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Association of *MMP-1, 9, 12* and *TIMP-1* gene polymorphisms in Malaysian male hypertensive subjects.

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Abstract

Background: Matrix Metalloproteinases (MMPs) plays an important role in hypertensive vascular stiffness, remodeling and dysfunction. Several studies have been reported the imbalanced MMP: TIMP (tissue inhibitors of metalloproteinase-1) ratio in hypertensive subjects, indicating the depressed systematic degradation of collagenase in etiology of hypertension. The main objective of this study was to determine the candidate gene polymorphisms involved in extracellular matrix metabolism among Malaysian male subject with Essential Hypertension (EH).

Methods: A total of 133 newly diagnosed EH subjects and 129 unrelated healthy individuals were included in this study. The genomic DNA was extracted and the genotyping was done by PCR-RFLP method.

Result: The demographic characteristic of the subjects such as age, body mass index, systolic blood pressure, diastolic blood pressure, low-density lipoprotein, triglyceride and cholesterol were shown to be significantly different ($p<0.05$) in case subjects when compared to controls. The allelic distribution of *TIMP-1* 372 T/C gene polymorphism was significantly associated with hypertension ($p<0.05$). While, *MMP-1*: *rs1799750, MMP-9: rs3918242* and *rs17576* and *MMP-12: rs2276109* polymorphisms did not differ significantly ($p>0.05$).

Conclusion: Hence, the *rs4898* polymorphism of *TIMP-1* may be considered as a possible genetic biomarker and a risk factor predictor for EH among Malaysian male subjects.

Keywords: *MMP1, MMP9, MMP12, TIMP1*, Essential hypertension, Polymorphism.

List of Abbreviations

MMPs: Matrix Metalloproteinases; TIMP: Tissue Inhibitors of Metalloproteinase-1; ECM: Extracellular Matrix; EH: Essential Hypertension; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.

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Introduction

Extracellular Matrix (ECM) act as network within the endothelial cells that provide elasticity and shape to the arteries [1]. Degradation of the ECM is fundamental in many aspects, both physiologically and pathologically [2,3]. The Matrix Metalloproteinases (MMPs) belong to the large family of the zinc-dependent endopeptidases involved in degradation of connective tissues and ECM proteins [4]. Their activity is well documented in major vascular complications particularly in hypertension [5]. The dysregulation can cause aggravated ECM degradation, which in turn provokes vascular smooth muscle cell migration, proliferation and inflammatory cells invasion to the vessel wall, bringing about vascular remodeling and stiffness [6]. Abnormal ECM metabolism has been reported in hypertensive subjects [5]. Where, decrease in MMP activity is accompanied by dysregulation of its inducer and activator proteins [7]. The plasma concentration of tissue inhibitors of metalloproteinase-1 (*TIMP-1*) was shown to be associated with arterial stiffness, greater systolic and diastolic dysfunction in hypertensive patients [8-11]. Multiple genetic and environmental factors involved in the development of hypertension [12]. Estimation of about 30-70% of genetic heritability believed to play a key role in blood pressure regulation [13]. Recently, some studies have evaluated the association of *MMP* gene polymorphisms with hypertension [14,15]. Several studies have been carried out on association between *MMP* gene single nucleotide polymorphisms (SNPs) and Cardiovascular Disease (CVD) in many populations, with conflicting results (Table 1). Some of these SNPs have been associated with higher Blood Pressure (BP) in hypertensive patients [16,17]. Data regarding *TIMP-1* 372 T/C

polymorphism are scarce. The study of ischemic patients showed this SNP effects the drug responsiveness to nitrates in men [18]. Furthermore, higher plasma concentration of *TIMP-1* was associated with *T* allele [19] in septic patients. To the best of our knowledge there were no former data available in respect to p *MMP-1*, *9, 12* and *TIMP-1* gene polymorphisms, in relation to EH in Malaysian population. This initiated us to determine the possible association of *MMP-1, 9, 12* and *TIMP-1* polymorphisms in Malay male EH subjects.

Materials and Methods

Study subjects

Upon the ethical approval from the National Medical Research Register of Malaysia (NMRR) (Ref. No.: NMRR-12-1062-12), the case subjects were recruited from "Klinik Kesihatan Senawang", Seremban. A total of 300 subjects were approached for sample collection, out of which 38 samples were eliminated during the analysis due to outlier or skewness and genotyping errors. Overall, 133 newly diagnosed hypertensive patients with SBP (Systolic Blood Pressure) ≥ 140 mmHg and/or DBP (Diastolic Blood Pressure) ≥ 90 mmHg, Malay male aged ≥ 18 were included in our study. Patients with history of acute myocardial infarction, renal failure, cardiac failure, diabetes or those with medical conditions that can cause secondary form of hypertension were excluded. Furthermore, 129 healthy cases with SBP/ DBP<140/90 mmHg and no family history of hypertension, diabetes, heart disease, high cholesterol, renal and kidney failure were considered as controls. The control subjects were selected from Seremban occupancies. The semi-assisted questionnaire prepared in Malay and English was given to each subject and based on their preference and the compliance to the inclusion criteria, informed consent was obtained from all the subjects. The socio-demographic information such as age, smoking habits and family history of disease were attained to assist the selection procedure. The blood pressure of the subjects were measured by Automated BP Meter (Omron, Japan), at the sitting position on the right arm after at least 5 min of sitting, however the average of two readings was calculated for the SBP and DBP.

Sampling and biochemical analysis

Five ml of peripheral blood was collected by a qualified phlebotomist and transferred to the EDTA tube (Becton Dickinson, NJ). The plasma was separated by centrifugation and stored under -80°C for further biochemical analysis. While, biochemical data of the patients were obtained from their medical records, lipid profile of the controls was measured by Roche Hitachi-911 Chemistry Analyzer (Hitachi, Japan) along with the kit supplied by Roche Diagnostics (Mannheim, Germany).

Genotyping methods

The FlexiGene® DNA kit (Qiagen Inc., Chatsworth, CA, USA) was used for genomic extraction from buffy coat. The quality and concentration of the extracted DNA was verified by the Q3000 UV Spectrophotometer and its analytical software V3.3.1 (Quawell, San Jose, CA, USA). Polymerase chain reaction technique was applied to amplify the genomic DNA using the primers synthesized by Focus Biotech Sdn Bhd (Table 2). The amplification of PCR products of *MMP-1-1607 1G/2G (rs1799750), MMP-9-1562C>T (rs3918242) and R279Q (rs17576), MMP-12-82A/G (rs2276109)* and *TIMP-1* gene 372 T/C (rs4898) polymorphisms were separately carried out in the total PCR reaction volume of 25 µl using PrimeG (Techne, Bibby Scientific, UK) thermo-cycler. The reaction mixture consisted of 7-8 µl of Prime Taq Premix (2X) (GenetBio, Korea), 0.5 to 0.7 µM of each prime, 100 ng of DNA. The amplified products were later digested with 3-4 Units of their respective REs (New England Biolabs, Beverly, USA) along with the provided NEB buffer in the total volume of 10 µl. The incubation of the RFLP product was done on 37°C for 30 min to 1 h according to the protocol provided by the manufacturer. The primers and enzymatic digestion specifications for screening of the variants using PCR- RFLP method are presented in Table 2. The sequencing result of the selected amplified products of the respective genes was confirmed with BLAST. These samples then served as positive controls in the later experiments. The non-template controls were also ran in all the experiments. The RFLP products were separated by 2-4% agarose gel in electrophoresis tank (Origins, Elchrom Scientific AG, Switzerland) at 100 v. The agarose gels were later stained by soaking into EtBr (0.5 µl/ml concentration; Bio-Rad, California, USA) and visualized by Alpha Imager (AlphaInnotech, San Leandro, USA). For validation, about 10% of randomly selected samples were genotyped using the same conditions and the results were 100% in concordance with previous results.

Results

Clinical characteristics

The clinical characteristics of the study subjects are represented in Table 3. The age of hypertensive subjects ranged between 23 to 65 years old with the mean of 49.10 ± 8.49 y, while the age of controls subjects varied between 18 to 63 y with the mean of 43.04 ± 12.24. The percentage of smoking in case and control subjects was 34.6% and 10.9%, respectively. The mean value of age, Body Mass Index (BMI), SBP, DBP, Low Density Lipoprotein (LDL), Triglycerides (TG) and Total Cholesterol (TC) were significantly higher in EH group as compared to the controls ($P<0.05$). Not much of difference could be seen when comparing the mean value of High Density Lipoprotein (HDL) between the case and control subjects ($P>0.05$).

Genotyping

All the *MMP* and *TIMP-1* gene variants were separated on 2-4% gel electrophoresis. Figure 1, illustrated the genotyping results of restricted fragments of the selected variants viewed under UV light.

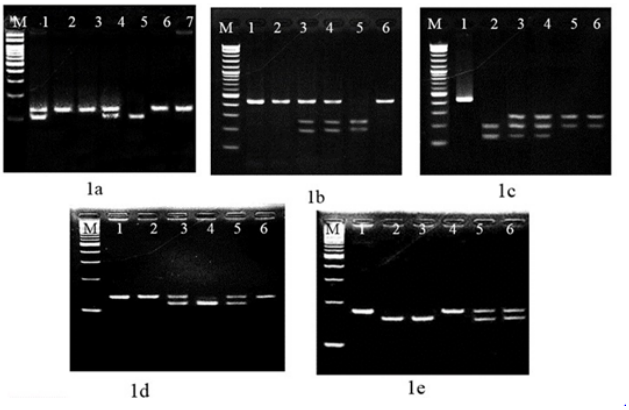

Figure 1. *Restricted fragment of MMP-1, 9, 12 and TIMP-1 variants. M represents 100 bp ladder (NEB). (a) Lane 5 represents the wild type (1G/1G-241 bp), lane 1 and 4 are heterozygous (1G/2G-241/269 bp) and lane 2, 3, 6 and 7 show mutant (2G/2G-269 bp) types of 16071G/2G Polymorphism of MMP-1 gene (b). Lane 1, 2 and 6 represent the wild type (CC-435 bp), lane 3 and 4 are heterozygous (CT-435/247, 188 bp) and lane 5 shows mutant types (TT-247 and 188 bp) for C1562T polymorphism of MMP-9 gene. (c) Lane 1 represent PCR product of 439 bp, lane 2 the wild type (RR-187 and 129 bp), lane 3 and 4 are heterozygous (RQ- 252, 187and 129 bp) and lane 5 and 6 show mutant types (QQ 252 and 187 bp) for R279Q variant of MMP-9 gene (1d) Lane 1, 2 and 6 are the wild types (AA-137 bp), lane 3 and 5 are heterozygous (AG-137/119 bp) and lane 4 shows mutant types (GG-119 bp) for 82 A/G polymorphism of MMP-12 gene (1e). Lane 1 and 4 are the wild types (TT-175 bp), lane 5 and 6 are heterozygous (TC 175 and 155 bp) and lane 2 and 3 shows mutant types (CC 155 bp) for 372 T/C polymorphism of TIMP-1 gene.*

16071G/2G Polymorphism of MMP-1 gene

Table 4, represents the genotype and allele frequencies of MMP and *TIMP-1* variants. There were no significant association in relation to genotype (p=0.954) and allele frequencies (p=0.925) between the cases and controls. We found high percentage of mutant (control: 46.5% and EH: 46.6%) and heterozygous (control: 45% and EH: 45.9%) in both the study groups.

C1562T and R279Q variants of MMP-9 gene

Concerning the C1562T variant, there could be no significant association detected in relation to genotype or allelic distribution of the subjects. The percentage of CC, CT and TT genotypes were 29.5%, 68.2% and 2.3% in controls compared to cases (33.1%, 63.9% and 3%). No significant differences in the genotypic distributions were observed in either group (p>0.05). Looking into genotypic and allelic frequencies of the R279Q polymorphism, the allelic frequency showed no significant difference between the hypertensive and control subjects (p=0.925). The percentage of the A (*R*) allele was 68.2% and 68.8% among controls and hypertensive subjects, respectively. Whereas, the percentage of the G (*Q*) allele was 31.8% in controls and 31.2% in cases (Table 4).

82A/G polymorphism of MMP-12 gene

Table 4, illustrates the genotypic and allelic distribution of the 82A/G polymorphism of *MMP-12* gene in the studied group. There was no significant difference in distribution of the genotypes and alleles between the hypertensives and controls. The percentage of 82A/G genotypes were 94%, 5.3% and 0.8% and 94.6% and 5.4% respectively for hypertensives and control subjects. We did not detect any GG in the controls. The percentage of *A* allele in controls and hypertensives were 97.3% and 96.9% respectively. Whereas, the percentage of *G* allele in control and the hypertensives were 2.7% and 3.4% respectively.

372 T/C polymorphism of TIMP-1 gene

The frequency of the T allele was 44.3% and 54.3% respectively in case and controls. Whereas, the frequency of C allele was 55.7% and 45.7% in case and controls, respectively. The allelic frequency of 372 T/C variant of *TIMP-1* gene was significantly different in case and control subjects (p=0.023).

Table 1. *Conflicting results of genetic variants of MMP and TIMP-1 genes in various populations.*

Gene variants	Diseases	Population/References	No. of subjects	p- value
MMP-9-C1562T	CAD	Brazilian Caucasian [20]		
	IS in T2DM	Tunisian [21]	388	NS
	CHD	Caucasian [22]	471	S
	Gestational hypertension	Czech Caucasian [23]	158	NS
	Hypertension	Northeastern Han Chinese [24]	1765	NS
MMP-9-C1562T	AF	Chinese Han [25]	881	S
	CAD	Polish [26]	180	NS

	CVD	Turkish [27]	209	NS
	EH	Turkish [15]	224	NS
	Hypertension	North-Eastern Han Chinese [24]	1765	NS
MMP-9- R279Q	AF	Chinese Han [25]	881	NS
	Stable CAD+ hypertension	Norwegians [28]	1205	NS
	Hypertension	Indonesian Javanese [14]	100	NS
MMP-12-82A/G	AAA	Italian [29]	846	NS
	IS in T2DM	Tunisian [21]	388	S
	CAD	Mexican Mestizo [30]	300	S
	Hypertension	Northeastern Han Chinese [24]	1765	S
TIMP-1-372 T/C	Ischemic patients	Czech [18]	537	S
	AAA	CAUCASIAN [31]	279	NS

NS: Non-Significant (p>0.05); S: Significant (p<0.05); CAD/CHD: Coronary Artery Disease; IS: Ischemic Stroke; AF: Atrial Fibrillation; AAA: Abdominal Aortic Aneurysm

Table 2. *The primers and enzymatic digestion specifications for screening of the variants.*

Gene: variant	Forward Primer (FP) Reverse Primer (RP)	Restriction enzyme	PCR product (bp)
MMP-1: rs1799750	FP-5'TGACTTTTAAAACATAGTCTATGTTCA-3'	*AluI*	269
	FP-5'TGACTTTTAAAACATAGTCTATGTTCA-3'		
MMP-9: rs3918242	FP-5′-GCCTGGCACATAGTAGGCCC-3′	*SphI*	435
	RP-5′-CTTCCTAGCCAGCCGGCA TC-3′		
MMP-9: rs17576	FP-5′-GAGAGATGGGATGAACTG-3′	*MspI*	439
	RP-5′-GTGGTGGAAATGTGGTGT-3'		
MMP-12: rs2276109	FP-5′-GTCAAGGGATGATATCAGCT-3′	*PvuII*	137
	RP-5′-CTTCTAAACGGATCAATTCAG-3′		
TIMP-1: rs4898	FP-5'-GCACATCACTACCTGCAGTC-3'	*BssSI*	175
	RP-5'-GAAACAAGCCCACGATTTAG-3'		

Table 3. *Clinical and biochemical parameter of EH patients and control subjects.*

Factor	EH patients (133)	Controls (129)	p-value
Age (years)	49.10 ± 8.49	43.04 ± 12.24	0.000*
BMI (kg/m^2)	28.96 ± 5.25	25.94 ± 5.28	0.000*
SBP (mm Hg)	154.91 ± 10.93	119.86 ± 9.32	0.000*
DBP (mm Hg)	95.28 ± 5.78	76.78 ± 8.64	0.000*
Smoking (Yes/No)	34.60%	10.90%	-
LDL (mmol/ L)	3.19 ± 0 .93	2.76 ± 0.95	0.000*
HDL (mmol/ L)	1.04 ± 0.22	1.05 ± 0.26	0.703*
TG (mmol/ L)	1.64 ± 0.73	1.40 ± 0.76	0.011*
TC (mmol/ L)	5.05 ± 1.11	4.74 ± 1.21	0.033*

Student-t test; EH: Essential Hypertensive; *Significant, p<0.05, Values shown as ± SD.

Table 4. *Genotypic and allelic distribution of MMP and TIMP-1 variants.*

Genotypes and alleles	Control subjects (n=129) n (%)	EH patients (n=133) n (%)	p- value	Odd ratio (95% CI)
MMP-1 1607 1G/2G				

1G/1G	11 (8.5%)	10 (7.5%)		
1G/2G	58 (45%)	61 (45.9%)		
2G/2G	60 (46.5%)	62 (46.6%)	0.954	
1G	80 (31.1%)	81(30.4%)		
2G	178 (68.9%)	185 (69.6%)	0.925	0.974 (0.672-1.412)
MMP-9 C1562T				
CC	38 (29.5%)	44 (33.1%)		
CT	88 (68.2%)	85 (63.9%)		
TT	3 (2.3%)	4 (3.0%)	0.751	
C	164 (64.1%)	173 (65%)		
T	94(35.9%)	93(35%)	0.856	1.041 (0.729-1.487)
MMP-9 R279Q				
RR	61 (47.2%)	69 (51.9%)		
RQ	54 (41.9%)	45 (33.8%)		
QQ	14 (10.9%)	19 (14.3%)	0.367	
R	176 (68.2%)	183 (68.8%)		
Q	82 (31.8%)	83 (31.2%)	0.925	1.027 (0.710-1.485)
MMP-12 82A/G				
AA	122 (94.6%)	125 (94.0%)		
AG	7 (5.4%)	7 (5.3%)		
GG	0	1 (0.8%)	0.614	
A	251(97.3%)	257(96.9%)		
G	7 (2.7%)	9 (3.4%)	0.801	0.796 (0.292-2.171)
TIMP-1 372 T/C				
T	140 (54.3%)	118 (44.3%)		
C	118 (45.7%)	148 (55.7%)	0.023*	0.672 (0.476-0.948)

*p-value<0.05 calculated through Chi-square (χ^2) test; CI: Confidence Interval.

Discussion

Clinical and biochemical characteristics

Hypertension is associated with abnormalities in lipid profile level resulted from lipid metabolism alteration [32]. Several studies have been reported the association of lipid profiles in the hypertensive subjects with disagreeing results [15,33,34]. We evaluated association of clinical and biochemical characteristics with EH, which are tabulated in Table 3.

We found significant difference in the mean of age, BMI, SBP, DBP, LDL, TG and TC ($p<0.05$) between hypertensives and control subjects. Our results are in line with the study of lipid profile measures in South Asian elderly hypertensive subjects [35]. However, we could not find a significance difference in HDL level of the study groups, where the mean difference were 1.04 ± 0.22 and 1.05 ± 0.26 for case and controls, respectively. Untreated hypertensive patients experience abnormalities in lipoprotein metabolism and have higher plasma LDL /HDL ratio [36,37]. It has been demonstrated that high triglyceride and low HDL cholesterol can serve as predictive value for CVD outcome and high TG/ HDL ratio is associated with arterial stiffness [38].

16071G/2G polymorphism of MMP-1 gene

A functional polymorphism resulting from an insertion of a guanosine at position-1607 in the promoter of *MMP-1* gene enhances transcriptional activity [23]. While, the 2G allele reduces the risk of CHD in Caucasians [22], it also has been associated with higher MMP1 antigen level in Polish patients with coexisting CHD and T2DM [39]. We found high percentage of mutant (control: 46.5% and EH: 46.6%) and

heterozygous (control: 45% and EH: 45.9%) in both the study groups. It may be explained by the fact that, there was high number of overweight and obese in both the study groups [40]. The percentage of obese and overweight was 22.5% and 26.4% in controls. While, in the cases the values were corresponding to 37.6% and 44.4%. This polymorphism was associated with BMI in Korean population aged above 50 and the *1G* allele depicted as protective effect against weight gain [40]. We could only find the similar results in Japanese and Iranians myocardial infarction subjects [41,42]. Furthermore, these findings are supported by the recent study conducted on North-eastern Han Chinese hypertensive subjects [24]. Collectively, data suggest that this polymorphism is unlikely to be a risk factor or a candidate gene for the development of hypertension in various populations.

C1562T and R279Q variants of MMP-9 gene

The *MMP-9*, also known as gelatinase B or 92-kDa type IV collagenase, is one member of the MMP family contributes to both normal and pathological tissue remodeling [42]. The polymorphism at position-1562 caused by a single base change C → T results in the loss of binding of a nuclear protein to this region of the *MMP-9* gene promoter and increased transcriptional activity [25]. The T allele was associated with higher blood pressure and arterial stiffness in hypertensives [17]. This polymorphism (rs3918242) was also studied in relation with AF, CAD and CVD [25-27]. Another polymorphism R279Q (rs17576) of *MMP-9* gene located at exon 6, is A to G substitution that results in change in amino acid arginine (R) to glutamine (Q) which lowers the catalytic domain activity of the enzyme [25]. The R279Q variant was evaluated in association with carotid artery [43,44] and hypertension [14,28]. Moreover, increased plasma level of *MMP-9* was associated with hypertension [45]. As a result, *MMP-9* gene polymorphisms may be considered as functional candidate genes for ischemic stroke and hypertension.

Concerning the C1562T variant, there could be no significant association detected in relation to genotype or allelic distribution of the subjects. The results are well in line with other populations where, there was no significant difference in Northeastern Han Chinese and Turkish hypertensive subjects [15,24]. However, not in accordance with other studies done in isolated systolic hypertension among Han Chinese [33]. This study was the first to evaluate the association of *MMP-9* C1562T polymorphism with hypertension among Malay males. MMP-9 enzyme level might be a risk factor for hypertension [45] but, C1562T variant shows no association ($p>0.05$) with EH susceptibility. Looking into genotypic and allelic frequencies of the R279Q polymorphism, No significant association could be seen in genotype or allele frequencies in either of the study groups ($p>0.05$). Our results are in contrast with the other study reported an association of *G* (*Q*) allele with hypertension [28]. Our results are in line with the study of arterial fibrillation in hypertensive heart disease patients of Chinese Han population [25] and Indonesian Javanese hypertensive subjects [14].

82A/G polymorphism of MMP-12 gene

The *MMP-12* enzyme secreted mainly by macrophages acts on variety of ECM substrates such as heparin, entactin, gelatin, fibronectin, elastin and type IV collagen [43]. This functional polymorphism located at position-82 causes an A to G substitution that affects the AP-1 binding site in the *MMP-12* gene, altering the expression of the gene [46]. This polymorphism had been studied in CAD [30], AAA [29] and ischemic patients [21] and it was suggested that, *A* allele was associated with smaller artery lumen in CAD patients with diabetes [46]. Despite of having diverse substrate specificity [43], data relation to this polymorphism are relatively inadequate. There was no significant difference in distribution of the genotypes and alleles between the hypertensives and controls. Our results are in line with the study conducted on carotid plaque susceptibility in Han Chinese population [47]. However, they failed to find any GG genotypes in either of their study groups. Nevertheless, the results are in disagreement with the findings of the latest study in North-eastern Han Chinese [24]. It may be concluded that, 82A/G variant of *MMP-12* gene was implausible to be related to EH in Malaysian male subjects.

372 T/C polymorphism of TIMP-1 gene

Tissue inhibitor of metalloproteinase-1, is a glycoprotein engaged in ECM degradation and has broad substrate specificity over most MMPs [2]. Circulating levels of *TIMP-1* were associated with Left ventricular diastolic impairment in hypertensives [11]. The *TIMP-1* 372 T/C polymorphism (rs4898) resulted from T to C substitution at position 372, was shown to be associated with survival rate in sepsis [19] and crohn's disease [48]. However, the association between *372 T/C* gene polymorphism of *TIMP-1*, was not determined in hypertensive subjects. The allelic frequency of *372 T/C* variant of *TIMP-1* gene was significantly different in case and control subjects ($p=0.023$).

Hitherto, this polymorphism was not analysed in relation to hypertensives and any other diseases in Malaysia. An emergent amount of studies reported imbalance in MMP activity/ inhibition in hypertension [49-51]. The *TIMP-1* gene is located on the X chromosome. Due to the nature of the analysis in X-linked gene polymorphisms, there might be larger or no difference seen in genotypic distribution of this variant regarding the gender [19,48]. In this study, the *372 T/C* polymorphism of *TIMP-1* was significantly associated with EH ($p=0.000$) among Malay males. However, future study is recommended to perceive whether the same results can be extrapolated in females as well.

Study Limitations

The current study has to be interpreted within the context of its limitations. The study provided only the evidences of the association between genetic polymorphisms of ECM metabolism genes. This study did not address the mechanism or the functionality of the variants for the selected genes. The

study groups were not age matched and our study was only focused on Malay male subjects. Age-matched association study needs to be done on the other ethnics; Chinese and Indians, as well to know the genetic risk factor for the development of hypertension in Malaysian population. It is necessary to investigate a large number of polymorphisms throughout the MMP, TIMP and the other genes, to perform association with hypertension and its complications. Also replication studies with larger samples and female subjects are strongly recommended to confirm the association of *MMPs* gene polymorphisms with hypertension.

Conclusions

The *TIMP-1* gene polymorphism (*372 T/C*, *rs4898*) can be considered as an independent risk factor or a candidate gene polymorphism for the development of hypertension among Malay male subjects.

Ethics Approval and Consent to Participate

Approved by National Medical Research Register of Malaysia (NMRR) (Ref. No.: NMRR-12-1062-12). Consent was obtained from all the subjects prior conducting this study.

Consent for Publication

Not applicable

Availability of Data and Materials

All relevant datasets supporting the conclusions of this article are available within the article.

Competing Interests

The authors declare that they have no competing interests.

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Author's Contributions

RV conceived the study and FAGT participated in the experimental design, data acquisition and analysis, interpretation of results, and drafted the manuscript. FAGT and RV interpreted the results, FH, SK, AE and PI critically reviewed the study for important intellectual content. All authors approved the final version of the manuscript.

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