Prevalence of some genetic polymorphisms among cardiovascular patients residing at high altitude and sea level

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A B S T R A C T

The role of high altitude in the etiology of cardiovascular diseases (CVD) is conflicting. No published conclusive data dealing with the association between genetic polymorphisms and CVD among high altitude populations. The aim of the present study is to assess the association between high altitude and some CVD related polymorphisms. One hundred and twenty CVD patients were enrolled in this study. Of them 60 patients had been living at the sea level (Mekkah group), while other 60 patients had been living at high altitude at 1,800 m above the sea level (Taif group). The examined polymorphisms are MTHFR C677T, β-Fibrinogen-455 G-A and PAI-1 4G/5G. There was high prevalence % and allele frequency of studied polymorphisms in the high altitude CVD patients in comparison to sea level patients. There was highly significant association between studied polymorphisms and CVD at high altitude. These results could be taken as indication to genomic response of high altitude residents to oxidative stress of hypoxia.

Keywords

High altitude, MTHFR C677T, β-Fibrinogen-455 G-A, PAI-1 4G/5G, CVD

Introduction

Several comparative studies have been conducted to prove the effects of high altitude residence on CVD as well as to evaluate this effects. Although it is hypothesized that residence at moderate to high altitude benefits the cardiovascular system, there are conflicting results about the role of altitude in the CVD morbidity and mortality. Higher negative correlation with cardiovascular mortality rate and high altitude was reported (Voors and Johnson, 1978). Reduction of coronary heart disease event rates at high altitude in comparison with sea level was observed and its might be related to lower levels of the atherogenic lipoprotein cholesterol, C-LDL (de Mendoza et al., 1979). Cardiovascular status was studied in 500 natives living at high to extreme altitudes of the Himalayas. No case of congenital heart disease, rheumatic heart...
disease, coronary artery disease, primary myocardial disease or hypertension was found (Sharma, 1990). Pasini and his coworkers (1999) performed a population survey in the Italian Valle Sabbia mountain community to estimate the prevalence of the main risk factors for coronary heart disease (CHD) among middle-aged men and women. Generally there are no differences were found between prevalence among high and low Italian land populations. Among Saudi population the average blood pressure (BPs) was significantly higher in highlanders compared with lowlanders (Khalid et al., 1994). While Fior and his colleagues (2000) reported that hypertension is more frequent in low altitude than moderate altitude and high altitude subjects from Central Asia. The distribution of risk factors and complications in patients with acute coronary syndrome (ACS) at high-altitude vs. low-altitude areas in Yemen was compared by (Al-Huthali et al., 2006), ACS occurs at a younger age at high altitudes. Patients who live in high-altitude regions are also more likely to have hyperlipidemia and a previous history of CAD. Stroke and reduced left ventricular ejection fraction (LVEF) occur more commonly in high-altitude ACS patients.

Most of these studies regarding the effect of high altitude on cardiovascular system due to physiological, environmental, diet and lifestyle not genetics responses. On the other hand, numerous genetic variants have been associated with cardiovascular health but in the different high-altitude populations, data published to date are inconclusive regarding these gene polymorphisms in CVD (Leon-Velarde and Mejia, 2008).

Rupert et al. (2003) compared the allele frequency of five polymorphic loci in genes encoding components of the renin-angiotensin system (RAS) among CVD patient in high and low altitudes. There was no evidence for an over-representation of the RAS alleles associated with cardiovascular fitness in the high-altitude Amerindian population when compared to the lowland Amerindian population. Deindl et al. (2003) investigated the effect of intermittent high altitude hypoxia on the mRNA expression of different cytokines and proto-oncogenes as well as other genes described to be regulated by hypoxia, in the left ventricle, the right ventricle, atria and the lung of adult rats after simulation of hypoxia in a barochamber. Intermittent hypoxia is a modulator of gene expression under physiological conditions. It differently regulates the expression of distinct genes not only in individual organs but even within one organ, i.e. in the heart. The aim of the present study is to evaluate the effect of high altitude on the distribution of some polymorphisms as a CVD genetic risk factors.

Materials and methods

Samples collection

A total of 120 CVD patients admitted to two hospitals at two distinct geographical locations were enrolled in this study: King Abdul-Aziz specialized hospital at Taif (which is more than 1,800 m above the sea level) and Al-nour hospital at Mekkah, KSA (which is at the sea level). Whole blood samples were collected into EDTA-anticoagulated vacutainer tube.

DNA extraction, PCR amplification and reverse hybridization

Analysis of Factor V G1691A (Leiden), Factor V H1299R (R2), Prothrombin G20210A and Factor XIII, V34L polymorphism was carried out via CVD Strip Assay (ViennaLab, Austria) (http://www.viennalab.com) was used according to the manufacturer’s instruction. The CVD
Strip Assay is based on the in vitro reverse-hybridization principle, and includes three steps: (1) DNA isolation (2) PCR amplification using biotinylated primers (3) hybridization of amplification products to a test strip containing allele specific oligonucleotide probes). Briefly, PCR amplifications were carried out in two separate reactions A (amplification mix A) and B (amplification mix B) that differ in primers pairs. PCR reactions A and B were carried out with the same thermal profile as follows: Initial step of 94°C for 2 min and followed by 35 cycles of 94°C for 15 s, 58°C for 30 s and 72°C for 30 s, final extension was at 72°C for 3 min. PCR products from reaction A and B were mixed together with hybridization buffer, incubated for 5 min at room temperature and hybridized to the detection test strip. Hybridization was accomplished at 45°C. After series of stringent washes (according to the protocol of provider), the reaction was detected by color development directly on test strip. Results were evaluated from test strips using provided scale included in the kit.

Test strips contain allele specific (wild type and mutation) oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin–alkaline phosphatase and color substrates.

Results and Discussion

To evaluate the effect of altitude on CVD, genotype and allele frequencies of three polymorphisms in genes that have alleles associated with CVD were determined and compared among two groups of CVD patients. The first group consists of sixty CVD patients collected from Taif governorate (1800 m above sea level) and other sixty. The second group was of sixty patients collected from Mekkah governorate (sea level). The studied polymorphisms were MTHFR C677T, β-Fibrinogen -455 G-A and PAI-1 4G/5G. CVD Strip Assay was utilized. For each patient one of three possible staining patterns may be obtained as follow; (1) Wild type probe only: Normal genotype, (2) Wild type and mutant probe: Heterozygous genotype and (3) Mutant type probe only: Homozygous mutant type. Genotype and allele frequencies as well as prevalence% of each studied polymorphism among Taif and Mekkah CVD patient are summarized in table 1.

Three genotypes CC, CT and were recorded for MTHFR C677T polymorphism among Taif group with prevalence% 48.3, 50 and 1.7 respectively. The C and T alleles frequency was 0.73 and 0.27. While the TT genotype was absent among Mekkah group and allele frequency was 0.9 and 0.1 for C and T alleles respectively.

The prevalence% of β-Fibrinogen -455 G-A was 73.3 and 35 among Taif and Mekkah groups. The frequency of G and A alleles were (0.63 & 0.37) among Taif and (0.82 & 0.18) among Mekkah group.

The prevalence% of 4G/4G, 4G/5G and 5G/5G for PAI-1 4G/5G polymorphism among Taif group were 8.3, 76.7 and 15. While among Mekkah group the two genotypes 4G/5G and 5G/5G were observed with prevalence % 35 and 65 respectively. The allele frequency of 4G and 5G were 0.47 and 0.53 among Taiif group in comparison with 0.18 and 0.82 among Mekkah group.

Prevalence and allele frequency of all studied polymorphisms were significantly different among Taif group from Mekkah group (Table 1).
In the present study allele frequencies for three polymorphisms MTHFR C677T, β-Fibrinogen -455 G-A and PAI-1 4G/5G were compared between CVD patients residing at high-altitude and sea level regions. Highly significant differences were detected between the two CVD patient groups.

Methylene tetrahydrofolate reductase (MTHFR) enzyme directs folate species either to DNA synthesis or to homocysteine (Hcy) remethylation (Ueland et al., 2001). Variants forms of MTHFR are associated with increased risk of several diseases such as CVD (Chandy et al., 2010). The common MTHFR C677T polymorphism affects the activity of the enzyme and hence folate distribution. The C677T mutation causes a valine for alanine substitution, which decreases MTHFR activity and tends to increase Hcy concentrations in individuals who are homozygous for the T/T genotype (Frost et al., 1995). The T/T genotype was, therefore, considered a common risk factor for CVD (Brattstrom et al., 1997). The results of the present work indicate high prevalence of MTHFR C677T polymorphism among CVD patients of Taif group than Mekkah group. The over representation of MTHFR C677T polymorphism among Taif group compared with Mekkah group could be taken as indication to genomic response of high altitude residents to oxidative stress of hypoxia. Lakshmi and his colleges (2013) reported that, there are association between oxidative stress and polymorphism that regulate folate uptake in etiology of coronary artery disease on the contrary, Rupert and his coworkers (2003) reported that no association between renin-angiotensin system (RAS) alleles and cardiovascular system. This is might be due to different in utilized techniques and/or race and ethnicity background.

An elevated plasma fibrinogen level has been established to be an independent predictor of coronary artery disease, stroke and peripheral vascular disease (Maresca et al., 1999; Kain et al., 2002) There is evidence that up to 51% of the variation in fibrinogen levels may be due to genetic factors, and a relation between the ββ Arg448Lys, β-455G/A and AlaThr312Ala polymorphisms and fibrinogen level has been reported (Behague et al., 1996; Kain et al., 2002). Variation of the β-fibrinogen gene (β-455G/A polymorphisms) was associated with the severity of cardiovascular disease (Scarabin et al., 1993; Behague et al., 1996; Martiskainen et al., 2011). The obtained data supported the association between high altitude and increment of the prevalence of β-455G/A polymorphisms among CVD patients. This is might be due to oxidative stress of hypoxia.

Plasminogen activator inhibitor-1 (PAI-1) is the major inhibitor of tissue type plasminogen activator (tPA). Reduced fibrinolytic capacity due to increased plasmaPAI-1 levels was postulated to play an important role in the pathogenesis of disorders associated with thrombosis (Kohler et al., 2000). A common functional deletion/insertion polymorphism (4G/5G) in the promoter of the PAI-1 gene located 675 bp upstream from the transcription start site was reported to result in the elevated expression ofPAI-1 gene (Dawson et al., 1993). Individuals homozygous for the 4G allele had increased plasma PAI-1 concentrations compared to the ones with 5G allele (Eriksson et al., 1995). This polymorphism has been studied extensively, and in some studies, the prevalence of 4G allele was found to be higher in disorders like coronary artery disease. The obtained results revealed significant increase of prevalence of PAI-1 4G/5G polymorphism.
among CVD patients at high altitude than CVD patient from sea level (Balta et al., 2002). Our findings showed that, the prevalence and 4G allele frequency of PAI-1 4G/5G polymorphism were high among Taif group in comparison to Mekkah group. These results might be due to effect of hypoxia. Hypoxia might enhance the expression of PAI-1 and hence suppress fibrinolysis under conditions of low oxygen tension (Dossenbach-Glaninger et al., 2003).

There was high prevalence % and allele frequency of studied polymorphisms in the high altitude CVD patients in comparison to sea level patients. There was highly significant association between studied polymorphisms and CVD at high altitude. These results might be elucidating the previously published reports in Arabian Saudi and Yemen populations (Khalid et al., 1994; Al-Huthali et al., 2006). Further larger studies are needed to evaluate the effect of high altitude on other CVD related polymorphisms.

**Table 1** Prevalence and allele frequency of the studies polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Population</th>
<th>Taif</th>
<th>Mekkah</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Genotypes</td>
<td>Prevalence</td>
<td>Allele frequency</td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td></td>
<td>CC (29)</td>
<td>48.3%</td>
<td>C 0.73</td>
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<tr>
<td></td>
<td></td>
<td>CT (30)</td>
<td>50%</td>
<td>T 0.27</td>
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<td></td>
<td></td>
<td>TT (1)</td>
<td>1.7%</td>
<td></td>
</tr>
<tr>
<td>ß-Fibrinogen 455 G-A</td>
<td></td>
<td>GG (16)</td>
<td>26.7%</td>
<td>G 0.63</td>
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<tr>
<td></td>
<td></td>
<td>GA (44)</td>
<td>73.3%</td>
<td>A 0.37</td>
</tr>
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<td></td>
<td></td>
<td>AA 0</td>
<td>0</td>
<td></td>
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<tr>
<td>PAI-1 4G/5G</td>
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<td>4G/4G (5)</td>
<td>8.3%</td>
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<tr>
<td></td>
<td></td>
<td>4G/5G (46)</td>
<td>76.7%</td>
<td>5G 0.53</td>
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<td></td>
<td></td>
<td>5G/5G (9)</td>
<td>15%</td>
<td></td>
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</tbody>
</table>

Reference


Maresca, G., Di Blasio, A., Marchioli, R.


