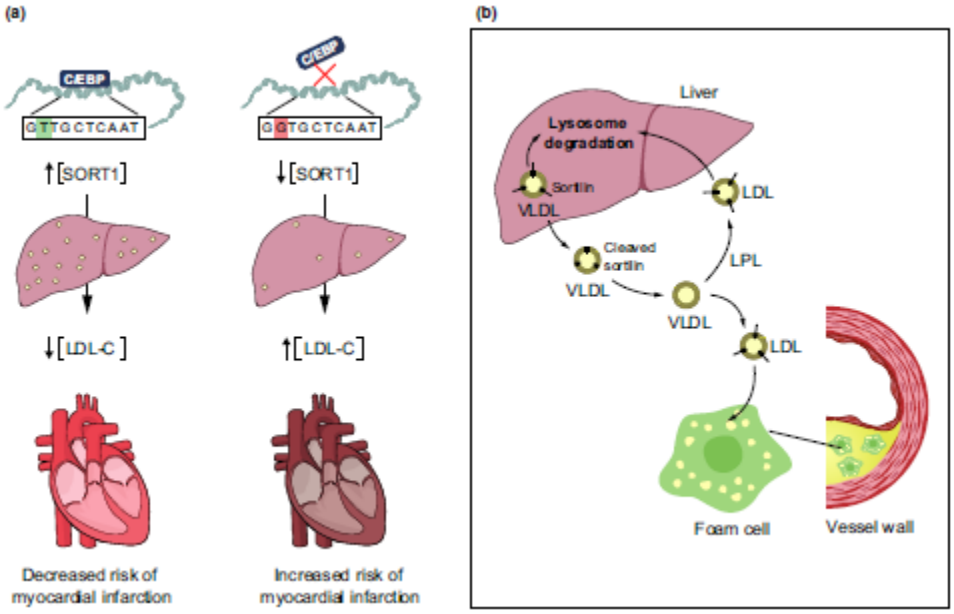

Figure (1) :- Identificational of sortiline1 via GWAS in human (Rajpathak *et al.*, 2009; Musunuru *et al.*, 2010; Preiss *et al.*, 2011; Orho-Melander, 2015; Patel *et al.*, 2015)

Methods:

The case-control study design. This study involved (140) samples collected from patients with MI and control group**.** The opt individual parity of 70 patients besides 70 control groups. All samples were collected from June 2019 till February 2020. They were selected from coronary care unit (CCU) department of Al-Imam Al-Hussein medical teaching City in Karbala Province. The blood five ml samples must be collected in

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1561-1574
Received 25 April 2021; Accepted 08 May 2021.

twofold different tubes, besides 3mL of coagulate blood use for lipid tests (biochemical analysis) besides two mL use for DNA tests (molecular analysis). Genomic DNA was extract by collect blood sample, besides specific primer was design for opt SNP **(*SORT1* gene -rs464218 polymorphisms)** besides Allele specific-PCR.

Primer of amplificated product was examined *SORT1* gene was amplified via use specific primer, are shown in table (1).

Table (1): Genotype technique, Primers Sequence, Annealing Temperature (AT) and Product Size of *SORT1* Gene.

***SORT1* Gene (C>T) or (G>A) SNPs**	
rs464218	
Forward primer (C allele)	CCACTTCTGTGTGTTCTGCATTGC
Forward primer (T allele)	CCACTTCTGTGTGTTCTGCATAGT
Reverse primer (common allele)	TGTGTGAGGAGCTGGTGTGCAGTG
Product size **Annealing Temperature (AT)** **Genotype technique**	218 base pair (bp) 64°C Allele Specific-Pcr

Results:

During this study process, 70 cases of Myocardial infarction Patients and 70 control groups was enrolled to study design (case-control) study. The **mean ± SD** age of patients besides control groups 58.4 ±10.7, and 58.771 ±10.9 and t-test were found to be 0.83, there was non-significant difference in age between control and MI patient groups. This age matching helps to eliminate differences in parameters. However, the clinical details were non-significantly difference between patients besides control groups on gender, BMI and TC, TG, LDL-C, VLDL-C parameters ($p > 0.05$); While the highly significant difference association with HDL-C between control and MI patient groups the p-value ($p < 0.01$) was shown in table (2).

Table (2): Demographic characteristics between Myocardial infarction Patients groups and Control groups.

Parameters	**Myocardial infarction Patients groups**	**Control groups**	**P value**

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1561-1574
Received 25 April 2021; Accepted 08 May 2021.

Gender(Male:female)	46/24	35/35	0.06
Age (years)	58.4 ±10.7	58.771 ±10.9	0.83
BMI (Kg / m^2)	27.775±4.779	28.327±3.3296	0.5666
HDL-C (mg/dl)	34.743±7.0919	39.752±8.1156	0.000*
TG (mg/dl)	172±61.051	170.21±91.05459	0.89278
TC (mg/dl)	159.72±27.807	153.35±38.38460	0.26801
LDL-C (mg/dl)	87.985±30.4039	83.877±28.1667	0.40832
VLDL-C (mg/dl)	33.417±13.345	34.042±18.2109	0.81708

***HS: Highly significant difference (P < 0.01).**
significant between Myocardial infarction Patients groups and Control groups. TC:Total cholesterol, TG:Triglycerides, SD:standard deviation, MI:Myocardial infarction, BMI:Body mass index.

Genotyping of *SORT1* gene there was GG polymorphism of *SORT1* gene is determine via allele specific-Pcr. The Pcr process was conducted used for each sample. Result of the first process which was conducted to determine the G allele and A allele, resulted in one bands for each allele, when visualized on agarose gel electrophoresis. These bands was 218 base pair (bp) represent presence of G allele or A allele or together GA, while absence of G allele (G>A) was assumed when 218 bp band was visualized in Homozygous mutation genotype, as shown in figure (2).

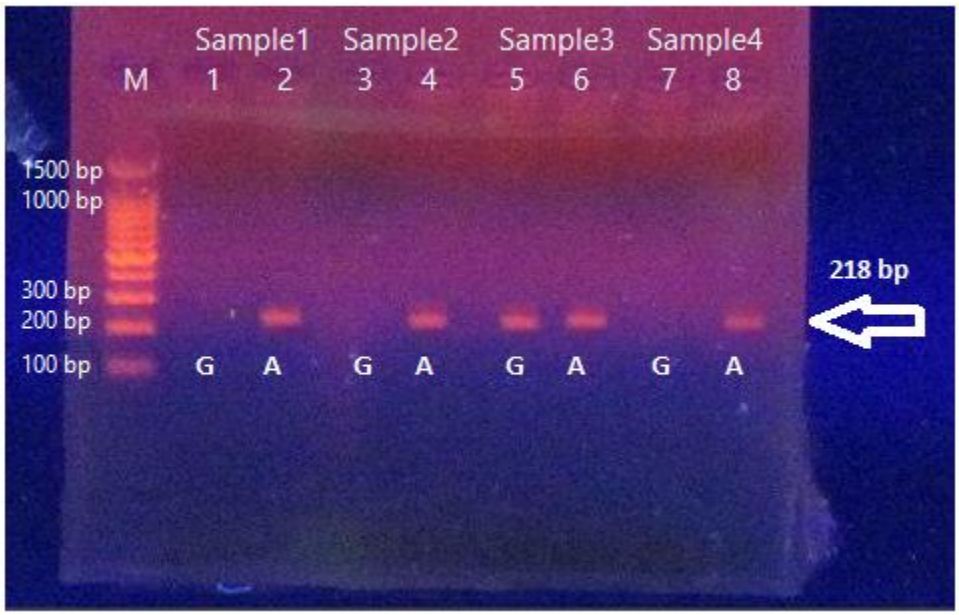

Figure (2): The electrophoretic pattern, PCR product of *SORT1* Gene (G>A) SNP in (1.5%) agarose gel electrophoresis, time (40 minute), voltage (55 V) besides 5µL of AS-Pcr product loaded in each well, where the lanes are:

- **Lane M: DNA size marker Ladder 100 bp.**
- **Lane 2,4, 5,6,8: PCR product (one band 218 bp).**
- **Lane 1 and 2 (Sample 1): no band (218 bp) for G and one band for A are homozygous genotype (AA).**
- **Lane 3 and 4 (Sample 2): no band (218 bp) for G and one band for A are homozygous genotype (AA).**
- **Lane 5 and 6 (Sample 3): Two bands (218 bp) for G and A, are heterozygous genotype (GA).**
- **Lane 7 and 8 (Sample 4): no band (218 bp) for G and one band for A are homozygous genotype (AA).**

The association of Genotype, in this research, **rs464218** polymorphism was carried out between the cases Myocardial infarction Patients groups and controls. Variant appear in HWE in together groups. Allele besides Genotype frequency of patients and control. GG, GA and AA are three genotype variant were detect in rs464218 (*SORT1*) gene. Genotypes distribution between Myocardial infarction Patients and healthy control groups, From documented in the table (3), can express the homozygous mutation genotype AA was recurrent in MI patient group, also the homozygouse mutation genotype AA was more abundant genotype in the control groups.

Table (3): Genotyping of *SORT1* gene polymorphism with allele frequency in Myocardial infarction Patients and Control groups.

Groups	Genotype of *SORT1*			Total	Variant Allele frequency	
	GG	GA	AA		G	A
Patients with MI	9	22	39	70	0.29	0.71
Control	12	0	58	70	0.17	0.83
Total	21	22	97	140	-	-

GG: Wild Homozygous genotype, GA: Heterozygous genotype and AA: Homozygous mutation genotype

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1561-1574
Received 25 April 2021; Accepted 08 May 2021.

The genetic influence was calculated, it characterizes the influence to detect significantly difference of ($p<0.05$) for *SORT1* gene. In general, these results are caused by relative small sample size (Ellis, 2010). Genotyping frequency of the *SORT1* gene was consistents by HWE ($p > 0.05$) both patients with MI patient groups. However, it is deviate by HWE ($p<0.05$) in healthy control groups, as documented in table (4). Deviation by HWE might be attribute to small sample size (Zintzaras, 2010).

The results not consistent with HWE, when the ($p<0.05$). The instruction to estimate the significantly of the findings, Chi-squared (X^2) value is use to examine Odds Ratio (OR) and significantly of genotype and variant allele frequencies, as documented in tables (4), (5).

Table (4): The observed genotype frequencies are consistent with results of Hardy Weinberg Equilibrium and Variant allele frequency for *SORT1* Gene (G>A) SNP genotypes in Patients with MI and the Controls Groups.

Groups with *SORT1* Gene	Chi-squared value (X^2)	P-value	Variant allele frequency
Patient with MI	3.703	0.0543	0.71
Control	70	0.000	0.83

The results consistent with HWE
p value >0.05, X^2: Chi-squar.

Relevance of *SORT1* Gene (G>A) Polymorphism with Myocardial infarction Patients and the Controls Groups: The genotypes distribution and frequency of *SORT1* gene (G>A) SNP as shown in Table (9). The analysis of results indicated that the *SORT1* gene (G>A) SNP genotype frequencies of wild homozygous genotype (GG) was 9 (12.9%), heterozygous genotype (GA) was 22 (31.4%) and homozygous mutation genotype (AA) was 39 (55.7%) in MI patients groups and 12 (17.1%), 0.5 (0.7%) and 58 (82.9%) in controls groups.

The heterozygous genotype (GA) of *SORT1* gene (G>A) SNP was found to be significantly higher (OR=**0.0169**, CI 95% = [**0.0009-0.315]**, P=**0.006**) the risk of MI with respect to those of the wild homozygous genotype (GG) of *SORT1* gene (G>A) SNP as reference. The homozygous mutation genotype (AA) of *SORT1* gene (G>A) SNP was found to be non-significantly higher (OR=**1.115**, CI 95% = **[0.429-2.898]**, P=**0.823**) the risk of MI with respect to those of the wild homozygous genotype (GG) of *SORT1* gene (G>A) SNP as reference. As documented in table (5).

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1561-1574
Received 25 April 2021; Accepted 08 May 2021.

Table (5):- Genotypes Distribution, Odd ratio, confidence interval (CI 95%) and Frequency of *SORT1* Gene (G>A) SNP in Myocardial infarction Patients and the Controls Groups.

Genotype of *SORT1*	MI patient	Control	O.R.	95% CI	p-value
Wild Homozygous genotype (GG)	9 (12.9%)	12 (17.1%)	Reference	Reference	Reference
Heterozygous genotype (GA)	22 (31.4%)	0.5 (0.7%)	0.0169	0.0009-0.315	0.006*
Homozygous mutation genotype (AA)	39 (55.7%)	58 (82.9%)	1.115	0.429-2.898	0.823
Total	70 (100%)	70 (100%)	-	-	-

* **Significantly difference (P < 0.05).**

In this research, heterozygous genotype (GA) *SORT1* gene (G>A) SNP was result significantly difference (P=**0.006**) higher the risk of MI with respect to those of the wild homozygous genotype (GG) of *SORT1* gene (G>A) SNP as reference, while the homozygous mutation genotype (AA) of *SORT1* gene (G>A) SNP was result non-significant (P=**0.823**) higher the risk of MI with respect to those of the wild homozygous genotype (GG) of *SORT1* gene (G>A) SNP as reference, its documented in table (5).

Table (6): Correlate between lipid profile, BMI and genotyping (Mean±SD) involve in the research.

***SORT1* gene (rs464218)**				
	GG	**GA**	**AA**	**P value**
Number	9(12.86%)	22(31.43%)	39 (55.7%)	_

BMI (kg/m^2)	26.9097±3.048	27.238±3.991	28.42±5.478	0.558
HDL-C (mg/dl)	32.1625±6.572	33.25±7.709	36.0762±6.707	0.187
TG (mg/dl)	142.55±40.984	167.248±82.507	180.5997±49.077	0.255
TC (mg/dl)	155.085±13.825	153.248±28.284	164.149±29.315	0.314
LDL-C (mg/dl)	94.4125±20.195	82.614±30.858	91.953±28.843	0.416
VLDL-C (mg/dl)	28.51±8.197	31.929±17.612	36.11995±9.815	0.213

The associate of rs464218 variant by combine parameters, the ANOVA analyse is carry out by research effect of (rs464218) variant on variance parameter. The analyse distribute of the variable around various genotype in select polymorphism. BMI was non-association with any of genotype in (rs464218) variant. Also the non-association in result with TG, TC, HDL-C, LDL-C, VLDL-C in (rs464218) *SORT1* gene (p=0.255), (p=0.31), (p=0.187) (p=0.416) and (0.213) were non-significantly associated variants respectively. The whole statistics of ANOVA analysed was shown in table (6).

Discussion:

The purpose of current research was to investigated confirm variant by GWAS study with Myocardial infarction patients and the controls groups in Iraqi population. As per the literature, this will be the initial study performed in the Iraqi populace. The genotypes were found to be non-associated with BMI, TC, TG, LDL-C, HDL-C and VLDL-C, where the correlation P-value was less than 0.05. The MI prevalence showed an increase and proved to be second reason for disability, it also proved to be fourth reason for death within the worldwide populace. But, problem with great amount is divided in developing countries for the reason that of greater populaces. The MI as a complex disease is extremely complicated to remove the contribution of the environmental and genetic effects. The previous researches showed the associations of genetics with MI. There are small number of genetic researches undertaken in Iraqi with MI. The determined the ***rs464218*** polymorphisms per male and female in patient and control groups.

The genetics contained in metabolism and synthesis of lipids in serum levels were intensely studied because of their quantifiability from serum and clinical significance (Alharbi *et al.*, 2018).

The ***rs464218*** polymorphisms was significant associate with genotype in Odd Ratio (O.R.) analysis ($p < 0.05$) in Heterozygous genotype (GA) of *SORT1* gene. The correlate in the middle of lipid profile, BMI and genotype have shown non association of rs464218 variant by combine parameters. The ANOVA analyse is carry out by research effect of (rs464218) variant on variance parameter. The analyse distribute of the variable around various genotype in select polymorphism. BMI was non-association with any of genotype in (rs464218) variant (p=0.558). Also the non-association in result with TG, TC, HDL-C, LDL-C, VLDL-C in (rs464218) *SORT1* gene (p=0.255), (p=0.31), (p=0.187) (p=0.416) and (0.213) were non-significantly associated variants correspondingly. These statistic with (rs464218) polymorphism are in agreement with previous research with (Alharbi *et al.*, 2018) in the correlation of parameter between lipid profile, BMI and genotype, but differ in TG, which is not associated with genotype in this research.

Presently, GWAS reported thousands of SNPs related with human disease have showed achievements in explaining pathophysiological mechanisms with genetic effect (Traylor *et al.*, 2017; Zheng *et al.*, 2017) The MI is affected through genetics and environmental factors is considered to be the fourth leading cause for death around the world (Terni *et al.*, 2015). Meta analysis, Genome wide linkage, GWAS studies concluded that variances of genetic are accountable for MI varied risk (Zhang *et al.*, 2017).

In Iraq, the epidemiological statistics on prevalence and incidence of CAD as evidence of awareness are limited due to the unavailability of suggestion founded national guideline for administration of cardiovascular disease (Traina *et al.*, 2017). The prior study in 2014, cardiovascular disease mortality was estimated to account for 33% in Iraq (GBD 2015 Eastern Mediterranean Region Cardiovascular Disease Collaborators, 2018). Numerous researches have recognized the relative between atherosclerosis and MI (Bhatt *et al.*, 2006) (Scheen, 2018).

The MI identify interruption supply of blood to a portion of heart, it is continually because of development of occlusive thrombus at position of erosion or rupture of an athermanous plague in coronaries artery affecting lacking handling infarct correlated arteries residues always blocked in (30%) of patient and heart cells to death (Boon, 2006).

The previous research via (Musunuru *et al.*, 2010) documented genetic relative associated using LDL-R level action combinding of shifting expression and a main transcription factor of *SORT1* gene included in intracellular protein transport. The *SORT1* gene (rs464218) polymorphism has been recognized via GWAS in East Asian, Europe, Southern Asian, Africa Americans and Middle Eastern Asian populaces (Ozaki *et al.*, 2002; Consortium, 2009, 2011; Gudbjartsson *et al.*, 2009; Saade *et al.*, 2011; Wang *et al.*,

2011; Buyske *et al.*, 2012). The acquired data with (rs464218) polymorphism are in association with researches undertaken by: (Jeemon *et al.*, 2011; Zhou *et al.*, 2015; Alharbi *et al.*, 2018) in heterozygous genotype (GA) in *SORT1* gene and the difference in correlation TG, which was not associated with genotype in this research, this might be related to the ethnicities.

In this research, heterozygous genotype (GA) in *SORT1* gene (G>A) SNP was result significantly difference (P=**0.006**) higher the risk of MI with respect to those of the wild homozygous genotype (GG) of *SORT1* gene (G>A) SNP as reference, while the homozygous mutation genotype (AA) of *SORT1* gene (G>A) SNP was result non-significant (P=**0.823**) higher the risk of MI with respect to those of the wild homozygous genotype (GG) of *SORT1* gene (G>A) SNP as reference, its documented the . The data with (rs464218) polymorphism are in association with (Alharbi *et al.*, 2018) researches, but be different in heterozygous genotype (GA) in *SORT1* gene, and this might be because the ethnicities

Conclusion:-

The research confirms the *SORT1* variant that was associated in Iraqi populaces. The present research were in association with previous research data shown through meta-analysis association and GWAS. The worldwide ethnic populace research must be achieved to cancel in all ethnicities in human hereditary diseases.

References

Alharbi, K. K. *et al.* (2018) 'Molecular genetic studies in Saudi population; identified variants from GWAS and meta-analysis in stroke', *Saudi journal of biological sciences*. Elsevier, 25(1), pp. 83–89.

Arvind, P. *et al.* (2014) 'CELSR2–PSRC1–SORT1 gene expression and association with coronary artery disease and plasma lipid levels in an Asian Indian cohort', *Journal of Cardiology*. Elsevier, 64(5), pp. 339–346.

Bhatt, D. L. *et al.* (2006) 'International prevalence, recognition, and treatment of cardiovascular risk factors in outpatients with atherothrombosis', *Jama*. American Medical Association, 295(2), pp. 180–189.

Boon, N. A. (2006) 'Davidson's principles and practice of medicine', in. Churchill Livingstone, p. 591.

Buyske, S. *et al.* (2012) 'Evaluation of the metabochip genotyping array in African Americans and implications for fine mapping of GWAS-identified loci: the PAGE study', *PloS one*. Public Library of Science, 7(4), p. e35651.

Consortium, C. A. D. (C4D) G. (2011) 'A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease', *Nature genetics*. Nature Publishing Group, 43(4), p. 339.

Consortium, M. I. G. (2009) 'Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants', *Nature genetics*. Nature Publishing Group, 41(3), p. 334.

Ellis, P. D. (2010) *The essential guide to effect sizes: Statistical power, meta-analysis, and the interpretation of research results*. Cambridge university press.

GBD 2015 Eastern Mediterranean Region Cardiovascular Disease Collaborators (2018) 'Burden of cardiovascular diseases in the Eastern Mediterranean Region, 1990–2015: findings from the Global Burden of Disease 2015 study', *International Journal of Public Health*. Springer, 63(S1), pp. 137–149. doi: 10.1007/s00038-017-1012-3.

Gudbjartsson, D. F. *et al.* (2009) 'Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction', *Nature genetics*. Nature Publishing Group, 41(3), pp. 342–347.

Hubáček, J. A. (2016) 'Genetic determination of dyslipidemia-What tell us the results of genome-wide association studies?', *Vnitrni lekarstvi*, 62(11), pp. 868–876.

Jeemon, P. *et al.* (2011) 'Implications of discoveries from genome-wide association studies in current cardiovascular practice', *World journal of cardiology*. Baishideng Publishing Group Inc, 3(7), p. 230.

Kathiresan, S. *et al.* (2008) 'Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans', *Nature genetics*. Nature Publishing Group, 40(2), pp. 189–197.

Kjolby, M. *et al.* (2010) 'Sort1, encoded by the cardiovascular risk locus 1p13. 3, is a regulator of hepatic lipoprotein export', *Cell metabolism*. Elsevier, 12(3), pp. 213–223.

Musunuru, K. *et al.* (2010) 'From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus', *Nature*. Nature Publishing Group, 466(7307), pp. 714–719.

Nascimento, B. R. *et al.* (2019) 'Implementing myocardial infarction systems of care in low/middle-income countries', *Heart*. BMJ Publishing Group Ltd and British Cardiovascular Society, 105(1), pp. 20–26.

Orho-Melander, M. (2015) 'Genetics of coronary heart disease: towards causal mechanisms, novel drug targets and more personalized prevention', *Journal of Internal Medicine*, 278(5), pp. 433–446. doi: 10.1111/joim.12407.

Ozaki, K. *et al.* (2002) 'Functional SNPs in the lymphotoxin-α gene that are associated with susceptibility to myocardial infarction', *Nature genetics*. Nature Publishing Group, 32(4), pp. 650–654.

Patel, K. M. *et al.* (2015) 'Macrophage sortilin promotes LDL uptake, foam cell formation, and atherosclerosis', *Circulation research*. Am Heart Assoc, 116(5), pp. 789–796.

Preiss, D. *et al.* (2011) 'Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy: a meta-analysis', *Jama*. American Medical Association, 305(24), pp. 2556–2564.

Rajpathak, S. N. *et al.* (2009) 'Statin therapy and risk of developing type 2 diabetes: a meta-analysis', *Diabetes care*. Am Diabetes Assoc, 32(10), pp. 1924–1929.

Saade, S. *et al.* (2011) 'Large scale association analysis identifies three susceptibility loci for coronary artery disease', *PloS one*. Public Library of Science, 6(12), p. e29427.

Samani, N. J. *et al.* (2007) 'Genomewide association analysis of coronary artery disease', *New England Journal of Medicine*. Mass Medical Soc, 357(5), pp. 443–453.

Scheen, A. J. (2018) 'From atherosclerosis to atherothrombosis: from a silent chronic pathology to an acute critical event', *Revue medicale de Liege*, 73(5–6), pp. 224–228.

Strong, A. *et al.* (2012) 'Hepatic sortilin regulates both apolipoprotein B secretion and LDL catabolism', *The Journal of clinical investigation*. Am Soc Clin Investig, 122(8), pp. 2807–2816.

Terni, E. *et al.* (2015) 'Genetics of ischaemic stroke in young adults', *BBA clinical*. Elsevier, 3, pp. 96–106.

Traina, M. I. *et al.* (2017) 'Coronary heart disease in the Middle East and North Africa: current status and future goals', *Current atherosclerosis reports*. Springer, 19(5), p. 24.

Traylor, M. *et al.* (2017) 'Genetic variation at 16q24. 2 is associated with small vessel stroke', *Annals of neurology*. Wiley Online Library, 81(3), pp. 383–394.

Wang, F. *et al.* (2011) 'Genome-wide association identifies a susceptibility locus for coronary artery disease in the Chinese Han population', *Nature genetics*. Nature Publishing Group, 43(4), pp. 345–349.

World Health Organization (no date) 'The Top Ten Causes of Death. WHO Fact Sheet'. Available at: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (Accessed: 17 January 2021).

Zeng, J., Racicott, J. and Morales, C. R. (2009) 'The inactivation of the sortilin gene leads to a partial disruption of prosaposin trafficking to the lysosomes', *Experimental cell research*. Elsevier, 315(18), pp. 3112–3124.

Zhang, H. *et al.* (2017) 'Association of GWAS-Supported Variants rs556621 on Chromosome 6p21. 1 with large artery atherosclerotic stroke in a Southern Chinese Han Population', *Neuromolecular medicine*. Springer, 19(1), pp. 94–100.

Zheng, J. *et al.* (2017) 'HAPRAP: a haplotype-based iterative method for statistical fine mapping using GWAS summary statistics', *Bioinformatics*. Oxford University Press, 33(1), pp. 79–86.

Zhou, Y.-J. *et al.* (2015) 'Association of variants in CELSR2-PSRC1-SORT1 with risk of serum lipid traits, coronary artery disease and ischemic stroke', *International journal of clinical and experimental pathology*. e-Century Publishing Corporation, 8(8), p. 9543.

Zintzaras, E. (2010) 'Impact of Hardy–Weinberg equilibrium deviation on allele-based risk effect of genetic association studies and meta-analysis', *European journal of epidemiology*. Springer, 25(8), pp. 553–560.