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Assessment of the Platelet Adenosine Diphosphate Receptor (ADP Receptor p2y12) Polymorphism in Coronary Heart Diseases Patients in Ismailia

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Abstract

***Background*: Cardiovascular diseases are the leading cause of death in developed countries. In Egypt mortality rates from coronary heart disease are one of the highest worldwide, also coronary heart diseases will be the leading cause of death in developing countries by the year 2020. P2Y12 ADP receptor gene polymorphism is now considered one important implication factor in atherothrombosis and coronary heart diseases. *Aim:* To assess the correlation between the platelet Adenosine Diphosphate receptor (P2Y12) polymorphism and coronary heart diseases patients in Ismailia. *Subject and Methods:* An analytical case control study; conducted on 100 volunteers divided into 2 groups; 50 for study (patient) group and 50 for control group at Ismailia Hospitals' Cardiology premises (clinics & inpatient department) to assess the correlation between the platelet ADP receptor (P2Y12) polymorphism and coronary heart diseases in Ismailia. The patients were subjected to an Interview Questionnaire, clinical examination and Laboratory Investigations and testing for P2Y12 gene polymorphism. *Results:* In the study group, 42% patients were hypertensive, 48% were diabetics, 60% were dyslipidemic and 66% were smokers. In the control group individuals, 26% were hypertensive, 20% were diabetics, 12% were dyslipidemic and 24% were smokers. DNA extraction and examination for P2Y12 gene polymorphism was negative in all participants. *Conclusion:* Our study revealed that there is No correlation between the platelet ADP receptor (P2Y12) gene polymorphism and coronary heart diseases in Ismailia.**

Keywords: Ischemic heart disease, Thrombophilia, Platelet glycoproteins, Atherothrombosis

Introduction

Epidemiological studies regarding cardiovascular disease; showed that despite the considerable advances in its treatment cardiovascular diseases remain an important cause of death in the developed countries. It is assumed that Coronary artery diseases (coronary heart disease) will be the first leading cause of death in the developing countries by year 2020. The mortality secondary to coronary heart disease in Egypt is rapidly rising; according to the WHO statistics, the mortality rates from the coronary heart diseases are from the highest all over the world(1). The crucial role of platelet aggregation as a key component for development of acute thrombosis in cardiovascular diseases had been repeatedly em-

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phasized[2]. The platelet membrane glycoproteins are surface glycoproteins found on the surface of platelets; the main role of them is Stable adhesion to Von Willebrand factor and platelet activation leading to their aggregation and thrombus expansion[3]. Genetic polymorphisms are the stable DNA sequence variations that occur in a percentage greater than 1% of chromosomes in the population. These polymorphisms may be silent or can alter the amino acids. Moreover, in the near past, many studies have been designed addressing the role of the inherited platelet risk factors in the acute ischemic coronary syndromes[2]. Polymorphisms of the adhesive molecules are being thought about as important risk factors for the arterial thrombosis due to many reasons. First, most of these are variations in the adhesion molecules and if the amino acid change affects function, it can affect pro-thrombotic tendency of platelet. Second, there is big heterogeneity among platelets from different donors. In spite of that many factors could affect this inter-individual variation; the genetic differences are the likely explanation[2]. Adenosine diphosphate (ADP) is one of the key molecules which enhances the platelet aggregation and that is released from the activated platelets. There are three known, main ADP receptors on the platelets: the P2X1 receptor (a ligand-induced ion channel) and the G-protein coupled receptors P2Y1 and P2Y12 ADP receptors[4]. The P2Y12 is the ADP receptor that has been cloned recently. It is the target of the thienopyridine anti-platelet drugs. The stimulation of P2Y12 receptor facilitates the binding of fibrinogen to the glycoprotein receptor IIb-IIIa[5]. Genetic polymorphisms of receptor P2Y12 (ADP receptor) have been identified, this may affect its activation by ADP or affect the response of patients to platelet inhibitors[6]. There is another frequent polymorphism which was found to be associated with a reduced clinical response to thienopyridines treatment, the 34°C > T polymorphism. A study by Fontana, et al. discussed that H2 haplotype of P2Y12 ADP receptor was significantly associated with an increased platelet aggregation[7]. Thus, it was postulated that P2Y12 polymorphisms are associated with less protection by these platelet inhibitors, which would be of good clinical relevance for patients with coronary artery disease. However, the majority of patients with CAD receive treatment with acetylsalicylic acid, which primarily inhibits the TXA2-dependent signaling cascade of the platelet activation process[8]. A crosstalk between ADP- dependent and TXA2-dependent pathways of platelet activation is confirmed (i.e. activation of platelet thromboxane receptors leads to ADP release and vice versa) however the role of response of P2Y12 polymorphism to acetylsalicylic acid is not clear so far. Do the mutations in the P2Y12 gene for the ADP receptor cause the general anergy of platelets, or do they alter the specific pathways of platelet activation? The study of P2Y12 gene mutations allows the investigation of a relationship between signaling of different activation pathways such as ADP and arachidonic acid. The authors suggested that the aspirin anti-aggregatory effects in patients carrying P2Y12 ADP receptor polymorphisms could be due to the predominance of the ADP-dependent aggregatory mechanisms. Recently personalized medicine in the cardiovascular field had been frequently acknowledged with a remarkable growth in scientific publication[9]. Like other polymorphisms in the clinical studies, findings related to P2Y12 polymorphism are conflicted, even the results of the meta-analyses were not the same: some studies have shown no link between the polymorphic p2Y12 genes and MI and/or CAD risk, whereas other studies have shown slight

but clear associations between these polymorphisms and the CAD risk with/without ischemic coronary events occurring after revascularization[10,11]. According to the best of our knowledge, no published data is available about the role of the platelet Adenosine Diphosphate receptor (ADP receptor P2Y12) polymorphism as a genetic risk factor in coronary heart disease patients in Ismailia. This work aims to assess the correlation between the platelet Adenosine Diphosphate receptor (P2Y12) polymorphism and coronary heart diseases patients in Ismailia.

Subjects and Methods

The present study is analytical case control study conducted on 100 individuals, 50 subjects and 50 controls, who attended the cardiology inpatient wards and outpatient clinic, Suez Canal University Hospital, Ismailia Insurance hospital, Ismailia General Hospital, Ismailia, Egypt. the mean age in MS group was (48.2 ±2) compared to the control group which was (44.72 ±2.14). Controls had 21 female and 29 males, while metabolic syndrome patients were 24 females and 26 males. Subjects were divided into two groups: *Group 1* included fifty patients who were diagnosed with CHDs or its variants. *Inclusion criteria were:* i) Patients diagnosed with acute myocardial infarction (MI) by meeting the following criteria (Rise or fall of cardiac biomarkers indicating pathology in the cardiac physiology; at least above or below the 99^{h} percentile of the upper or lower reference limit and with at least one of the following: Symptoms of ischemia, ECG changes indicative of new ischemia including new significant ST-T changes or new left bundle branch block; Development of pathological Q waves in the electrocardiogram; Imaging evidence of new loss of viable myocardium, or new regional wall motion abnormality. ii) Patients diagnosed with prior myocardial infarction by meeting the following criteria (Development of new pathological Q waves in the ECG with or without symptoms and Imaging evidence of a region of loss of viable myocardium that is thinned and failed to contact). *Exclusion criteria were:* Clinical signs of heart failure, Severe arrhythmia and conduction defects of the heart, Familial forms of hypercholesterolaemia or hyper-triglyceridaemia, Hepatic and renal failure, Neoplastic diseases, Pregnancy and/or lactation at screening, or on contraception. *Group 2* (Control group) included fifty apparently normal and healthy subjects attending any of the fore mentioned settings for routine medical check-up, or attending the blood bank for blood donation were included in the study; they were age and gender matched with the study group. All participants were subjected to the following 1- interview questionnaire that included personal, present, medical and family history. 2- General examination including general appearance, vital signs, head, neck, heart, chest, and abdomen examinations. 3- Lab investigations including CBC, Liver function tests, kidney function tests, lipid profile, fasting & postprandial blood sugar, glucosylated haemoglobin (HbA1C). 4- DNA isolation and genotyping for the platelet Adenosine Diphosphate receptor (ADP receptor P2Y12) polymorphism: Genomic DNA was isolated from whole blood according to manufacturer's instructions using a commercial kit QIAamp DNA Blood Mini Kit (Quiagen, Hilden, Germany).. The extracted DNA was extracted and purified according to the slandered protocol. Genotyping of ADP receptor (P2Y12) for gene polymorphism was performed to detect the *P2Y12* T744C gene polymorphisms.

Results

The participants' ages ranged between 30 and 65 years old, most of them are around

60 years old, which is typical with the common age for CHDs. The mean age of the study group was (53.5 ± 9.07) years, while for the control group was (49.9 ± 13.1), moreover 71% of the whole study population were males and 29% of population were females. As for dyslipidemia in the two studied groups, about 60% of the case (study) group had dyslipidemia while only 12% of the control group had dyslipidemia, and 83.3% of the dyslipidemia cases was belonging to the group of cases which is considered relevant in our study subject (P <0.05) (Table 1). As for hypertension in the two studied groups, it was found that about 42% of the case-study group had hypertension and only 26% of the control group was hypertensive. (Table 2).

Table 1: Dyslipidemia among the study population

Variable	Groups		Total	Odds ratio (95% CI)	p-value
	Control N=50	Cases N=50			
Dyslipidemia					
• No					
Count	44	20	64	11 (3.95-30.61)	<0.0001*
%	68.8%	31.3%	100.0%		
• Yes					
Count	6	30	36		
%	16.7%	83.3%	100.0%		

* = Significant, p-value < 0.05

Table 2: Hypertension among the study population

Variable	Groups		Total	Odds ratio (95% CI)	p-value
	Control N=50	Cases N=50			
Hypertension					
• No					
Count	37	29	66	2.061 (0.885-4.8)	0.091
%	56.1%	43.9%	100.0%		
• Yes					
Count	13	21	34		
%	38.2%	61.8%	100.0%		

* = Significant, p-value < 0.05

Molecular assessment in the form of DNA isolation and genotyping of the platelet ADP receptor (P2Y12) polymorphism for both groups was performed after PCR-based restriction fragment length polymorphism (RFLP) analysis of the samples. The primers used were designed by Analysis for life technology (Invitrogen), i.e. specific for the P2Y12 gene. All samples showed negative results for polymorphism of the P2Y12-gene using Afa I (Rsa I) restriction enzyme reaction mix clarifying that no polymorphic P2Y12 genes existed in samples of patient and control samples. (Table 3). The final run for all the fifty samples of case (patient) group is shown in figure1. None of the samples of either patients or control groups showed polymorphic ADP receptor (P2Y12) gene sequence

Table 3: Results of Genotyping & assessment of P2Y12 gene polymorphism

Variable	Groups		Total
	Control N=50	Cases N=50	
P2Y12 Gene Polymorphism			
• Present			
Count	0	0	0
%	0	0	100.0%
• Absent			
Count	50	50	100
%	50%	50%	100.0%

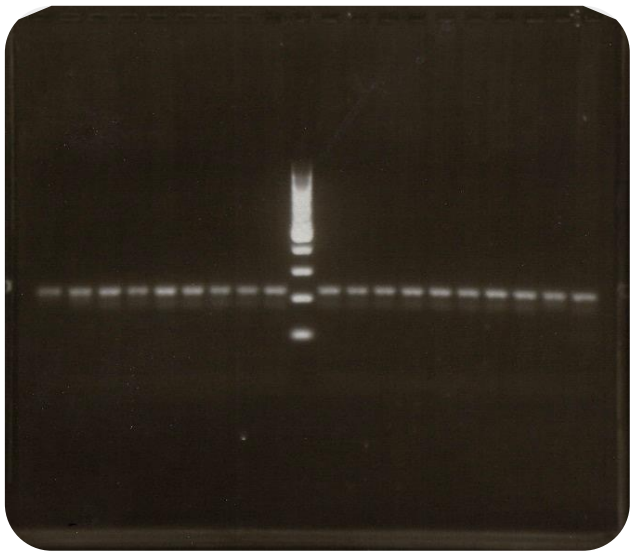

Figure 1: Gel electrophoresis using Primers and Afa I (RsaI) restriction enzyme reaction mix (Patient samples)

Discussion

Atherosclerosis is a multifactorial disease and involves both environmental and genetic factors. Therefore, the low penetrance of a single functional polymorphism may not always lead to a clinical phenotype as this may be obscured by environmental factors. This may explain why the H2 haplotype of P2Y12 gene had its strongest association with CAD in non-smoking individuals[12]. Therefore, this is also suggesting that the P2Y12 gene is an independent risk factor for coronary artery disease in these subjects. Another author concluded that conventional cardiovascular risk factors were more common in patients with CAD[12]. Several recent meta-analyses suggest that the effect of individual genes on the risk of complex traits such as coronary heart disease may be weak. Therefore, attempts should be made to improve the power to detect genetic associations[13]. In our study, about 60% of the case (study) group had dyslipidemia while about 12% only in the participants of the control group had dyslipidemia, and 83.3% of the dyslipidemia cases was belonging to the group of cases which is considered relevant in our study subject (P-value <0.05).Regarding the prevalence of hypertension, it was found that about 42% of the case-study group had hypertension and

only 26% of the control group was hypertensive. On the other side, in a study by Zoheir, et al. (2013) 40% of the case (study) group were hypertensive, and 30% of the control group were hypertensive[14]. Those results are very close to the results obtained in our study. By performing the DNA extraction and examination for P2Y12 gene polymorphism, the results were negative in all the participants. Although negative control samples as well as Positive control samples (PW2000 plasmid DNA sequence) were run by Gel electrophoresis with the study samples, this current study revealed no positive results; this indicates that there is no correlation between the platelet ADP receptor (P2Y12) polymorphism and the coronary heart diseases in this study population. Similar to our study, in a study of Bierend, et al., platelet aggregation of CAD patients was not significantly affected by polymorphism of the ADP receptor (P2Y12) gene "H2 haplotype"[15]. These findings is in contrast to another study by Fontana, et al. who found enhanced aggregation response to ADP in healthy untreated volunteers who were carriers of the H2 haplotype, this might put patients at increased risk of thrombotic complications despite ongoing antiplatelet therapy[7]. Also Angiolillo, et al. found no differences between carriers and non-carriers of the P2Y12 gene polymorphism "H2 haplotype"[16]. Indeed, a significant association between this functional P2Y12 gene polymorphism and atherosclerosis was shown by Fontana, et al.[7]. Bierend, et al. also concluded that P2Y12 ADP receptor polymorphisms are more frequent in patients with vascular disease[15], the majority of those patients received aspirin. An association between the minor H2 haplotype of the P2Y12 gene and presence of significant coronary artery disease was also demonstrated by Ugo Cavallari, et al.[12]. In fact, this polymorphism has shown to be associated with peripheral arterial disease, while its association with coronary artery disease has been poorly explored according to Fontana, et al.[7]. One prior report has assessed the association between minor H2 haplotype of the P2Y12 gene and long term complications of coronary artery disease (cardiac death, myocardial infarction and refractory angina requiring revascularization) but failed to find any association[17]. Angiolillo et al. compared between T744C polymorphism genotypes [wild allele TT and C allele (heterozygous CT, homozygous CC)] in ACS patients regarding clinical data (age, Sex, hypertension, smoking, Diabetes), they revealed no statistically significant difference between the two groups, also showed that there were no statistically significant differences in platelet count, haematocrit or mean platelet volume between carriers and non-carriers of C-allele[16]. A recent study, an association between the T744C polymorphism of the P2Y12 ADP receptor gene and platelet reactivity was found. Carrying C allele at this position is associated with an increased platelet activation response to ADP. Identification of this genotype effect partly explains the inter-individual variation in platelet response to ADP and may have clinical implications with regard to risk of arterial thrombosis and individual response to certain anti-platelet agents[14].

Conclusion

Our study revealed the absence of a correlation between the platelet adenosine diphosphate receptor (P2Y12) gene polymorphism and coronary heart diseases in patients in Ismailia.

Conflict of interest:
Authors declare no conflicts of in interest

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