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Multiplex Apo E, Apo C III and Ace II Genes Quantification with Anthropometric, Clinical, Socio-demographic and Dietary Risk Factors in Myocardial Infracted (MI) Patients from Southern Punjab, Pakistan

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Abstract

Introduction: Myocardial infarction (MI) is 3rd leading disorder with worldwide prevalence. Males have more death risk because of hypoxia to cardiac myocytes due to thrombus formation resulted from cholesterol or fatty acid deposition. This blockage induces blood flow interruption causing atherosclerosis and embolism. Dysregulation in triglycerides, cholesterol and fatty acid increases the risk for MI in these individuals.

Materials and methods: A study was designed with real time (rt) mRNA measurement (RT-PCR) using LUX™ primer for Apolipoprotein E, Apolipoprotein C III and ACE II genes/products. Study looks for uncontrollable factors - sex, age and family history and controllable factors - Body Mass Index (BMI), BSF, Diabetes, duration of diabetes, physical activity, hypertension, blood pressure, smoking, education level, socioeconomic status, dyslipidaemia, sleeping/ awaking habit, consumption of fruit, use of vegetables and consumption of meat, number of tea/coffee cups, were looked at.

Results: Results indicates that long duration of diabetes (p=0.005) diabetes mellitus (p=0.042), and consumption of meat (chicken) (p=0.032) pose significant association MI and the genes. Apolipoprotein E has shown to effect myocardial infracted male patients in age group 23-33 years, Body Mass Index (BMI) (>16 kg/m2), diabetes mellitus, creatinine (170-190 mmol/L), systolic blood pressure (90-120 mmHg), low levels of triglyceride can be independently assistant to MI. In the study pan /naswar show high statistical significance with previous heart attack (p=0.001) and sleeping /awaking time (p=0.001) pattern.

Conclusion: In nutshell, duration diabetes, quantities of triglyceride, age, systolic blood pressure and interestingly sleeping/awaking time may be of great importance to draw a mathematical model.

Keywords: Apolipoprotein CIII; Apolipoprotein E; ACE II; Transcriptomics; Myocardial infarction; Awaking time; Exercise

Introduction

Cardiovascular diseases (CVDs) have emerged as a complex medical encroachment of this century with a forecast of being leading cause of death by 2020 [1]. Since last two decades, around 16.7 million deaths took place with Myocardial Infarction (MI) all around the world [2]. Two thirds of these cases of MI transience occur in developing countries. Anticipation for future suggests that there would be a rapid increase till 2020 [3]. Higher risk of MI is documented in Southeast Asia in recent past [4]. From these data it is also eminent that population is at more threat from indigenous outfit [4,5]. According to one investigation, spanning 25 years, MI has reached with mortality of around ~50% in Australia, Canada, France and United States [1,2,4]. In Japan it gets to 60% [6]. Data have shown that Indo-Asian population has a repressive Coronary Artery Diseases (CAD) leading to MI episode [3,6,7]. This double specificity has appeared as foremost source of bereavement to this illness in the Indo-Pakistan subcontinent [7,8]. In south Asia (Pakistan, India, Bangladesh, Nepal, and Sri Lanka) that represent more than a quarter of world populace produces strong pretention to argue MI prevention in the region [8]. Today nucleotide polymorphism in MI influenced genes are enlisted in patient gene typing and single gene amplification expression studies [9,10]. Number of studies have highlighted many genes associated to MI i.e., Matrix metalloproteinase 3a (MMP3a) [11], Lipoprotein lipase protein (LPL) [12], Chemokine C-C motif receptor 5 (CCR5) [13], Apolipoprotein B (Apolipoprotein B) [7,8], Apolipoprotein E (Apolipoprotein E), Angiotensin II type 1 receptor (AGT R1) [14], AGT (Angiotensinogen), Angiotensin 1 converting enzyme (ACE 1) [8,15], C-reactive protein (CRP) [16], Fibrinogen β chain (FGB) [17], Thrombomodulin (THBD) [18], Platelet-endothelial cell adhesion molecule 1 (PECAM 1) [19] Atrial Natriuretic Peptide (ANP), Brain Natriuretic Peptide (BNP), C-type Natriuretic Peptide (CNP) [8], Heat Shock Protein A70-1 (HSP 70 A1)

[20], Selectin P (SELP) [21], CD 14 [22] are few in the long list [8,23]. To fulfil above notion on cardiovascular risks, a hypothesis was drawn on MI patients for demographic, dietary, socioeconomic, habitual and clinical observations for the first time in south this area population. In this respect three genes Apolipoprotein E, Apolipoprotein C III and ACE II were selected for transcriptomic quantification using real time LUX™ primers with other listed factors.

Materials and Methods

Collection of specimen and processing

Total of 45 myocardial samples of infracted patients were including. Nearly all specimens were collected from Combined Military Hospital (CMH), Multan, Pakistan. Some other samples were collected from Chaudhary Pervaiz Elahi Institute of Cardiology (CPEIC), Multan, Pakistan. A detailed questionnaire was developed using standard methods. Data was recorded for anthropometric and clinical review on the proforma including sex, age, height/weight BMI (kg/m2), Blood sugar (Fasting) and lipid profile. Other sociodemographic characteristics caste/race, monthly income, dietary habits, sleeping and awakening pattern, blood group, and education was asked from the patients. Clinical parameters like diabetes, smoking addiction, family history disease, tobacco used, and hypertension were also recorded to the above feedback form.

DNA extraction

Blood and serum samples were obtained after venous puncture (5.0 mL) from patients after 12 hours fast. From each above specimen 2.0 mL blood was added to Na2 EDTA vacuum tubes (BioVac, China) and stored at – 20°C in a freezer till future use (Orient, Pakistan). All samples were brought under cold chain to Micro/Molecular Biology Laboratory (MMBL), Institute of Pure and Applied Biology, BZU, Multan, Pakistan. All solutions used in this protocol to be sterilized at 121°C, 15 lbs pressure for 15 minutes autoclaving (Hirayama, Japan).

Defabricate blood (300 µL) from each patient was washed with 900 µL RBCs lysing solution (155 mM NH4Cl, 12 mM NaHCO3, 0.1 mM EDTA) [10]. Total RNAs were extracted with 330 µL TRIzolTM LS Reagent (Invitrogen, USA) [24,25]. The upper colourless phase in each case was precipitated with equal volume of 2-Propanol (Merck, Germany). RNA isolation solutions were kept at – 20°C for 2 hours or preferably overnight. Next day, tubes were centrifuged at 16 kg for 10 min (Eppendorf, Germany). The RNA pellets were washed once with 70% ethanol (DEPT water). The pellets were air dried first and then on heating block at 45°C for 30 minutes. The dried pellets were again dissolved in 200 µL DEPT – H2O and re-precipitated with half column Ammonium Acetate (5 M–pH 4.5) and 3 volume of absolute Ethanol (Merck, Germany). Samples were again dried in air and then at 45°C for 30 minutes. All samples were stored at – 20°C till further use [4,26]. Individual total RNA sample was reverse transcribed with RT - MaximeTM Random Hexamer kit (Intron Biotech, S. Korea). Reactions were performed on conventional thermocycler (Eppendorf, Germany). The program was written as 16°C for 10 minutes, 25°C for 10 minutes, 37°C for 45 minutes, 42°C for 10 minutes. It was followed with 95°C denaturation step.

qPCR Primer design

Real time LUXTM primers were designed from FASTA sequences (www.ncbi.org) for Apolipoprotein E (NM 000041.2), Apolipoprotein C III (NM 000040.1) and ACE II (NM 021804.2) with 50% GC constant, Tm target 63°C and 150-180 bp amplicon using QuantPrime® software (Max Plank Institute, Germany) (Table 1). The qPCR Platinum master mix with UDG (Invitrogen, USA) was optimized at 1.0 µL template in a 20 µL PCR reaction with 1.5 µL each gene LUXTM primers (10 µM) mix (forward and reverse primers). Real time multiplex quantifications were carried out on either Smart® Thermocycler (Cepheid Innovation, USA) or BioRad C1000 with CFX 96 real time detector (BioRad Laboratories, USA) for 45 cycles.

S. no	Target	Primer sequence	Amplicon	Modification
1	Apo E -F	5¢-TGGTCACATTCCTGGCAGGAACCA-3¢	206	3¢- JOE
2	Apo E -R	5¢-CTGCTCAGACAGTGTCTGCA-3¢		
3	Apo CIII -F	5¢-CATTGCAGGGTTACATGAAGCAC-3¢	150	3¢-FAM
4	Apo CIII- R	5¢-GTAGGAGAGCACTGAGAATAC-3¢		
5	ACE II-F	5¢-AGCGCCCAACCCAAGTTCAAAGGCTA-3¢	121	3¢- ROX
6	ACE II -R	5¢-AGGAGCCAGGAAGAGCTGGACATC-3¢		
Apo E - Apolipoprotein E, Apo CIII - Apolipoprotein CIII and ACE II - Angiotensin II sequences with LUX™ dye in modification column				

Table 1: Associated genes and LUX™ primers used in study.

Statistical Analysis

Statistical analysis of all parameters and gene quantifications (repeated thrice) were used to get two-way Analysis of Variance (ANOVA) with SPSS 19.0 version (SPSS Inc., USA). Significance of Pearson's correlation was also looked in with all parameters along with genes of the study.

Results

In Table 2 all highlights are outlined for significance with Diabetes mellitus (DM) (p=0.042), duration of Diabetes mellitus (DDM) (p=0.005) and interestingly consumption of meat (CM) with p values of 0.032 at 95% Confidence Limit (CL). On the contrary, Table 3 provides significance of anthropometric variables that are studied with Apolipoprotein E when its Ct value is 0 ≤ 10 and 10 ≤ 20 levels. Diabetes Mellitus shows borderline significant trend (p=0.09) value when Ct values of Apolipoprotein E are highly significant at 0 ≤ 10 and 10 ≤ 20. Furthermore, BMI less than 16 kg/m2 (underweight) show highly significant correlation to ACE II at ≤ 10 and 10 ≤ 20 (lower age) values. Creatinine (CTN) levels are highly associated to both ACE II at 0 ≤ 10, 10 ≤ 20, 20 ≤ 30, and 30 ≤ 40 ages while Apolipoprotein E is highly significant to 0 ≤ 10, 10 ≤ 20. Results on systolic blood pressure,

90-120 mm Hg, signify borderline relationship (p=0.092) to Apolipoprotein E, at 0 ≤ 10 and 10 ≤ 20 (Table 4). There have been significance values of p=0.025 that link meat consumption to Apolipoprotein C III gene estimations (Ct values) (data not shown). Lipid profile, especially higher triglyceride has higher significance to both Apolipoprotein C III (Table 4). Results on gene quantification (Ct) show this with the increase in age. On the other hand, Apolipoprotein E have very high significant correlation to ACE II (p=0.002 at 99% CL) and to Apolipoprotein C III (p=0.004) also. Here, it is important that they all are concomitant underweight individuals in the study (Table 3). It is captivating that family history (fhMI) is just correlated significantly to Apolipoprotein C III with Pearson correlation 0.317 (p=0.046). Patient's education level, as expected, has a very high inverse correlation to previous heart attack with p ≤ 0.000 (Table 5). The consumption of pan/naswar/others (PNO) all show correlated significant to previous myocardial infraction with p=0.002 at 99% CL (Table 5). Another important factor in these patients is their previous heart attack which specifically correlates to patient's physical activity (p=0.001 at 99% CL). Amazingly, time to get to bed has been associated to previous heart attack (p=0.001 at 99% CL), as well as to PNO (p ≤ 0.000 at 99% CL) and physical activity (p ≤ 0.000. In further analysis, morning awaking time show significant association to physical activity (Table 5). Again, in Table 4 and Table 5, represent some other important significances of risk factors that are correlated to quantified genes. Very exciting observation of these is that DM is negatively associated with sex (p=0.042). Furthermore, grippingly outcome on diastolic blood pressure (p=0.038 at 95% CL) show good correlation to age (Data not shown). In the same finding, on the other hand, hypertension is inversely association to systolic blood pressure (p ≤ 0.000). In Table 4 also systolic blood pressure is highly statistically significant to BMI kg/m2 (p ≤ 0.000 at 99% CI) also. In toto, linking these parameters, we can interpretate from these finding that diastolic blood pressure is linked to expressive transcripts of Apolipoprotein C III and Hypertension (p=0.004 at 99% CL). This in turn is a new finding. These multiplex gene quantifications also significances on the lipid profiling of these individuals. In Table 4, such correlations are decorated with total cholesterol and triglyceride that are highly significant (p=0.003). As expected, HDL is negative associated to total cholesterol (p=0.001) and triglyceride (p=0.005).

Parameters		Sig. (p Value)
DM	Between Groups	0.042
	Within Groups	
	Total	
DDM	Between Groups	0.005
	Within Groups	
	Total	
CM	Between Groups	0.032
	Within Groups	
	Total	

Note: DM (Diabetes mellitus), DDM (Duration of Diabetes mellitus), and CM (Consumption of Meat)

Table 2: ANOVA table.

Parameters		Sig. (p value)
Threshold	Sex=Male	0.998
	Apo E=0£ <10	0
	Apo E =10£ <20	0
Threshold	Age=23	0.007
	Age=24-33	0.023
	Apo E =10£ <20	0.034
Threshold	DM=exist	0.999
	Apo E=0£ <10	0
	Apo E =10£ <20	0
Threshold	BMI ≤ 16	0.028
	ACE II=20£ <30	0.038
	ACE II=30£ <40	0.038
Threshold	CTN=170-190	0.06
	ACE II=0 £ <10	0
	ACE II=10£ <20	0
	ACE II=20£ <30	0
	ACE II=30£ <40	0
	Apo E=0£ <10	0
	Apo E =10£ <20	0
Threshold	Systolic=90-120	0.092
	Apo E=0£ <10	0
	Apo E =10£ <20	0
Threshold	CM=1	0.025
	Apo CIII=20£ <30	0.014
	Apo CIII=30£ <40	0.034
Threshold	TG=1	0.015
	Apo CIII=0£ <10	0
	Apo CIII=10£ <20	0
	Apo CIII=20£ <30	0
	Apo CIII=30£ <40	0
	Apo E=0£ <10	0
	Apo E =10£ <20	0

Note: Apo CIII – Apolipoprotein CIII, Apo E – Apolipoprotein E, ACE II – Angiotensin Converting Enzyme II, BMI – Body Mass Index, CTN - Creatinine. p>0.05 not significant, p<0.01 Significant, p<0.001 highly significant.

Table 3: There is a significant association of different Ct values of genes to parameters with 95% confidence interval with lower and upper bound.

Variables		ACE II	Apo CIII	Apo E	TG	TC	HDL
ACE II	Pearson Correlation	1					
	Sig. (2-tailed)						
Apo CIII	Pearson Correlation	0.504**	1				
	Sig. (2-tailed)	0.001					
Apo E	Pearson Correlation	0.479**	0.448**	1			
	Sig. (2-tailed)	0.002	0.004				
TG	Pearson Correlation		0.490**		1		
	Sig. (2-tailed)		0.001				
TC	Pearson Correlation				0.454**	1	
	Sig. (2-tailed)				0.003		
HDL	Pearson Correlation				-0.487**	-0.433**	1
	Sig. (2-tailed)				0.001	0.005	
LDL	Pearson Correlation				-0.576**		0.764**
	Sig. (2-tailed)				0		0
Note: TG (Triglyceride), TC (Total Cholesterol), HDL (High Density Lipoprotein) and LDL (Low Density Lipoprotein).							

Table 4: Correlation of ACE II, Apo CIII and Apo E with biochemical parameters.

Variables		ACE II	Apo CIII	DM	PHA	EDUC	PNO	PA	ST
ACE II	Pearson Correlation	1							
	Sig. (2-tailed)								
Apo CIII	Pearson Correlation	0.504**	1						
	Sig. (2-tailed)	0.001							
Apo E	Pearson Correlation	0.479**	0.448**						
	Sig. (2-tailed)	0.002	0.004						
DDM	Pearson Correlation			-0.702**					
	Sig. (2-tailed)			0					
fhMI	Pearson Correlation		0.317*						
	Sig. (2-tailed)		0.046						
EDUC.	Pearson Correlation				-0.586**	1			
	Sig. (2-tailed)				0				
PNO	Pearson Correlation				0.479**		1		
	Sig. (2-tailed)				0.002				
PA	Pearson Correlation				0.504**		0.511**	1	
	Sig. (2-tailed)				0.001		0.001		
ST	Pearson Correlation				0.499**		0.659**	0.639**	1

	Sig. (2-tailed)				0.001		0	0	
AT	Pearson Correlation				0.757**		0.772**	0.723**	0.823**
	Sig. (2-tailed)				0		0	0	0

Note: DM (Diabetes Mellitus), DDM (Duration of Diabetes Mellitus), fhMI (family history of MI), PHA (Previous Heart Attack), EDUC.(Education), PNO (Pan/ Naswar/ others), PA (Physical Activity), ST (Sleeping Time), AT (Awaking Time).

Table 5: Pearson's Correlation of ACE II, Apo CIII and Apo E and physical parameters in the study.

Discussion

Myocardial infarction results coronary arteries damage/or blockage that cause hypoxia to heart muscles [27]. Several medico-epidemiological studies have documented that dyslipidaemia as one main reason of such event [28,29]. This dysregulation in lipids metabolism could be a consequence of some genetic factors/ polymorphisms in several genes [29] along with vulnerability to environmental factors [28,30]. Our group outlined a study to get insight into excess amounts of gene transcripts, Apolipoprotein E, Apolipoprotein C III and ACE 2, that could influence with other risk factors in myocardial infarcted individuals. This outlined study could also answer/identify early assessment biomarker for multifactorial risks process within these families. This Pakistani study is similar to RAG system of Proforma Risk Assessment System as reported earlier [31].

Age and sex

The study reveals data on sex and age that most MI patients occur in male (45-55 years) group (57.5%). A similar pattern is presented in the study of Roncaglioni and associates [28]. Global data from developing countries, as well as of Pakistan, indicates that young people are at higher risk in developing MI as compared to other developed parts of the world [32,33]. This is well cited in age related male reports [22]. Additional to this, presence of previously heart attack has emerged as a major risk factor for these individuals (data not presented). In our study, selected MI patients, 32.5%, were retained under these criteria. Data indicates that study population comprises of uneducated (55%) or/and lesser knowledge about the disease creates a trend with a higher risk. This has again been highlighted by other evidential studies reported elsewhere [22,24,33].

Sleeping/awakening, hypertension and others

In our final analysis an interesting conclusion is drawn about previous history of MI, sleeping pattern and awaking time is highly significant along with physical activity in this Pakistani patient collection (Table 4). To our knowledge this investigation is probably first of its kind where morning awaking time is more significantly placed than to the sleeping time (Table 4). Some of the findings are already been stipulated in other prior studies with daily daylight biorhythmic cycles [4]. Our data also indicates that 37.5% MI patients in category are hypertensive. Volumatous data citations support our finding where hypertension is considered as major cause of mortality in MI patients [34].

LUX Primer™ Gene Quantification

Interpretation to the gene quantification for ACE II and Apolipoprotein (both Apolipoprotein E and Apolipoprotein C III) indicates that they are interconnected to systolic and diastolic blood pressure (hypertension) (Table 4). It is proposed that in hypertension the quantities of Apolipoprotein C III are influenced by the hepatic uptake of triglyceride (TG) rich lipoproteins (TRLs) and their remnants [5,30,35]. The presence and quantities of remnants is now considered as determinants in the lifestyle causes that include diet, obesity, alcohol intake, and physical activity [36]. Similar outcome is also been portrayed very well in our study also. Recent literature has focused on these remnant lipoproteins, especially with reference to Apolipoprotein C III, which have been named to create extremely atherogenic deposits and contributes to specific CHD risk assessment. This signifies dysregulated lipid metabolism [34,35]. The Apolipoprotein C III is also shown to prevent lipoprotein lipase in catabolism of accumulated TG rich lipoproteins (Table 4) [12,35]. Literature provides an ample portentous evidence in its assembly that secretion of this gene product is a metabolic deferment [15]. In our study, Apolipoprotein C III gene quantification also indicates similar association to our MI patients in family history with an overall genetic predisposition for 22.5% which has been well addressed in number of excellent papers [7,12].

Apolipoprotein C III

In a recent research survey on relative risks of MI with Apolipoprotein C III without (0.66 times) and with HDL-C (1.18 times) showed that there is high significance of Apolipoproteins in general [9]. Molecular function of Apolipoprotein C III, a 79 amino acid protein, is assigned to the surface of some lipoproteins complexes which in turn enthused an inflammatory event that engross an atherosclerotic deposit [34,37]. It has been enlisted in literature that Apolipoprotein C III gene sequence polymorphism at 3'-untranslated region relates to elevated triglyceride in several populations [10]. From our data, we have inferred that interaction of Apolipoprotein C III depict a genetic disorder in these patients which we studied in our study (data not shown) [2]. Previously also, this has been singled out with an allelic copy that sufficiently increases probability of atherosclerotic and amassing of fat in vessels [35]. Recently separation of HDL-C in accordance to Apolipoprotein C III is an eye opener where two types of HDL with opposing associations with risk of Coronary Heart Disease (CHD) and MI [37,38]. The proatherogenic effects of Apolipoprotein C III, as a component to VLDL and LDL, may also reasonably be extended to levels of HDL also [9], as indicated in our study. Previous work on Apolipoprotein C III quantities has shown that higher levels have related to several pathological conditions like hyperbilirubinemia [6], type 2 diabetes, kidney functional deficiency [13] which we also extra Apolipoprotein from our data to some extent (Table 2 and Table 4) [2]. Molecular perturbation on Apolipoprotein C III is shown to result in receptor mediated degradation of circulating TRLs which could develop hypertriglyceridemia [36], myocardial ischemia and carotid artery atherosclerosis [39]. Statistical data in Apolipoprotein C III expression

(data not presented) can influence by both type of blood pressure i.e., systolic and diastolic, through Body Mass Index parameter [16]. It could be concluded from our, as well as from other recent years data, that quantification of Apolipoprotein C III (gene)/Apolipoprotein C III (protein) is a reliable marker for lipoprotein concentration associated CAD risk that lead to MI [5,40]. In our study, one other finding with Apolipoprotein C III show that increased gene expression relates to young individuals negatively (Table 4). This trend represents same worldwide trend where young age group individuals develop hypertension [30]. It has also been demonstrated that Apolipoprotein C III promote pancreatic β-cell death, while its antisense activity delays the onset of type 1 diabetes [41,42]. These findings provide us an important gist that they are relatively less protected than the aged individuals and put them at more risk to MI. Same conclusion has also been drawn by Flint and associates in preceding work [1].

Apolipoprotein E

Our study wields on statistical methods that showed that Apolipoprotein E transcripts (protein) along with Apolipoprotein C III may serve as predictor for next cardiovascular episode, especially in these selected individual [s] in this study (Table 4). Literature suggests that an increase in Apolipoprotein E content relates to VLDL whereas Apolipoprotein C III on lipid fractions and catabolism [17,29]. This interpretation helps us to understand the association of triglycerides and cholesterol which is also reflected and infer from our study (Table 4). In this context, Mendivil and associates [29] has rightly proposed that Apolipoprotein C III can be a low risk marker for CHD and MI [43]. Some other studies on mouse models, overexpression of Apolipoprotein C III produces hyperlipidaemia that increases atherosclerotic lesion [22].

Apolipoprotein E gene has shown that it has a positive and significant connection to other two genes of study i.e., Apolipoprotein C III and ACE II. Apolipoprotein E has been an important gene in lipoprotein metabolism especially for LDL-cholesterol and Apolipoprotein E receptors isoforms [1,37,44]. Apolipoprotein E mutations have been linked to many other diseases as well as inactivation of specific metabolism [45,46]. Involvements of Liver X receptors and recently identified lipoprotein (a) are risk factor that have effect with Apolipoprotein E product [34]. Recently genetic variant alleles were studied in Genome Wide Association Study [GWAS] which implicate familial hyperlipidaemia with elevated levels of triglycerides in Mexicans population [23,37]. We infer from our data that there is significant existence of Apolipoprotein E as of chylomicrons and VLDL remnants in our studied group (Table 4). As shown in other citations, its role is also connected to antioxidant and modulative neurotropic factor [47-49]. Lipid profiling in study show that it is positively association to LDL-Cholesterol and Triglyceride (Table 3]. From our analysis HDL – Cholesterol and LDL-Cholesterol are, however, negative association to Apolipoprotein E [9,49]. This again provide a plausable explanation that they are two separate pathways in lipid abnormality of metabolism [5,7,49].

The expression levels of Apolipoprotein E 4 (ε4) allele show that higher levels of total cholesterol and LDL cholesterol are regulated [32]. This tends to create an unusual risk for CHD. On the contrary, Apolipoprotein E 2 (ε2) isoform associates to the opposite (i.e., protective) in Caucasian populations [1,49]. Though we didn't do gene polymorphism study nor can extract any significance on present data to address isoform specific connotation. This needs further investigation on the specific isoform concentrations. On the above the disagreements, use of Apolipoprotein A and Apolipoprotein B ratio (Apo A/Apo B) is one that is an offshoot for risk assessment marker in recent presented studies [42-44]. In our opinion, quantified fraction of the Apolipoprotein E (ε4) to Apolipoprotein E (ε2) could be important for future evaluation of these individuals that could create difference in disease and its types. At present hypertensive individuals show similar pattern as simulated to other many global investigations [6,27,30,49]. In recent conclusions, we know that all these receptor – isoforms interactions are able to excite separate specificities that influence triglyceride – cholesterol metabolism [1,30]. Change in lifestyle, diet therapy appears to be very much essential to reduce these and other associated risks including glucose levels [1,50-52].

ACE II

ACE II is a result of cleavage of parent peptide that becomes activated. The activated peptides control maintenance of ionic concentrations in blood [53]. Higher concentrations of ACE II have also shown to limit pulmonary blood flow to embryonic life effecting myocardial cell death [29]. There are literature citing that indicates that ACE II gene expression influences osmotic balance of body fluid [54]. In our exploration to find how ACE II it has effect on low BMI (<16 kg/m^2). Table 3 show considerable correlation to ACE I gene expression. Our collective data on ACE II and Apolipoprotein E suggests that it relates to creatinine concentration also in these subjects (Table 3). ACE II, Apolipoprotein E and Apolipoprotein C III joint analysis show that Apolipoprotein E plays an impact on total LDL cholesterol levels, because of heart muscle osmotic regulation [53]. This in turn increases risk for atherosclerosis and then cardiovascular disease in this group of Pakistani individuals [35,38,55].

In the last but not the least, our investigation suggest that more meat consumption may cause increased risk of heart attacks. These findings on Pakistani MI patients highlights contradiction to other such previous work [35]. From our study we can also summarise that persons who do not consume meat have lower or non-MI episode [p=0.032] and their after. In this inquiry it also designates use of tobacco, Pan/Naswar/Huqqa/Beedis containing tobacco etc. effect MI outcome, like demonstrated in previous studies (Table 3 and Table 4) to heart condition as indicated in our results [56]. This above notion of ours has been well supported by Ali's study in this respect [51]. Other supporting studies have shown that training and exercise in these individuals creates a safe and value addition to these MI condition. This will definitely influence their systolic dysfunction also [57]. Further work on the cell signalling on the heart muscle is needed to understand completely the association of these three gene products and their interactions for the influence on other biomolecules.

Conclusion

Our data outline points to some new interesting possessions i.e., longer duration of diabetes, consumption of chicken meat creates greater risk to have myocardial infarction. These gene quantifications also demonstrate and reinforce our belief that physical activity in addition to morning awaking time significantly effect myocardial infarction or any other outcome. Higher levels of transcripts of ACE II and Apolipoprotein III are associated to hypertension, a major impact on these patients. ACE II alone can draw our focus to the involvement in MI with lower BMI subjects. From the previous data and this data, we can infer that Apolipoprotein C III quantification appears to be mutated form that lack triglycerides catabolism. In last, this multiplex quantification does provide an insight in the prospective outcomes in

these MI patients and Apolipoprotein CIII and ACE II genes. Use of Apolipoprotein E, however, could provide valuable information about dysregulation in lipid metabolism.

Conflicts of Interest

There are no conflicts of interest for the present study.

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