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Research Article

Association of Glyoxalase 1 Gene Variations and Altitude Residency in Taif, Saudi Arabia

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Abstract

Altitude residency is associated with hypoxic stress. Hypoxia is one of the major stresses affecting human body. Human living in altitude have adopted genetically and physiologically to this stress to permit their health, survival and reproduction in such environment. Many gene variations are associated with living in altitude. This study aims to investigate the association between Glo1 gene variations and living in altitude. In total 80 blood samples were collected from altitude city (Taif) and 80 blood samples from sea level city (Jeddah). Glo1 copy number and Glo1 SNP were investigated after the DNA extraction. The genotyping of Glo1 polymorphism was analysed by PCR and RFLP and then digested using SfaNI digestive enzyme. The copy number of Glo1 was determined by TaqMan® Copy Number Assays. The genotype differences between both populations was not significant and no association has been identified between Glo1 gene variations and living in altitude. Further studies are needed to investigate this association using samples from people living consistently in higher altitude than Taif city and other variation including epigenetics variations.

Keywords: Hypoxia; Altitude; Glyoxalase 1; Genetics; Taif

Introduction

Altitude residency is associated with hypoxic stress. Human living in altitude have adopted genetically and physiologically to this stress to permit their health, survival and reproduction in such environment. Interestingly, it has been reported that people who are not adopted to living in altitudes have shown increase in haematocrit levels and number of erythrocytes, whereas, in most of highlanders who lives in altitude such as Tibetan maintain the level of haematocrit comparable to populations living at the see level [1]. This physiological adaptation process could be beneficial, however, the increase of haematocrit levels and number of erythrocytes might increase blood viscosity and hence impair blood flow and oxygen delivery [2].

Previous studies in Taif city have found that altitude can have several effects on wellbeing [3,4]. ISG-15 is an important immune modulator and can have several effects on innate and adaptive immune response and living in altitude can affect the levels of this protein as our previous studies have shown [4-6], also, smoking in altitude and hypoxia has effected the levels of αβ and γδ T-cells [7].

Genetically, the master regulator of oxygen homeostasis is Hypoxia Inducible Factors (HIFs). HIFs have several subunit in which some are encoded by EPAS1 [8]. Mutations and dysregulation of EPAS1 are associated with anaemia and polycythaemia [2]. In addition, there is association between SNPs of EPAS1 loci and haemoglobin level in Tibetans [9]. Hypoxia has been linked with variations of other genes such as Glyoxalase 1 (Glo1). It has been reported that the combination of hypoxic stress and dicarbonyl stress induced copy number variation of the gene in cells and in patients [10].

Glo 1 is important gene in the glyoxalase system, which plays a protective role against the accumulation of methylglyoxal and some other dicarbonyls in biological system [11]. Two variations have been reported in Glo1 including Glo1 polymorphism (C419A) and Glo1 copy number. Glo1 SNP causes the substitution of Ala111Glu amino acid. As this substitution can cause protein modification hence it was predicted to be tolerant. The association of this SNP with autism was not confirmed [12,13] nor the association with cardiovascular complication of diabetes [14]. However, a correlation was reported between Glu111Glu homozygote and increased prevalence of vascular diseases in renal failure [15].

Up to our knowledge, the association of both variation with living in altitude has never been studied. Therefore, the aim of this study is to find out the possibility of the association between living in altitude and Glo1 copy number variation and Glo1 SNP. This may improve our understanding of the possible adoption way of living in altitude.

Materials and Methods

Subjects

This study was performed in High Altitude Research Center, Taif university and approved by Taif University Ethical Committee and used of written informed consent. The participants number was 80 subjects aged between 19-25 years, all those participants lives in Taif city and the surrounding areas for most of their lifetime, where Taif region is 1800 to 2500 meters above sea level. The control samples (80) were collected from 80 subject living for their lifetime in Jeddah city (at sea level) aged 22-30. Both groups were healthy with no chronic diseases and non-smokers. Venous blood (3ml) were collected from every one of the both groups in EDTA containing tubes under complete aspect condition. These samples were used for DNA extraction from whole blood.

Glo1 copy number variation

DNA was extracted and purified from peripheral whole blood using Thermo Scientific DNA isolation kit (Thermo Scientific, UK). In order to detect the copy number, TaqMan® Copy Number Assay was performed using a reference gene (TERT, telomerase reverse transcriptase) according to the protocol of the manufacturer (PN 4397425, Applied Biosystems, Paisley, UK). The software used was CopyCaller TM® for data analyses.

Glo1 polymorphism

Genotyping of Glo1 polymorphism was performed by PCR (polymerase chain reaction) for amplification of the templet and then by RFLP (restriction length fragment polymorphisms) as described in [16]. The primers used for C419A genotyping were forward primer (5'-TCAGAGTGTGTGATTTCGTG-3') and reverse primer (5'CATGGTGAGATGGTAAGTGT-3'). The protocol of the PCR is shown in table 1.

Step		Temperature (°C)	Time
DNA template denature		95	2 min
40 cycles	Denaturation	94	45 sec
	Annealing	56	45 sec
	Extension	72	1 min
Final extension		72	2 min

Table 1: PCR protocol for Glo1 polymorphism genotyping.

The PCR product was then digested using SfaNI enzyme for 1 hour at 37°C. The digested PCR product was electrophoresed using 2% agarose gel. The fragments in the gel were stained with ethidium bromide and visualized under UV light. The SNP was identified as the presence of A 111 allele showed 453 bp and 260 bp fragments, while, the presence of E 111 allele showed 713 bp fragment.

Statistical analysis

SPSS program version 19 was used for data analysis. The data normality of distribution testing was performed by Kolmogorov-Smirnov test. The deference in variance was performed by F-test. Student's t-test was used for the means significance difference in two parametric data groups of independent samples and Spearman`s test was used for testing the correlations. The comparisons and the correlations were considered as statistically significant when $P < 0.05$.

Results

Demographic study

This study included 160 subjects and were classified as 80 persons from Taif city (altitude area) and 80 persons from Jeddah city (sea level area), out of which, 40 male and 40 female in each group. The main objective of this study was to find out the possibility of the association between living in altitude and Glo1 copy number and Glo1 SNP. This may improve our understanding of the possible adoption way of living in altitude.

Glo1 copy number

The relevance of Glo1 copy number variation in association with living in altitude was assessed in this study. In 80 control subjects from sea level, Glo1 copy number was 2.00 ± 0.19. In 80 subject living in altitude, Glo1 copy number was 2.00 ± 0.23. The data from people living in altitude was indistinguishable statistically from the controls. Therefore, Glo1 copy number variation prevalence was not associated with altitude.

Glo1 polymorphism

The relevance of Glo1 SNP in association with living in altitude was assessed in this study and presented in both groups for all subjects in table 2. The deference of the genotypes between both groups was statically insignificant. However, the occurrence of heterozygous CA genotypes was higher in subjects from the sea level, while, homozygous AA and CC were more in altitude area. The allele distribution was almost same in both groups.

	Subjects of Taif (80)	Subjects of Jeddah (80)	P value
Wild type CC	24 (30%)	22 (27.5%)	0.102
Heterozygous CA	29 (36%)	34 (42.5%)	0.81
Homozygous AA	27 (34%)	24 (30%)	0.89
C Allele	77 (48%)	78 (49%)	0.43
A Allele	83 (52%)	82 (51%)	0.41

Table 2: The relevance of Glo1 SNP in association with living in altitude (Taif) and in sea level (Jeddah).

Discussion

Hypoxia is one of the major stresses affecting human body. People living in altitudes have shown physiological adoption signs [1]. In addition, genetical adaptation is one of the ways that human body response to the hypoxic stress. This adaptation includes several gene variation including polymorphisms and copy number variation [9,17]. In the previous studies, gene variations have been assisted genome wild, but up to our knowledge, no study have investigated the detailed association between Glo1 1 gene variations and living in altitude. Thus, this study aimed to investigate the relevance of Glo1 gene variation in association with living in altitude.

Glo1 has shown increase in copy number in relation to hypoxic stress *in vivo* and *in vitro* [10]. Moreover, the copy number variation has been linked to diseases including cancer [18], this might be due to the hypoxic stress in the cancerous mass. Also, it has been linked to diabetes and specifically to the patient who have diabetes and at the end stage renal failure where they suffer from hypoxic and dicarbonyl stresses [19].

In this study, no association between Glo1 gene variations and living in altitude. This might be due to the hight of Taif city which is 1800 meter above the sea [4], if samples were collected from people living in higher altitude could show different results. Also, the improved transportation in Saudi Arabia let people travel easily between cities hence the hypoxic stress is not consistent.

Conclusion

This study has investigated the association between living in altitude and Glo1 gene variation. Two variations were examined including Glo1 polymorphism (C419A) and Glo1 copy number variation. No association between these variations and living in altitude was found. Further studies are recommended to investigate people living consistently in higher altitude than Taif city and other variation including epigenetics variations.

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