International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Assessment of Matrix Metalloproteinase-9 Polymorphism in Acute Coronary Syndrome

Adel M Agha[1], Mona M Elbehisy[2], Amr E Elnagar[3], Basma M. Elnagar[4]

Clinical & Chemical Pathology, Faculty of Medicine, Benha University, Egypt

Abstract: ***Background: Matrix metallopeptidase-9 (MMP-9) plays a pivotal role in vascular remodelling and development of atherosclerotic lesion. The potentially functional MMP-9 polymorphisms may contribute to the susceptibility of Acute Coronary Syndrome (ACS). Objectives: Our aim was to examine whether MMP9-1562C/Tpolymorphism is associated with susceptibility to acute coronary syndrome (ACS) in the Egyptian population. Methods: This case-control study was composed of 80 ACS patients and 40 control subjects. The ACS group included 40 patients with Acute Myocardial Infarction (AMI) and 40 patients with Unstable Angina Pectoris (UAP). The genotypes of MMP-9 -1562 C/T polymorphism was determined by the method of polymerase chain reaction and restriction fragment length polymorphism (RFLP-PCR). The relationship between the polymorphism of the MMP-9 gene and Acute Coronary Syndromewas analysed. Results: The genotype frequencies for CT+TT genotypes and the −1562T allele were significantly higher in the ACS group than in the control group (25% vs. 0.0% and 20.4% vs. 0.0%, P=0.001and P=0.004, respectively). The T allele carriers had an approximately 1.51 -fold higher risk of developing ACS than those with the CC homozygote (OR=1.51; 95% CI, 1.33–1.72). While there was no statistically significant difference between patients with acute myocardial infarction and unstable angina pectoris regarding genotypes and allele frequencies (P > 0.05). Conclusion: MMP-9-1562C>T polymorphism is associated with the susceptibility to ACS in the Egyptian population.But there was no significant difference between the AMI and UAP subgroups.***

Keywords: Matrix metalloproteinase-9, -1562C/T, Acute coronary syndrome, polymorphism, RFLP

1. Introduction

Coronary artery disease (CAD) is a multifactorial, complicated disease and is the leading cause of mortality in most low- and middle-income countries ***(1)***.The clinical manifestation is "the acute coronary syndrome (ACS)" which encompasses unstable angina, non- ST-elevation myocardial infarction (NSTEMI) to ST segment elevation myocardial infarction(STEMI)***(2)***.

The pathogenic mechanism of ACS is most often based on thrombosis secondary to plaque rupture in atherosclerosis. ACS is mainly caused by coronary atherosclerotic plaque rupture or erosion and subsequentintracoronary thrombus formation ***(3)***.

Age, gender, smoking, hypertension, hypercholesterolemia, diabetes mellitus, obesity and sedentary lifestyle are reported to be associated with ACS ***(4)***, but the exact mechanism of ACS is still not clear. There is a genetic association between polymorphic variants in candidate genes and atherosclerosis. The matrix metalloproteinase (MMP) family is one of the potential candidate gene systems ***(5)***.

Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of the extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis***(6)***. Henney et al reported that genetic change which affects the expression of MMPs may contribute to the occurrence of cardiovascular disease. Matrix metalloproteinase 9 (MMP-9), also known as 92 k Da type IV collagenase, 92 k Da gelatinase or gelatinase B , is an enzyme that in humans is encoded by the MMP9 gene ***(7)***.

MMP9 is highly expressed in the vulnerable regions of atherosclerotic plaques and has been suggested to be causally involved in plaque rupture***(8)***. Dysregulation of MMP9 expression is characteristic of several pathologic conditions, including metastasis, vascular and cardiac remodeling, atherosclerotic plaque rupture, and acute coronary syndrome***(9)***. Studies have indicated that higher plasma concentrations of MMP9 may be predictor of cardiovascular disease risk,***(10)*** and plasma MMP9 Concentrations are elevated in patients with acute MI***(11)***.

Studies showed that functional genetic variations of MMP-9 might contribute to the susceptibility and progression of cardiovascular disease***(12)***. One of these polymorphisms is MMP- 9 −1562C>T which is included in this study. The association between the polymorphism of MMP- 9 −1562C>T and CAD had been reported in many countries including USA, Korea and China ***(2); (8);(13);(14)***.Due to the disparity of results among different populations, we aimed at studying such association in Egypt. The study was designed to clarify the relationship of polymorphism of MMP-9 −1562C>T and susceptibility to ACS in an Egyptian case control study.

2. Subjects and Method

2.1. Study Design

Case-control study both descriptive and analytic.

2.2. Subjects

This study was conducted on 120 subjects, 94 males and 26 females attending Cardiology Department ofBenha University Hospital from March to December 2015. The participants were divided into 3 groups; 40 in the MI group, 40 in the UAP group and 40 in the control group. Acute

Coronary Syndrome (STEMI, UA/NSTEMI) was diagnosed according to 2007 American Heart Association (AHA) definitions of myocardial infarction ***(15)***and UA/NSTEMI***(16)***. All individuals had age at ≥18 years while any patients with stable angina or had any associated liver or kidney diseases were excluded.

2.3.1. Patients were subjected to the following

1) History taking and clinical assessment.
2) Twelve lead electrocardiogram (ECG) at admission to determine acute coronary syndrome and its type.
3) Transthoracic Echocardiography was done for every patient on admission.
4) Laboratory investigations including; Fasting Serum glucose level, Urea, Creatinine, ALT, AST, Troponin I, CK-MB, HS-CRP and CBCs count.
5) Polymerase chain reaction, restriction fragment lengthpolymorphism (PCR-RFLP) technique, for the detection of MMP9_1562C >T polymorphism.

2.3.2. MMP-9 genotyping

Blood samples were collected under complete aseptic precautions, and were put into EDTA-containing tubes. DNA was extracted from peripheral vein blood leukocytes using aGene JET Whole Blood Genomic DNA Purification Mini Kit 250 preps (Thermo Scientific, EU). MMP9-1562C >Tpolymorphism primers were designed by *(Biosearch technologies, USA)*.

The reaction was performed in a50 µl final volume and contained 1 µl each primer,25 µlMyTaq Red Mix (2X)*(Bioline, UK)* and 200ng genomicDNA. The PCR products of −1562C>T polymorphism site was digested with the restrictionenzyme(SphI-HF)*(New England Biolab, UK)*at 37°C for 10 minutes, separated by electrophoresis on a 1.3 % agarose gel, and visualized by ethidium bromide.The MMP-9-1562C allele was not cut; it produceda 435-base pair (bp) fragment, and the MMP-9 -1562Tallele was cut into fragments of 188 and 247(bp). Detailed descriptions of themethods are summarized in **[table 1], [table 2]** and**[figure 1].**

Table 1: PCR primer sets and conditions for the MMP-9 gene.

Polymorphism (dbSNP No.)	Primer sequence	Initial denature	Denature	Annealing	Extension	Cycles	Final extention	Cooling
−1562C>T	F:(5-GCC TGG CAC ATA GTA GGC CC-3) R: (5-CTT CCT AGC CAG CCG GCA TC-3)	95°C 1 minute	95°C 15 seconds	58°C 15 seconds	72°C 10 seconds	40 cycles	72°C 15 minutes	4°C 5 minutes

Table 2: Restriction enzymes, conditions and product lengths for analysis of the MMP-9 gene

Polymorphism	Restriction enzyme	Conditions	Fragment length (bp)
−1562C>T	(SphI-HF)	37 °C for 10 minutes: PCR product 1 µg,10×buffer 5 µl (1X), SphI1 µl, in total Rxn volume 50 µl	CC one band at 435 bp. TT two bands at 188, 247 bp. CT three bands at 188, 247 and 435 bp.

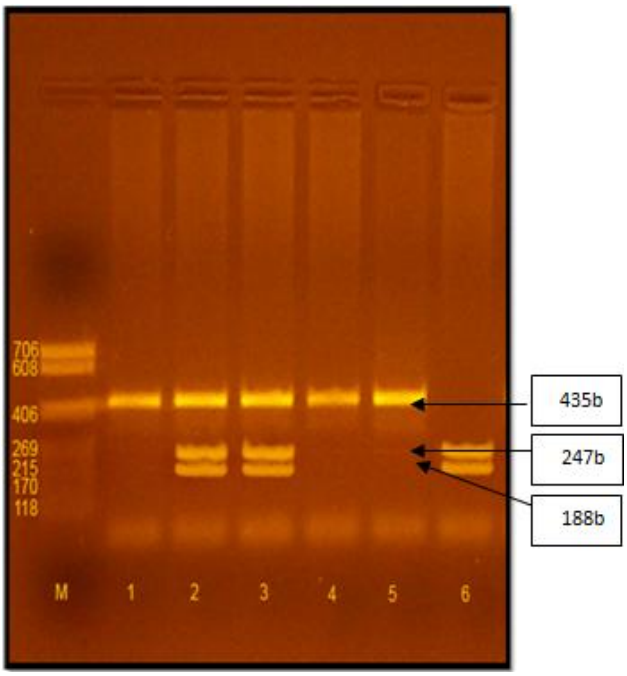

Figure 1: Genotyping of the MMP9gene -1562C >Tpolymorphism. Lane M: PCR marker **(**DNA ladder**)**.Lane 6: showshomozygous (T/T) with two bands at 188 and 247bp.Lanes 1 , 4 and 5 : show homozygous (C/C) with one band at 435bp.Lanes 2 and 3: showheterozygous (C/T) with three bands at 188, 247and 435bp.

2.4. Statistical Methods

Data were statistically described in terms of mean ± standard deviation (±SD), frequencies (number of cases) and percentages when appropriate. Student's *t*-test and Mann-Whitney test were used to compare mean of two groups of quantitative data of parametric and non-parametric respectively.Inter-group comparison of categorical data was performed by using chi square test (X^2-value) and fisher exact test (FET). Odds ratio (OR) and 95% confidence interval (95%CI) were used toquantify the risk among study group compared with the control group. p values less than 0.05 were considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL,USA) version 16 for MicrosoftWindows.

3. Results

3.1 Characteristics of the Study Participants

The comparison of clinical characteristics between the ACS group and the control group is shown in Table 3. There were

no significant differences in age, sex,AST, ALT, Urea, Creatinine and WBCs (P>0.05). However, Fasting glucose, Troponin I, CK-MB, HS-CRP were significantly higher in the ACS group than in the control group (P<0.001), whereas Ejection Fraction percentage was significantly lower in the ACS group than in the control group (P<0.001). There were significantly higher percentages of smokers and patients with diabetes mellitus, hypertension in the ACS group (P<0.001)**[table 3]**.

3.2. MMP-9−1562C>T and allele frequency and genotype distribution

The frequencies of C/C, C/T and T/T of MMP-9 (−1562C>T) polymorphism were75%, 22.5% and 2.5% in the ACS group, and 100%, 0.0% and 0.0% in the control group. TheCT+TT genotype were more frequent in the ACS group versus control group [25.0 % and 0.0 %, respectively (P=0.001)] and the −1562T allele frequency was significantly higher in the ACS group than in the control group[20.4 % and 0.0%, respectively (P=0.004)].

We found that the patients with CT or TT genotype had a higher risk of ACS (vs. CC genotype; CT+TT: OR=1.67). Patients with T allele had an increased risk of ACS (OR=1.51)**[table 4]**.

While analysis of subgroups revealed that there was no statistically significant difference between patients with acute myocardial infarctionand unstable angina pectoris regarding genotypes and allele frequencies (P >0.05)**[table 5]**.

Relation of risk factors to −1562C> T genotypes is shown in Table 6. There was no statistically significant association between the different variables and−1562C > T. Also there was no association between the values of different studied laboratory parameters (Fasting Serum glucose level, Urea, Creatinine, ALT, AST, Troponin I, CK-MB, HS-CRPand WBCs count) and MMP9 polymorphisms' genotypes (P>0.05)**[table 6]**.

3.3. Association between LVEF and different genotypes and cardiac markers

According to the results of the present study, there was no significant association between MMP-9 (−1562C>T) genotypes and LVEF (P>0.05). While correlation tests have revealed highly significant negative correlation between LVEF and both CK-MB and Troponin I levels (r = - 0.643, P < 0.001) and (r = -0.564, P < 0.001) respectively **[figure 2]** and **[figure 3].**

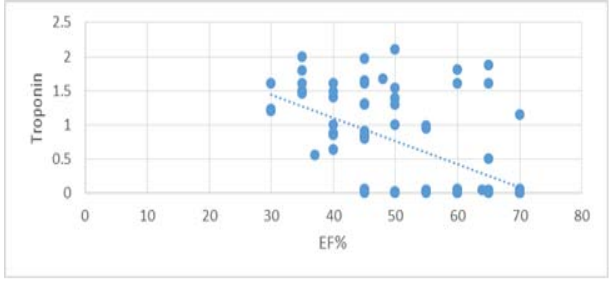

Figure 2: Correlation between EF% and troponin in patient group.

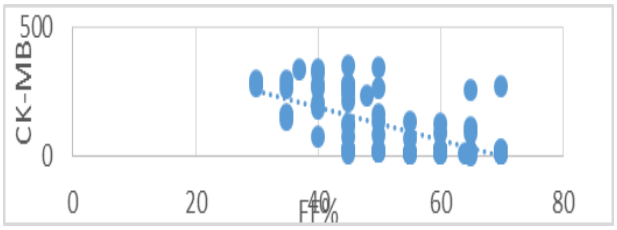

Figure 3: Correlation between EF% and CKMB in patient group

Table 3: Clinical data of the ACS group and the control group

Gr. / variable		ACS (n=80) Mean	ACS (n=80) S.D	Control (n=40) Mean	Control (n=40) S.D	t	p-value
Age(years)		53.44	6.30	51.7	5.23	1.5	0.113
Sex (no.%)	females	no=17	21.2%	9	22.5	0.025	0.88
	males	no=63	78.8%	31	77.5		
Fasting glucose (mg/dl)		191.88	91.41	84.53	9.91	7.39	<0.001**
AST(U/L)		27.33	7.38	24.55	8.03	1.89	0.062
ALT(U/L)		23.96	6.01	23.58	5.19	0.348	0.729
Urea(mg/dl)		29.23	7.59	27.48	4.46	1.35	0.181
Creat(mg/dl)		0.87	0.18	0.82	0.21	1.41	0.162
WBCs		9.46	2.45	9.01	2.09	0.992	0.323
HSCRP(mg/L)		5.35	2.44	0.87	0.31	t= 11.54	<0.001**
Trop.I (ng/ml)		0.67	0.71	0.02	0.01	Z= 5.9	<0.001**
CKMB (U/L)		110.69	115.6	12.7	5.4	Z= 4.74	<0.001**
EF%(Ejection Fraction)		52.86	11.73	66.75	4.74	7.19	<0.001**
Smoking (no. %)		no=52	65.0%	9	22.5	X^2=19.27	<0.001**
Hypertension (no. %)		no=52	65.0%	4	10.0	X^2=32.41	<0.001**
Diabetes mellitus (no. %)		no=51	63.8%	0	0.0	X^2=44.35	<0.001**

Table 4: MMP-9−1562C>T allele frequency and genotype distribution in the ACS group and the control group:

Gr. / variable	Cases (ACS) (n=80) No	Cases (ACS) (n=80) %	Control (n=40) No	Control (n=40) %	X^2	p-value	OR (95%CI)
Genotypes CT+TT CC	20 60	25.0 75.0	0 40	0.0 100	12.0	0.001**	1.67 (1.42-1.96)
Alleles T C	20 78	20.4 79.6	0 40	0.0 100	7.97	0.004**	1.51 (1.33-1.72)

Table 5: Comparison of genotypes and allele frequencies between subgroups

Gr. / variable	AMI (n=40) No	AMI (n=40) %	UAP (n=40) No	UAP (n=40) %	Test of sig.	p-value
Genotypes CT+ TT CC	11 29	27.5 72.5	9 31	22.5 77.5	X^2=0.267	0.606
Alleles T C	11 39	22.0 78.0	9 39	18.7 81.3	FET =0.022	0.88

Table 6: Comparison of the genotype groups regarding different clinical data

		CC genotype (n=60)		CT+TT genotype (n=20)		Total		Test of sig.	p-value
		No.	%	No.	%	No.	%		
Age (Mean ± SD)		52.75±6.47		55.5±5.36				t= 1.71	0.091
Sex	Female	3	15.0	14	23.3	17	21.2	FET= 0.224	0.636
	Male	17	85.0	46	76.7	63	78.8		
Smoking Status	NS	23	38.3	5	25.0	28	35.0	X^2=1.17	0.279
	S	37	61.7	15	75.0	52	65.0		
HTN	HTN	39	65.0	13	65.0	52	65.0	X^2=0.0	1.0
	NON	21	35.0	7	35.0	28	35.0		
DM	DM	41	68.3	10	50.0	51	63.8	X^2=2.18	0.14
	NON	19	31.7	10	50.0	29	36.2		

4. Discussion

IN the present study we found that the – 1562 C>T polymorphism in the promoter region of MMP-9 gene has a significant role in the development of acute coronary syndrome. In our study population, the genotype frequencies of −1562C>T polymorphism for CC, CT and TT were 75%, 22.5% and 2.5% respectively in the ACS patients, and 100%, 0.0% and 0.0% in the control subjects respectively. The genotype frequencies for CT+TT genotypes and the −1562T allele were significantly higher in the ACS group than in the control group (25% vs. 0.0% and 20.4% vs. 0.0%, *P*=0.001and *P*=0.004, respectively). The T allele carriers had an approximately 1.51 -fold higher risk of developing ACS than those with the CC homozygote(OR=1.51; 95% CI,1.33–1.72).

These results were in accordance with a study done by***(Wang et al., 2011)(5),***who reported that MMP-9-1562C>T polymorphism is associated with the susceptibility to ACS in the Uygur population of China. He found that the genotype frequencies of CT+TT genotypes and the −1562T allele were significantly higher in the ACS patients than in the control subjects.

Also ***(Xu et al., 2013)(17)*** found that carriers of the T allele ofMMP9 were more susceptible to CAD than C homozygous carriers. In addition, the CT genotype was also associated with an increased risk of CAD in the Chinese Han population. Similarly a study performed by ***(Saedi et al., 2012)(18)***, in Tehran population of Iran, reported that that individuals carrying the1562 T allele of the MMP-9 gene are predisposed to developing early CAD. In addition, ***(Zhi et al., 2010)(12)***reported that −1562 CT/TTgenotypes may contribute to CAD in a Chinese population. Also, a strong association between −1562CT polymorphism in the MMP-9 promoter and AMI was found in a study done by***(Koh et al., 2008)(19)***, in Korean population.

On the other hand, a study performed by ***(Wu et al., 2009)(13)***, on 1373 Chinese subjects found that the *MMP9 -1562C/T* polymorphism was not associated with an increased risk of CHD, AMI, or ACS. Also, a study carried out by ***(Han, 2012)(20)***, demonstrated that the functional1562C > T polymorphism is not a risk contributor to CAD susceptibility. Similarly, a study conducted by***(Kaplan et al., 2008)(21)***, in American population; found that the MMP9 haplotypes and SNPs were not significantly associated with myocardial infarction or stroke.

Recently a meta-analysis by ***(Wang and Shi2014)(2)*** including 16 potentially eligible articles involving 11032 CAD patients and4628 non-CAD controls tried to find an answer to whether MMPs polymorphisms increase the risk of CAD. This meta-analysis found a significant association between MMP-9 C1562T polymorphisms and CAD and MI in overall population, but this association varies in different ethnic populations.

A possible explanation is that the human MMP9 gene is located on chromosome 20q12.2-13.1, and is functionally implicated in the process of infarct healing. A number of MMP9 single nucleotide polymorphisms in the promoter, coding, and untranslated regions have been reported. Among them, promoterC-1562Tpolymorphism with acytosine to thymi- dines transitions the most studied and functional studies indicate that this polymorphism has an allele-specific effectonMMP-9 transcription***(8)***. The variant T allele of MMP-9C-1562T polymorphism has been associated with an increase in expression of the gene and higher MMP9 levels, due to preferential binding of the transcriptional repressor protein to the C allele (binding weaker to the T allele), and over expression of MMP9 was found in human atherosclerotic plaques and involved in rupture of the plaques ***(22)***.

Few epidemiologic studies have investigated associations between MMP9 and CAD onset.***(Setianto et al., 2012)(23)***demonstrated that the MMP9 C-1562T polymorphism is associated with high serum MMP9 levels in patients with segment elevation MI. ***(Jefferis et al.,2010)(24)***identified that serum MMP9 is associated with risk of MI and stroke.

As thrombosis is generally accepted as the most common pathogenic pathway of ACS, It has been reported that MMP9 brings about destabilizing structural changes invulnerable atherosclerotic plaques and may promote cellular infiltration of plaques, weakening the fibrous cap of the atherosclerotic plaque, and increasing the size of the lipid core ***(25)***. These processes render the plaque susceptible to ruptured due to reduced mechanical strength and hence increase the probability of atherothrombotic ischemia ***(8)***.

From the results of the present study, we suggested that−1562 CT/TT genotypes and T allele are associated with an increased risk of ACS in the Egyptian population. The

results are consistent with the notion that MMP-9 plays an important role in the development of atherosclerotic lesion and arterial plaque rupture***(26),*** making MMP-9 a desirable target for both therapy and diagnosis of atherosclerotic cardiovascular diseases. MMP inhibition appears to be a good direction to follow in order to develop satisfactory strategies to prevent ACS and the development of post infarction heart failure***(27).*** We expect to learn of more reports about theMMP9 -1562C/T polymorphism, which can help to prevent and cure ACS patients.

In the present study, regarding analysis of sub groups, there was no statistically significant difference between patients with acute myocardial infarction and unstable angina pectoris regarding genotypes and allele frequencies (P > 0.05). These results were in agreement with ***(Wang et al., 2007) (28)***who found that the-1562C/T MMP-9 polymorphism may be susceptible to ACS but there was no significant difference between the AMI and UAP subgroups (x 2=0.073, P=0.788).

5. Conclusions

In conclusion, this study suggests that the-1562C/T polymorphism in the MMP-9 gene can be used as a novel genetic method to detect susceptibility to acute coronary syndrome in Egyptian population. Further study on a larger population will be required to confirm these findings. Meanwhile, the race selection should be paid more attention since the pathogenesis of the disease might have different bases in different racial population groups.

6. Recommendations

There were several limitations in our study that are worth mentioning. First, the findings of our study are based on a sample size of 120 participants, and our results should be confirmed by further study of a larger population. Second, we did not measure plasma MMP-9 in this study soconcomitant measurement of MMP9 level in patients´ sera and coronary arteries is recommended. Third, because of the case–control study design, in which the study participants were not recruited prospectively, we could not exclude the possibility of a selection bias. Therefore, these findings need to be confirmed in cohort studies.

References

[1] **Zheng X, Wang S, Ni M (2016):** Association between interleukin 17A gene polymorphisms and risk of coronary artery disease. Genetics and molecular research: GMR; 15(151).

[2] **Wang X and Shi L (2014):**Association of matrix metalloproteinase-9 C1562T polymorphism and coronary artery disease: a meta-analysis. J Zhejiang UnivSci B; 15(3):256-263.

[3] **Badimon L, Padró T and Vilahur G (2012):**Atherosclerosis, platelets and thrombosis in acute ischaemic heart disease.Eur Hear journal Acute Cardiovasc care; 1(1):60-74.

[4] **Huma S, Tariq R, Amin Dr. F, et al. (2012):**Modifiable and non-modifiable predisposing risk factors of myocardial infarction -A review.J Pharm SciRes ; 4(1):1649-1653.

[5] **Wang L, Ma Y-T, Xie X, et al. (2011):**Association of MMP-9 gene polymorphisms with acute coronary syndrome in the Uygur population of China. World J Emerg Med; 2(2):104-110.

[6] **Chaudhary AK, Pandya S, Ghosh K, et al. (2013):** Matrix metalloproteinase and its drug targets therapy in solid and hematological malignancies: an overview. Mutat. Res; 753: 7-23.

[7] **Henney AM, Ye S, Zhang B, et al. (2000):Genetic** diversity in the matrix metalloproteinase family. Effects on function and disease progression. Ann N Y AcadSci; 902: 27-38.

[8] **Juan Z, Wei-Guo Z, Heng-Liang S, et al. (2015):**Association of matrix metalloproteinase 9 C-1562T polymorphism with genetic susceptibility to myocardial infarction: A meta-analysis. CurrTher Res - ClinExp; 77:40-45.

[9] **Rodríguez-Pérez, J. M., Vargas-Alarcón, G., Posadas-Sánchez, R, et al. (2016):** rs3918242 MMP9 gene polymorphism is associated with myocardial infarction in Mexican patients. Genetics and molecular research: GMR; 15(1).

[10] **Blankenberg S, Rupprecht HJ, Poirier O, et al. (2003):**Plasma **concentrations** and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation; 107:1579–1585.

[11] **Squire IB, Jon Evans, Leong L Ng, et al. (2004):** Plasma MMP-9 and MMP-2 following acute myocardial infarction :correlation with echo cardio graphic and neurohumoral parameters of left ventricular dysfunction. Journal of cardiac failure; 10(4): 328–333.

[12] **Zhi H, Wang H, Ren L, et al. (2010):** Functional polymorphisms of matrix metallopeptidase-9 and risk of coronary artery disease in a Chinese population. MolBiol Rep; 37(1):13-20.

[13] **Wu N, Lu X, Hua Y, et al. (2009):**Haplotype analysis of the stromelysin-1 (MMP3) and gelatinase B (MMP9) genes in relation to coronary heart disease. Ann Hum Genet; 73(4):404-410.

[14] **Li J, Lu H, Tao F, et al. (2013):**Meta-Analysis of MMP9–1562C/T and the Risk of Coronary Heart Disease. Cardiology; 124(1):53-59.

[15] **KristianThygesen, Joseph S. Alpert, Harvey D (2007):**Universal Definition of Myocardial **Infarction**. White Circulation; 116:2634-2653.

[16] **Jeffrey LA, Cynthia DA, Elliott MA, et al. (2007):** ACC/AHA 2007 guidelines for the management of patients with unstable angina/non–ST-elevation myocardial infarction. J Am CollCardiol; 50:1–157.

[17] **Xu X, Wang L, Xu C, et al. (2013):** Variations in matrix **metalloproteinase**-1, -3, and -9 genes and the risk of acute coronary syndrome and coronary artery disease in the Chinese Han population.Coron Artery Dis; 24(4):259-265.

[18] **Saedi M, Vaisi-Raygani A, Khaghani S, et al. (2012):** MMP-9 functional **promoter** polymorphism 1562C>T increased risk of early-onset coronary artery disease. MolBiol Rep; 39(1):555-62.

[19] **Koh YS, Chang K, Kim PJ, et al. (2008):** A close relationship between functional polymorphism in the **promoter** region of matrix metalloproteinase-9 and acute myocardial infarction. Int J Cardiol; 127(3):430-432.

[20] **Han YZ (2012):** Correlation between the **matrix** metalloproteinase-9 gene single nucleotide polymorphim and coronary artery stenosis degree. Chin J Gerontol; 1:5–6.

[21] **Kaplan R, Smith N, Zucker S, et al. (2008):**Matrix metalloproteinase-3 (MMP3) and MMP9 genes and risk of myocardial infarction, ischemic stroke, and hemorrhagic stroke. Atherosclerosis; 201(1):130-137.

[22] **Heo SH, Cho CH, Kim HO, et al. (2011):**Plaque rupture is a determinant of **vascular** events in carotid artery atherosclerotic disease: involvement of matrix metalloproteinases 2 and 9. Journal of Clinical Neurology; 7(2):69-76.

[23] **Setianto BY, Mubarika S, Irawan B, et al. (2012):**Association Between High Serum Matrix Metalloproteinase-9 and MMP-9 (-1562C>T) Polymorphism in Patients With ST-**Elevation** Acute Myocardial Infarction. Cardiology Research; 3(5):222-229.

[24] **Jefferis BJ, Whincup P, Welsh P, et al. (2010):** Prospective study of matrix metalloproteinase-9 and risk of myocardial infarction and stroke in older men and women. Atherosclerosis; 208(2):557-563.

[25] **Reis ST, Leite KR, Piovesan LF, et al. (2012):**Increased expression of MMP-9 and IL-8 are correlated with poor prognosis of Bladder Cancer. BMC urology; 12(1):18.

[26] **Wang Y, Xu F, Chen J, et al. (2011):**Matrix metalloproteinase-9 induces cardiac fibroblast migration, collagen and cytokine secretion: inhibition by salvianolic acid B from Salvia miltiorrhiza.Phytomedicine; 19:13–19.

[27] **Ketelhuth DF and Bäck M (2011):** The role of matrix **metalloproteinases** in atherothrombosis. Current atherosclerosis reports; 13(2):162-169.

[28] **Wang L, Zhu T and Li Y (2007):** Relationship between Matrix metalloproteinase-9 polymorphism and acute coronary syndrome.Journal of Nanjing Medical University; 21(3):147-150.