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Original Article

Endothelial markers in high altitude induced systemic hypertension (HASH) at moderate high altitude

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ABSTRACT

Background: Chronic intermittent hypoxia is known to induce systemic arterial hypertension whereas chronic hypoxia causes pulmonary arterial hypertension. High altitude (HA) induced systemic hypertension (HASH) in previously normotensive lowlanders following acclimatisation and prolonged stay at moderate HA is a commonly encountered medical problem. HASH has been attributed to increased sympathetic discharge. Endothelial dysfunction (ED) is implicated in hypertension in the plains hence this study was conducted in HA. This is relevant especially because of the established role of ED in the aetiopathogenesis of HA illnesses. Since hypoxia may induce ED, we aimed at studying the association of endothelial dysfunction with HASH in temporary residents at HA.

Methods: In this case-control single-centre study, we evaluated ED, by measuring endothelial molecular markers, soluble intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (VCAM-1), vascular endothelial growth factor (VEGF) and endothelial selectin (E-Selectin) in 24 cases with HASH and 25 age, sex matched normotensive controls at moderate high altitude (11,500 ft).

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Results: The levels of sICAM-1 (patients: $214.3 \pm 34.2 \mu\text{g/L}$, controls: $196.2 \pm 28.5 \mu\text{g/L}$; $p = 0.049$) and VCAM-1 (patients $766.1 \pm 123.4 \text{ ng/mL}$, controls: $668.6 \pm 117.6 \text{ ng/mL}$; $p = 0.007$) were statistically higher in the patient group. However, VEGF and E-Selectin were not significantly different between the groups. sICAM-1 significantly correlated with levels of systolic and diastolic blood pressure ($r = 0.401$, $p = 0.003$ and 0.486 , $p = 0.000$) respectively. **Conclusion:** HASH is associated with endothelial dysfunction in form of raised levels of sICAM-1 and VCAM-1.

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Introduction

Chronic intermittent hypoxia (CIH), as in obstructive sleep apnoea (OSA), is known to cause systemic arterial hypertension (HTN), whereas, continuous hypoxia in healthy humans, as at high altitude (HA), is known to mediate pulmonary arterial hypertension.^{1–3} Amongst lowland sojourners at HA, systemic blood pressure (BP) was found to be elevated both in the un-acclimatised and the well acclimatised state.⁴ This increase during the initial exposure to HA is a part of the acclimatisation process to ensure sufficient tissue oxygenation in hypobaric hypoxia and subsides with in the first week of arrival to normal levels.⁴ The submaximal and maximal exercise was found to further elevate the systemic BP at HA.⁵ This elevation of BP has been shown to be due to increased sympathetic drive and could be attenuated but not abolished by the α -adrenergic blockade. The increase in sympathetic activity is known to be mediated by hypoxic chemoreflex mechanisms.⁶

Endothelial dysfunction (ED) can be determined by evaluation of endothelial markers, flow mediated dilation intimal media thickness and lately by intra-arterial ultrasonography.^{7,8} Cellular adhesion molecules (CAM) play a major role in causation of atherosclerosis mediated by Selectins (causes rolling of leukocytes on endothelium) and vascular cell adhesion molecule-1 (VCAM-1)/soluble intercellular adhesion molecule-1 (sICAM-1) (causes binding of circulating leukocytes to vascular endothelium, and aids in leucocyte migration to sub-endothelial spaces).⁹ Its levels increase in various conditions leading to atherosclerosis such as dyslipidaemia, diabetes mellitus, and chronic inflammatory states. The role of VCAM-1, endothelial-leucocyte adhesion molecule-1 (E-selectin) and sICAM-1 in the biology of atherosclerosis are well characterised.^{10–13} On immunohistochemical studies, these molecules have been expressed variably but consistently within the atherosclerotic plaque.^{14,15} Serum levels of these CAM have been elevated during inflammatory conditions with increased expression on endothelial cells and other tissue types, though their origin is still unclear and proposed to be secondary to shedding or proteolytic cleavage from endothelial cells.¹⁶

Endothelial dysfunction has been implicated in various HA illness (HAI) including high altitude pulmonary oedema (HAPE) and chronic mountain sickness (CMS).^{17,18} However causal relation in high altitude systemic hypertension

(HASH) is yet to be established. ED as a common contributor to hypoxia associated ailments at HA (such as HAI, HASH, CMS) is an attractive and plausible hypothesis. The findings of normalisation of urinary catecholamines by 90 days of stay at HA, the inability of a α -adrenoceptor blocker to obviate HTN on ascent/sojourn in chronic hypoxia and the likely involvement of angiotensin converting enzyme (ACE) in HASH – all suggest mechanisms in addition to sympathetic stimulation in the causation and/or sustenance of HASH.

It is suggested that the level of these endothelial markers increase on induction to HA owing to hypoxic stress. So, we hypothesise that the rise in these endothelial markers leads to increased systemic hypertension which was never studied in the past. Hence, we propose to measure various markers of ED (sICAM-1, VCAM-1, vascular endothelial growth factor (VEGF) and E-Selectin) in a patient with HASH. The aim was to study the level of ED markers (sICAM-1, VCAM-1, VEGF, and E-Selectin) and its association with HASH.

Material and methods

Study and control groups

This is a case–control single-centre study. All cases and controls were lowlanders sojourning at HA. The study was conducted in the Medicine department at a secondary care centre located at HA (11,500 feet) during the study period of 5 months (Aug 2012 – Dec 2012). All consecutive patients who underwent consultation for HASH meeting the inclusion criteria were included in the study (i.e., freshly diagnosed hypertension by joint national committee (JNC) VII criteria, age less than 40 years, not of Ladakhi/Tibetan ethnicity, and not Hypertensive before induction to HA). Exclusion criteria included (a) patients with less than 30 days duration of stay (from arrival at HA to symptom onset or detection of systemic HTN) (b) presence of any inflammatory states such as occurrence of acute HAI (HAPE, AMS and HACE on initial or re-ascent in the last 6 months), febrile illness and organ dysfunction.

An equal numbered age, sex, and ethnicity matched healthy controls with similar duration/altitude of stay at HA formed the control population. Study patients and controls were studied for their baseline epidemiological/laboratory parameters. The study protocol was approved by the institutional

ethics committee and performed in accordance with the Declaration of Helsinki.

Blood pressure evaluation

Blood pressure of all the study participants was taken at sea level prior to induction as part of pre-induction medical examination (within 7 days of gaining altitude). Any individual found to be hypertensive as per the JNC VII criteria were not allowed to be inducted to high altitude.¹⁹ The individuals were subjected to this pre-induction BP screening every time they travelled from sea level to high altitude. Office blood pressure of all patients and controls (Omron HEM 907 digital blood pressure monitor) were reconfirmed by 24 h ambulatory intermittent blood pressure (AIBP) (Schiller BER 102 ABP monitor and recorder). The 24 h AIBP was used owing to its superiority in correctly identifying HAI as proven in the literature.^{20,21}

Endothelial markers levels

All laboratory staff was blinded to the study-group assignments. After 12 h of fasting, 10-cc blood samples were collected in EDTA vacutainers for endothelial markers; plasma samples obtained by centrifugation at 3500 rpm for 5 min were bio-banked at -80°C . The biobanked samples were analysed within a period of 6 months and for internal quality control (QC), the serum protein levels were assessed randomly in each batch. These blood samples were obtained before beginning treatment for clinical findings. The levels of all endothelial markers were measured with use of an ELISA Kit (RayBiotech, Inc.; Norcross, Ga) and an enzyme-linked immunosorbent assay method. Results were recorded in terms of ng/mL for VCAM-1, pg/mL for E. Selectin, $\mu\text{g/L}$ for s-ICAM-1 and pg/mL for VEGF. Thermo Fischer ScientificTM was used for the ELISA reading.

Statistical analysis

The data were analysed using of SPSSver16.0 and JMPver13.0 software; the results are shown as mean $\pm$ SD. Mann–Whitney *U* and Kruskal–Wallis tests were used for statistical analysis, and a *p* value of <0.05 was accepted as significant. We did multivariate analysis (MANOVA) followed by univariate analysis to identify any correlation of the markers with the laboratory parameters as no previous studies were available for guiding the same.

Results

A total of 35 patients and 30 controls were screened for the study. Only 24 patients and 25 healthy volunteers finally underwent ED evaluation. A patient flow diagram shows screening and follow-up of the patients (Fig. 1).

The baseline characteristics of these individuals are described as in Table 1. The altitude at which the individuals were stationed prior to hospitalisation were comparable in both the groups ($11,316.04 \pm 1205.45$ ft in the hypertensives, $11,660.21 \pm 796.05$ ft in healthy controls, $p=0.247$). The total duration in the HA prior to hospital admission in the patients and controls was 134 ± 11.9 and 119.6 ± 7.9 weeks respectively with no statistical difference ($p=0.109$). This total duration includes absence from HA in the form of descent to lower altitude. The duration of stay in the HA during current hospital admission episode (without descent to altitudes < 8000 ft) in the patients and controls was 52.29 ± 7.3 and 49.22 ± 7.01 weeks respectively with no statistical difference ($p=0.697$). The office systolic/diastolic blood pressures were significantly different in both the groups, emphasising correct classification of the groups (Table 2). On laboratory evaluation, the levels of total cholesterol, LDL, and urea were significantly higher in the hypertensive group. Whereas other laboratory parameters

Fig. 1 – Consort Diagram showing the selection of the patients, controls among those screened for inclusion and depicting the reason/number of patients excluded (*AIBP – ambulatory intermittent blood pressure monitoring, HAI – high altitude illness).

Table 1 – Baseline characteristics of both the groups.

Variables	Hypertension		Healthy controls		p value
	Mean	SD	Mean	SD	
Age (years)	37.1	9.3	33.9	5.8	0.171
Body mass index (kg/m ²)	24.1	2.3	23.8	2.9	0.621
Urea (mg/dL)	29.1	4.8	25.0	4.4	0.004
Creatinine (mg/dL)	0.87	0.09	0.84	0.13	0.408
Total cholesterol (mg/dL)	188.7	14.6	164.3	28.8	0.001
Triglycerides (mg/dL)	150.3	31.1	151	14.6	0.914
LDL (mg/dL)	112.5	20.8	91	23.4	0.001
HDL (mg/dL)	40.3	5.4	40.7	4.5	0.797
VLDL (mg/dL)	30.4	6.4	30.1	3.2	0.829
Blood sugar fasting (mg/dL)	87.8	6.2	90.3	11.3	0.345
Haemoglobin (g/dL)	14.5	0.9	15	0.5	0.044

LDL – low density lipoproteins, VLDL – very low density lipoproteins, HDL – high density lipoproteins. The differences which are statistically significant are marked in bold.

Table 2 – Cardiac parameters of both the groups.

Variables	Hypertension		Healthy controls		p value
	Mean	SD	Mean	SD	
Blood pressure (systolic – mm Hg) Prior to Induction to HA	124.3	9.42	120.1	8.5	0.414
Blood pressure (diastolic – mm Hg) Prior to Induction to HA	81.3	6.74	80.3	4.3	0.824
Blood pressure (systolic – mm Hg)	154.3	11.16	129	9.8	0.000
Blood pressure (diastolic – mm Hg)	100.2	8	83.6	5.7	0.000
Heart rate (/min)	90.2	16.9	80.4	8.2	0.012

such as triglycerides, HDL, VLDL, creatinine, and blood sugar levels were comparable in both groups (Table 2).

The levels of sICAM-1 and VCAM-1 were statistically different between the two groups, with levels being higher in the hypertensive group. There was no significant difference in VEGF and E-Selectin levels between groups (though the mean values of VEGF were higher in control group and E-Selectin higher in the hypertensive group). The levels of endothelial markers in the two groups are enumerated in Table 3. The medians with ranges are enumerated in Fig. 2A–D (and means with 95% CI in Suppl Fig. 1A–D). We assessed correlation amongst various endothelial markers i.e., VCAM, sICAM, E-selectin, and VEGF (Suppl appendix Table 1, Suppl Fig. 2A–D). There was significant correlation only between VCAM-1 and sICAM-1 (Pearson's correlation coefficient, $r = 0.437$, $p = 0.002$). Correlation of endothelial markers to various baseline characters was studied which is presented in the supplement (Suppl appendix Table 2, Suppl Fig. 3A–H).

Discussion

HASH is a type of secondary hypertension where in HA stay acts as the predisposing factor. There is a paucity of data on HASH as lowlanders staying for prolonged periods in HA (2–3 years) in great numbers outside the military or occupational reasons is unusual. There is no literature available as on a date to explain what happens to these individuals with HASH who continue to stay in HA with/without treatment. Kumar et al. also found a greater BMI in eighteen subjects who developed HASH at 3300 m compared to 28 who did not. Conventional risk factors for atherosclerosis would, therefore, appear to have a role in high altitude induced systemic hypertension (HASH). In addition, they reported a significantly greater expression of the ACE D allele in subjects of HASH. Thus factors other than sympathetic activation may contribute to this multifactorial disorder.²²

Table 3 – Endothelial markers in both the groups.

Variable	Hypertension		Healthy controls		p value
	Mean	SD	Mean	SD	
E. Selectin (pg/mL)	4653.3	899.7	4314	1117.2	0.249
VCAM-1 (ng/mL)	766.1	123.4	668.6	117.6	0.007
s ICAM-1 (μ g/L)	214.3	34.2	196.2	28.5	0.049
VEGF (pg/mL)	1387.4	525.3	1631.8	891	0.251

Fig. 2 – (A–D) Box-whisker plots representing median with ranges of endothelial markers in hypertensive and non-hypertensive groups.

Endothelial dysfunction and HASH

The very occurrence of systemic hypertension at HA places the individual at increased risk of adverse health events, reduces his functional capacity and taxes the resources of the organisation. Also, since ED is known to accentuate the risk of cardiovascular morbidity, the occurrence of ED in hypertension at HA needs to be elucidated both as a mechanism of disease and a risk factor. ED can be determined by evaluation of endothelial markers, flow mediated dilation, intimal media thickness and lately by intra-arterial ultrasonography.^{7,8,23}

Our study findings

In our study, we found that the sICAM and VCAM levels were significantly higher in the patients with systemic hypertension when compared to the controls with similar duration of stay in HA. This rise could be either secondary to the inherent effect of atherosclerosis in these individuals or the effect of the HA on unmasking the underlying ED. As we did not study the levels of CAM at sea level in these individuals so it is not possible to answer this question. The only conclusion which can be drawn from these results includes that there is a significant difference in the above-mentioned markers between patients with HASH from controls. There are reports of

raised ED markers in the high-altitude illnesses, but this could not have modified our results as we excluded such patients from analysis (as mentioned in the exclusion criteria above).²⁴

Also, there is no difference in the levels of VEGF and E-Selectin in patients with and without HASH in our study. It is very difficult to comment on the reason for this differential raise in sICAM/VCAM vis-à-vis VEGF/selectins in the patients of HASH, as the study wasn't designed for the same. These results need to be validated in larger cohort along with prospective long-term evaluation in a chronological order at different altitudes to correctly determine the relevance of this raise.

Induction to HA combine with overweight/obesity, other risk factors such as smoking, dyslipidaemias, and hyperhomocysteinemia are likely to cause endothelial dysfunction in a significant number of sojourners. Whether changing levels of endothelial markers, a molecular component of endothelial dysfunction contributes to the occurrence of HASH is the subject of this study.

Other studies on ED in high altitude

Markers of ED such as reduced circulating endothelial progenitor cells have been reported at HA.²⁵ Hypoxia impairs endothelial function in HAPE susceptible subjects detected by attenuated ACh-induced vasodilation secondary to decreased

bioavailability of nitric oxide.¹⁷ E-Selectin levels rise significantly after exposure to hypoxia and re-oxygenation, similarly sICAM also increased on exposure to hypoxia, but did not achieve statistically significant levels. In a study by Grissom et al., they have hypothesised endothelial cell activation secondary to hypoxic altitude illness by showing an increase in plasma E-Selectin levels in hypoxic climbers with AMS and HAPE.²⁴ Klein et al. did not find any changes in E-Selectin, sICAM-1, and VCAM-1 under simulated hypoxic conditions. The study was limited by experimental settings and restricting its changes at 7 h and 24 h.²⁶ Similarly, Pavlicek et al. did not find any upregulation of VEGF in HAPE as an effect of hyperbaric hypoxia. Furthermore, they suggested lack of evidence of any relevant inflammation after initial exposure to hypoxia in the pathogenesis of HAPE.²⁷ Marks et al. reported that increase in CAMs reported in pre-eclampsia are not reported in pregnant women at HA.²⁸

Limitations of the study

Limitations are that the study included only males who were previously healthy at sea level prior to induction to HA and who underwent regular screening medical examination at monthly intervals during their stay at HA. This limitation led to sex bias and also selection bias of patients with milder disease. It also limits generalisation of findings to the individuals with comorbidities. A major problem area of the study is that it is not known for how long systemic hypertension existed before patients were diagnosed as cases of HASH as hypertension is an asymptomatic state and it was diagnosed in all the patients in this study by routine screening only.

Conclusion

This study shows that levels of sICAM and VCAM were significantly higher in patients with HASH. Also, there is no difference in the levels of VEGF and E-Selectin in patients with and without HASH in our study.

Conflicts of interest

The authors have none to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.mjafi.2017.09.010](https://doi.org/10.1016/j.mjafi.2017.09.010).

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