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Investigating the link between MCP-1 A-2518G, RANTES G-403A, CX3CR1 V249I and MTHFR C677T gene polymorphisms and the risk of acute myocardial infarction among Egyptians

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ABSTRACT

Background: Acute myocardial infarction (AMI) is one of the leading causes of death among Egyptians. Monocyte chemoattractant protein-1 (MCP-1), regulation on activation normal T cell expressed and secreted (RANTES) and fractalkine (FKN) are chemokines that act as components of inflammatory response while methylenetetrahydrofolate reductase (MTHFR) is important enzyme in folate metabolism essential for homocysteine metabolism. Hyperhomocysteinemia has been linked to AMI. MCP-1 A-2518G, RANTES G-403A, CX3CR1 V249I and MTHFR C677T are important polymorphisms identified in MCP-1, RANTES, CX3CR1 and MTHFR genes respectively. There are conflicting data in the literature about their association with AMI. Therefore, the aim of the current study was to investigate the contribution of these gene variants to risk of AMI among Egyptians.
Subjects and methods: The study comprised 200 subjects; 100 AMI patients and 100 age-matched healthy controls. The MCP-1, RANTES, CX3CR1 and MTHFR genotypes were determined by restriction fragment length polymorphism (PCR-RFLP).
Results: Genotypes distributions for RANTES, fractalkine and MTHFR genes were significantly different between AMI patients and controls ($p = 0.0221$, 0.0498 and 0.0083) while those results in MCP-1 were not significantly different. A significant risk for AMI with concurrent presence of RANTES (AG/AA), fractalkine (VV) and MTHFR (CT/TT) genotypes was observed.
Conclusions: 1 - Each of MTHFR 677T, RANTES-403A and CX3CR1 249V alleles is considered an independent risk factor for AMI. 2 - Concurrent presence of high risk genotypes of RANTES (AG/AA), fractalkine (VV) and MTHFR (CT/TT) increases risk of AMI more than their individual risks. 3 - MCP-1 polymorphism is not associated with AMI among Egyptians.

1. Introduction

AMI is a significant cause of mortality and morbidity in the world. The AMI is a consequence of a cascade of thrombotic events following atherosclerotic plaque rupture causes occlusion of the coronary artery, interrupting blood supply and oxygenation to myocardium (Boateng and Sanborn, 2013).

These events are mediated by the interaction between leucocytes and endothelium. In response to chemoattractant signals (chemokines), monocytes and T-cells adhere to inflamed endothelium where they recognise oxidised low-density lipoproteins (ox-LDL) and acquire an activated phenotype in the vessel wall (Rahman et al., 2015).

Monocyte chemoattractant protein-1 (MCP-1 or CCL2 in the newest nomenclature) is a chemokine secreted by a variety of cells as a response to several proinflammatory stimuli (Marsillach et al., 2005; Kiyici et al., 2006). MCP-1 triggers activation, chemotaxis and transendothelial migration of monocytes or macrophages to inflammatory lesions by interacting with the membrane receptor CCR2 in monocytes (O'Hayre et al., 2008).

While RANTES (regulation on activation normal T cell expressed and secreted); also known as CCL5; is another chemokine which belongs to the CC chemokine family (Appay and Rowland-Jones, 2001). RANTES is expressed in macrophages, endothelial cells, lymphocytes, vascular smooth muscle cells and atherosclerotic plaques suggesting its contribution in the development of atherosclerosis (Tavakkoly-Bazzaz et al., 2011).

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Fractalkine (FKN) is a unique proinflammatory member of the CX3C chemokine family that exists in both soluble and membrane-bound forms. It is expressed by vascular endothelial cells of the brain, heart and peripheral blood vessels. CX3CR1; the specific receptor of FKN; is expressed on the cell membrane of monocytes, NK cells and T cells. The interaction between FKN and CX3CR1 results in inflammation (Bazan et al., 1997).

In coronary artery disease (CAD) platelets aggregation and clot formation were also linked to elevated homocysteine levels (Deeparani et al., 2009). Elevated plasma homocysteine levels may arise from genetic factors autosomal recessive severe deficiency of methylenetetrahydrofolate reductase (MTHFR) (Goyette et al., 1995) and nutritional factors as deficiency of folate, vitamin B_6 or vitamin B_{12} (Kang et al., 1991). MTHFR is an important enzyme for the folate metabolism. Specifically, it converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate in a multistep process, in which MTHFR converts the amino acid homocysteine to another amino acid, methionine which can be used by the body as a building block for proteins and other important compounds (Goyette et al., 1995).

It has been shown that many patients with history of myocardial infarction do not have any conventional risk factors, suggesting the contribution of an uncharacterized genetic component 3a. Previous studies from our lab demonstrated the link between AMI in Egyptians and several variants in genes relevant to cardiovascular homeostasis (Gad et al., 2012; Hashad et al., 2014; Abdel Rahman et al., 2015).

In the current study 4 single nucleotide polymorphisms (SNPs), MCP-1 A-2518G, RANTES G-403A, CX3CR1 V249I and MTHFR C677T, were investigated for their association with the incidence of AMI in Egyptians.

MCP-1 A-2518G is a SNP in the regulatory region of the MCP-1 promoter while RANTES G-403A is a SNP that has been identified in the promoter of RANTES gene. Both MCP-1 and RANTES genes are localized on chromosome 17. MCP-1 A-2518G is suggested to increase the level of MCP-1 expression and circulating levels in response to inflammatory stimuli (Rovin et al., 1999; McDermott et al., 2005) while RANTES G-403A might have a functional effect increasing the RANTES expression (Liu et al., 1999; Nickel et al., 2000).

V249I SNP is one of 2 common polymorphisms identified in the CX3CR1 gene. Some studies reported the association of the CX3CR1 I249 allele in homo- and heterozygote conditions with a marked reduction in risk of acute coronary events (Fong et al., 1998; Moatti et al., 2001; Singh et al., 2012; Pucci et al., 2013).

MTHFR C677T gene polymorphism was identified in the MTHFR gene and the homozygosity for this SNP was associated with decreased specific enzyme activity, increased thermolability, and elevated homocysteine levels (Frosst et al., 1995), mainly in subjects with low levels of plasma folate (Jacques et al., 1996). Several studies have tried to explore the association between MTHFR C677T gene polymorphism and the incidence of CAD but the results were inconsistent.

To our knowledge no association studies were performed to investigate the association of any of these SNPs and the incidence of CAD in the Egyptian population.

2. Materials and methods

2.1. Study population

Random unrelated 100 healthy controls were recruited for the study from the volunteers attending the blood bank at 57357 Hospital in Cairo, Egypt. Out of the controls, 40 were females, aged between 41 and 55 years, and 60 were males, 42 to 55 years of age. On the other hand, random unrelated 100 AMI patients, divided into 45 females (age range 45 and 55 years) and 55 males (age range 44 and 55 years) were recruited from the intensive care unit of the National Heart Institute, Imbaba, Giza.

Patients were included if they had a diagnosis of an acute single or multi-vessel CAD verified by clinical presentation, ECG changes, and/or cardiac markers elevation. Written informed consent was obtained from each participant in the study that abided by the Helsinki declaration. Information on personal and family medical history and health-relevant behaviours, including exercise and diet was obtained by a routine questionnaire filled in by blood donors at the time of venesection. Exclusion criteria for both patients and controls included any concomitant acute or chronic severe diseases such as renal failure, hepatic insufficiency, cardiovascular disease, other than MI, and diabetes mellitus.

2.2. Specimen collection

Fasting blood samples (4 ml) were collected in EDTA-coated vacuum tubes stored at 4 °C for DNA extraction.

2.3. Purification of DNA from human blood by spin protocol

DNA purification was done using QIAamp DNA Blood Mini Kits (Qiagen, Germany). DNA eluted in buffer AE was ready for direct addition to PCR reaction. The purified DNA was free of protein, nucleases, and other contaminants or inhibitors (Greenspoon et al., 1998; Fahle and Fischer, 2000).

2.4. Determination of the MCP-1 A-2518G polymorphism

The determination of the MCP-1 A-2518G polymorphism was carried out by PCR-RFLP. The PCR was performed under the following cycling condition: 95 °C for 5 min, followed by 95 °C for 20 s, 65 °C for 20 s, and 72 °C for 45 s for 15 cycles, followed by another 20 cycles of 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 45 s, with a final extension step at 72 °C for 7 min.

Forward 5-CCGAGATGTTCCCAGCACAG-3primer and a reverse 5-CTGCTTTGCTTGTGCCTCTT-3primer were used for the PCR reactions.

The resulting PCR product size was 930 bp. The nucleotide substitution corresponding to position −2518 (A/G) creates a *Pvu*II (New England Biolabs) restriction site. Digestion resulted in 708- and 222-bp fragments for the G allele (Fig. 1) (Ramasawmy et al., 2006).

2.5. Determination of the RANTES G-403Apolymorphism

Determination of the RANTES G-403Apolymorphism was carried out by PCR followed by restriction digestion (PCR-RFLP)·The PCR reaction was performed in a final volume of 25 μl containing 1 μl genomic DNA, 3.5 pmoles of each primer, 2 mM of $MgCl_2$, 1× buffer, 0.2 mM

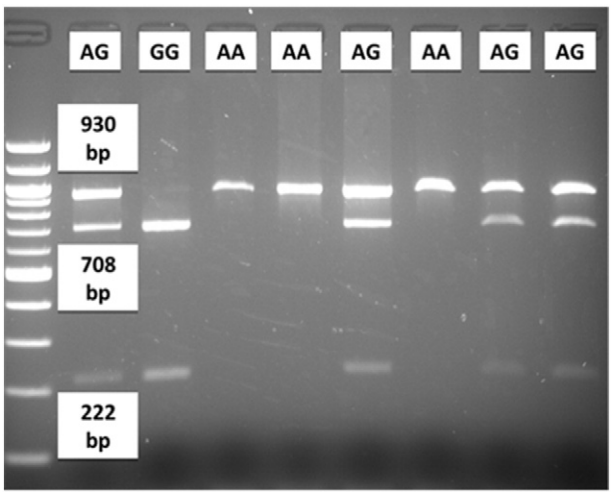

Fig. 1. Representative of 2% agarose gel electrophoresis by *PvuII* RFLP of MCP-1 gene.