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Life Sciences 260 (2020) 118408

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Subclinical elevated B-type Natriuretic Peptide (BNP) indicates endothelial dysfunction contributing to hypoxia susceptibility in healthy individuals

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ARTICLE INFO

Keywords:
B-type natriuretic peptide
Endothelial dysfunction
High altitude pulmonary edema
Hypoxic pulmonary vasoconstriction
COVID-19

ABSTRACT

Aims: Baseline elevated B-type Natriuretic Peptide (BNP) has been found in high altitude pulmonary edema susceptible population. Exaggerated pulmonary vascular response to hypoxia may be related to endothelial dysfunction in hypoxia susceptible. We hypothesize that baseline BNP levels can predict hypoxia susceptibility in healthy individuals.

Main methods: The pulmonary vascular response to hypoxia was compared in 35 male healthy individuals divided into two groups based on BNP levels (Group 1 $\leq$ 15 and Group 2 $>$ 15 pg/ml). Acute normobaric hypoxia was administered to both the groups, to confirm hypoxia susceptibility in Group 2.

Key findings: Unlike Group 1, Group 2 had elevated post hypoxia BNP levels (26 vs 33.5 pg/ml, $p = 0.002$) while pulmonary artery pressure was comparable. A negative correlation with tissue oxygen consumption (delta pO_2) and compartmental fluid shift was seen in Group 1 only. Endothelial dysfunction in Group 2 resulted in reduced vascular compliance leading to elevation of mean blood pressure on acute hypoxia exposure. BNP showed a positive correlation with endothelial dysfunction in Group 2 and has been linked to pre-diabetic disorder (HbA1c 6 $\pm$ 0.44%) and may additionally represent a lower cross-sectional area of vascular bed related to vascular remodeling mediated by chronic hypoxia.

Significance: Hypoxia susceptibility in healthy individuals may be related to endothelial dysfunction that limits vascular compliance during hypoxic stress. BNP level showed positive correlation with HbA1c ($r = 0.49$, $p = 0.04$) and negative correlation with delta pO_2 ($r = -0.52$, $p = 0.04$) can predict reduced microvascular compliance due to endothelial dysfunction contributing to hypoxia susceptibility in healthy individuals. BNP levels $\leq$ 15 pg/ml at sea level is indicative of hypoxia resistance.

1. Introduction

Hypoxia is an important environmental stressor faced by people visiting high altitude (HA). Various compensatory mechanisms become active above 2500 m and bring immediate and long-term modifications however, susceptible individuals fail to acclimatize leading to the development of high-altitude maladies like acute mountain sickness (AMS), high altitude pulmonary edema (HAPE) and high-altitude cerebral edema (HACE) etc. Various studies have shown that hypoxia susceptibility is related to vascular remodeling mediated by chronic hypoxia leading to a rise in basal vascular tone and exaggerated pulmonary vascular response to acute hypoxia [1,2]. HAPE-Susceptible (HAPE-S, episode of HAPE in past) individuals showed augmented sympathetic activation on hypoxia exposure or during exercise [3]. HAPE is hydrostatic edema which occurs in susceptible individuals on a rapid ascent to altitude involving physical exertion [4]. Elite athletes show exercise-induced pulmonary edema. It has been shown that chronic hypoxia is a constant feature in marathon runner and physical exertion of severe intensity causes an exercise-induced rise in pulmonary capillary pressure leading to stress failure at sea level similar to HAPE [4,5]. A large number of studies widely described elevated ($>$ 100 pg/ml) BNP level as a cardiac biomarker for congestive heart

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https://doi.org/10.1016/j.lfs.2020.118408
Received 24 June 2020; Received in revised form 31 August 2020; Accepted 4 September 2020
Available online 12 September 2020
0024-3205/

failure (CHF) and is released secondary to myocardial stretch having a complementary function in regulating blood volume [6]. However, chronic hypoxia alone, even in the absence of cardiac dysfunction is sufficient to increase BNP levels which might be by counteracting pulmonary vasoconstriction through autoregulation of hemostasis and vessel tone [7]. Our previous studies showed a correlation of BNP with mean pulmonary artery pressure (mPpa) and are capable to predict HAPE susceptibility (AUC 0.85) in non-mountaineers [8] and have an association with severe AMS [9]. It is important to identify individuals susceptible to hypoxia at sea level before induction to HA as the prevalence of AMS on passive ascent to an altitude of 3000–3500 m is up to 40% [10]. Previous studies on BNP and hypoxia susceptibility were conducted on army personnel [8,11] and mountaineers [9] having high physical fitness. Physical exertion during climbing mountain itself can precipitate HAPE in mountaineers, also the prevalence of high-altitude maladies is low in Army troops as they follow acclimatization schedules which involve a graded increase in physical activity for initial few days of induction to high altitude. Therefore, the present study was conducted on healthy individuals having average physical fitness at sea level to explore the screening ability of BNP for hypoxia susceptibility. BNP values at sea level were validated on sojourners who were passively inducted (by air) to high altitude (3200 m) and did not follow acclimatization schedule and suffered HAPE, unlike previous studies on mountaineers and army personnel [9,12]. We hypothesize that hypoxia susceptibility is related to endothelial dysfunction which correlates with subclinical elevation of BNP levels ($>$ 15 pg/ml). The aim of this study is to examine whether BNP can screen hypoxia susceptible among healthy individuals at threshold hypoxic stress (FiO_2 = 0.15) ~ 2600 m when acclimatization mechanisms become important as HAPE occurs above 2500 m [13]. Our previous study on army troop has shown the association of BNP $\leq$15 pg/ml with hypoxia resistance [8,14]. Similar studies on mountaineers at extreme altitudes have shown lower baseline BNP levels with hypoxia resistance [9]. Therefore, we divided individuals into two groups based on their baseline BNP levels. BNP $\leq$15 pg/ml, Group 1 and BNP $>$ 15 pg/ml, Group 2 were subjected to normobaric hypoxia (FiO_2 = 0.15) for 1 h. Hemodynamics, biochemical and hormonal parameters were compared at normoxia and during hypoxia between two groups. To validate BNP levels for hypoxia susceptibility we also measured BNP at high altitude in HAPE patients and controls.

Endothelial dysfunction has been implicated in HAPE susceptibility as baseline and HA levels of endothelin-1 (ET-1) were elevated in mountaineers and HAPE-S showed reduced systemic and vascular endothelial nitric oxide (NO) levels on acute hypoxia exposure at sea level [15,16]. Endothelial function is very preciously regulated by a large number of biological pathways. However, BNP has been found to be an independent predictor of endothelial dysfunction [6] since important physiological action of BNP is to regulate vascular tone via binding to natriuretic peptide receptor-A causing endothelium-dependent vasodilation via NO production [17].

Our previous studies have shown evidence of chronic inflammation in HAPE-S [18]. Chronic inflammation caused a significant reduction in FVC to $<$ 84% predicted which might contribute to HAPE susceptibility [11]. A chronic condition like prediabetes HbA1c (5.7–6.4) is highly prevalent (14%) in a healthy Indian population [19], which is a systemic inflammatory disorder that causes endothelial dysfunction leading to microangiopathy and can contribute to hypoxia susceptibility. Microvasculopathy indicate decreased blood flow through microvasculature (capillaries) leading to hypoxia of peripheral tissue. Normally peripheral vasculature dilates in response to hypoxia however microvasculopathy leads to reduced oxidative phosphorylation and lesser oxygen consumption i.e. delta pO_2 (change in pO_2 during hypoxia) of peripheral tissue. Therefore, we correlated BNP with HbA1c and delta pO_2 in order to determine endothelial dysfunction.

BNP is a known marker for vascular and cardiac remodeling and can be associated with various comorbid conditions associated with high fatality due to COVID-19 infection [20,21]. Interestingly, individuals with NT-pro BNP $>$ 88.64 pg/ml and BNP $>$ 100 pg/ml at admission were found to be associated with a greater number of intubations and grave prognosis in COVID-19 patients [22,23]. Whether vascular remodeling primes pulmonary vasculature for inflammatory storm leading to acute lung injury and multi-organ failure due to SARS CoV2 infection has also been discussed in the present study.

2. Material and methods

2.1. Study participants

The study was conducted on thirty-five healthy male individuals. Individuals who were smokers, hypertensive, cardiopulmonary disorder or on any medication were excluded from the study. None of the participants visited high altitude area within six months of commencement of study in Delhi, India. Participants were divided into two groups based on their baseline BNP levels: Group 1 $\leq$ 15 (n = 25) and Group 2 $>$ 15 pg/ml (n = 10) in accordance with our previous studies. To validate on sojourners, in another subset BNP levels and physiological parameters were measured at the time of admission in patients diagnosed for HAPE (n = 9; 2 Female and 7 male) along with their companions as controls (n = 15; 6 Females and 9 males) who were age, height and weight-matched with similar duration of stay. i.e. first three days of induction at HA. All experimental protocols were approved by Defence Institute of Physiology and Allied Sciences Ethics Committee for scientific experiments and for HA studies were approved by SNM Hospital, Leh. Informed written consent was obtained from all participants before enrolment in the study. All methods were carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. Testing procedure

All the participated individuals were studied in normobaric normoxia (Fi O_2 = 0.21) and normobaric hypoxia (Fi O_2 = 0.15) in a supine resting position for 60 min. GO2 altitude®, hypoxicator (Biomedtech, Australia) was used for creating Fi O_2 = 0.15 hypoxia which correlates with 2500 m of altitude. Inhalation was made using a tight face mask. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) was measured using an automatic blood pressure monitor (Omron, Model: HEM-8712, Vietnam). Peripheral oxygen saturation (SpO_2) and heart rate (HR) were recorded using pulse oximetry (Radiometer, TCM-400, Denmark). The body fluid compartment was measured using a bioelectrical impedance analyzer (BIA-101, Anniversary, Germany). All the measurements were recorded before and at the end of 60 min of hypoxic stress.

Pulmonary artery systolic pressure (sPpa), stroke volume (SV), and cardiac output (Q) were measured non-invasively using echocardiography (My LAB 60 alpha, Esaote India). Standard apical and parasternal two-dimensional views were obtained and color flow directed pulse wave doppler measurements of transvalvular flows and continuous wave Doppler measurements of tricuspid regurgitant flow were obtained.

Pulmonary artery systolic pressure (sPpa) was calculated as follows

$$sPpa = [4(TR_{vel})^2] + RAP \quad (1)$$

where TR_{vel} is tricuspid regurgitation jet velocity and RAP is the estimated right atrial pressure based on respiration variation in inferior vena cava size.

Calculation of Mean pulmonary artery pressure

$$Ppa = 0.61\ sPpa + 2\ mmHg \quad (2)$$

Cardiac output (Q) was calculated as follows

$$Q = [P(D/2)^2]Ao_{vti}\ HR \quad (3)$$

where $P(D/2)^2$ is the cross-sectional area of blood flow into the aorta, Ao_{vti} is the velocity-time integral through the aorta, and HR is the heart rate.

2.3. Measurement of Pulmonary Function Test (PFT) [2]

Spirometery and single breath diffusion capacity were performed using Easy one Pro system (NddMedizintechnik AG CH-8005 Zurich, Switzerland).

2.3.1. Measurement of fractional exhaled nitric oxide (FeNO)

Measured using NObreath® (bedfont®, England) measured in parts per billion using the manufacturer's protocol.

2.4. Venous blood gas and biochemical parameters

2.4.1. BNP measurement

BNP was measured in the venous blood sample taken at baseline level and after acute hypoxia exposure at resting supine position. At high altitude, samples were collected when the patient was admitted and controls were who accompanied the patient. BNP was measured immediately using the BNP test cartridge of i-STAT System cartridges (Abbott, USA) using iSTAT Handheld Analyzer (Abbott, USA). The BNP range reported by the cartridge is 15-5000 pg/ml. There might be changes observed in BNP value if detection range was broadened i.e. can be measured from 0 to 15 pg/ml. We used BNP cartridge i-STAT System in the present study due to following reasons: first is that the previous study have shown no change in BNP levels (< 5 pg/ml in 6 of 7 subjects, while 7th subject showed change in BNP from 18.1 to 18.9 pg/ml) on acute severe hypoxia exposure ($SpO_2 = 62.3\%$) in healthy individuals [24] and second is based on our previous study, where army troops showed association of BNP ≤ 15 pg/ml with hypoxia resistance. Although BNP cartridge i-STAT System is insensitive (lowest reportable value is 15 pg/ml) however it is user friendly, easy, portable, give results in minutes and can be used in remote high-altitude locations to screen hypoxia susceptibility where medical facility is limited compared to using a more reliable and sensitive investigation which required lab facility and skilled manpower.

2.4.2. Biochemical analysis

Venous Blood samples were taken two times first at normobaric normoxia and another sample was collected at the end of 60 min of hypoxic stress. Whole blood was used for blood gas parameters like Lactate, pH, PCO_2 (partial pressure of carbon dioxide), PO_2 (partial pressure of oxygen), TCO_2 (Total carbon dioxide), HCO_3 (Bicarbonate), and sO_2 (Oxygen saturation) measurement by CG4 i-STAT System cartridges (Abbott, USA), parameters like Glucose (Glu), Hematocrit (Hct) and Hemoglobin (Hb) by CHEM 8 i-STAT System cartridges (Abbott, USA), using iSTAT Handheld Analyzer (Abbott, USA). All tests were performed based on standard operating procedures (SOP) of the i-STAT System provided in the system manual.

2.4.3. Blood gas analysis for calculation of p50

Immediately two drops of blood were transferred to i-STAT cartridge CG4 (blood gas analyzer i-STAT Abbott, USA) for estimation of venous blood gas parameter.

Calculation of P50 [2].

$$
\begin{aligned}
&P50\ (Partial\ pressure\ of\ oxygen\ where\ hemoglobin\ is\ 50\%\ oxygenated)\\
&= Log\ po2\ (7{\cdot}4)\ =\ \log po2\ (observed\ value)\\
&\quad -\ [0{\cdot}5\ (7{\cdot}4\ pH\ observed\ value)]\ Log\ 1/k\\
&= anti\log\ [2{\cdot}7\log po2\ (7{\cdot}4)]\ \times\ [100\\
&\quad -\ SaO2\ (observed)]\ Estimated\ p50\ value\\
&= anti\log\ [(\log 1/k)\ \div\ 2{\cdot}7]\ (at\ pH\ =\ 7{\cdot}4,\ Temperature\ =\ 37\ ^{\circ}C)
\end{aligned}
\tag{4}
$$

where pH, Po_2 (partial pressure of oxygen), SaO_2 (saturation of oxygen) in venous blood.

For ELISA studies T3 (Calbiotech, T33797T, USA) range of the assay was 0–9 ng/ml, T4 (Calbiotech, T4224T, USA) range of the assay was 0–25 ng/dL, TSH (Calbiotech, TS227T, USA) range of the assay was 0.5–40 μIU/ml and GLUT-1 (Elabscience, *E*-EL-H1822, USA) the sensitivity of the assay was 0.1 ng/ml were used.

2.4.4. Quantification of hemoglobin fraction using high-performance liquid chromatography (HPLC)

3 ml of whole blood for quantification of hemoglobin fraction by high-performance liquid chromatography (HPLC, Bio-Rad D10) system. HbA1c values were measured in 10 participants from Group 1 and 5 participants from group 2.

2.5. Statistical analysis

Results are presented as mean ± SD, p-value < 0.05 is considered as significant. Data was analyzed by the student's *T*-Test. Corrected p values were used for multiple comparisons within and between two groups for normally distributed data. The normality of data was examined using skewness, kurtosis and the Shapiro–Wilk test. An independent sample Student's *t*-test, or the Mann–Whitney *U* test was used when conditions of normality were not met. For subjects with a BNP below the limit of detection of the assay (15 pg/ml), a value of 15 pg/ml was assigned for the purposes of statistical analysis. Receiver operator characteristic (ROC) analysis was used for BNP and sensitivity and specificity were calculated. All the analysis was done using SPSS Statistics version23 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Baseline anthropometry and pulmonary functions

Both groups were matched for their anthropometric variables. Table 1 confirms no significant differences in age, height, and weight

Table 1
Baseline anthropometry and Pulmonary Function Test in Group 1 and Group 2 subjects. Values are presented as Mean ± SD. $p < 0.05$ is considered as significant.

Parameters	Group 1 (BNP ≤ 15) $n = 25$	Group 2 (BNP > 15) $n = 10$	p value
AGE (yr)	37 ± 12	38 ± 8	0.67
HEIGHT (cm)	170.12 ± 7.62	171.58 ± 5.49	0.61
WEIGHT (kg)	78 ± 12	73 ± 9	0.23
BMI (kg/m^2)	27 ± 3	25 ± 3	0.11
FVC (litre)	3.78 ± 0.74	4.22 ± 0.61	0.11
FVC %Pred	93.6 ± 11.72	102.9 ± 14.22	0.05
FEV1 %Pred	90.44 ± 16.16	101.5 ± 14.81	0.07
FEV1/FVC (%) %Pred	99.6 ± 8.11	102 ± 5.14	0.39
DLCO (ml/kg/mmHg) %Pred	104.38 ± 15.27	99.43 ± 18.37	0.50
Dladj (ml/kg/mmHg) %Pred	102.13 ± 14.69	97.29 ± 18.25	0.50
VA sb %Pred	108.94 ± 15	116.29 ± 28.35	0.42
DLCO/VA %Pred	105.81 ± 13.37	96.57 ± 10.91	0.12
TLC sb %Pred	109 ± 15	116 ± 27	0.42

Table 2
Hemodynamic response to acute hypoxia in Group 1 and Group 2 subjects. Values are presented as Mean ± SD. HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; Ppa: mean pulmonary artery pressure; Q: cardiac output; SpO_2: peripheral oxygen saturation; SV: stroke volume; MAP: mean arterial pressure FeNO: fractional exhaled nitric oxide. a1: $p < 0.05$ (Group 1 versus Group 2 under normoxia). a2: $p < 0.05$ (Group 1 versus Group 2 under hypoxia), *$p < 0.05$ (normoxia versus hypoxia), ** $p < 0.01$ (normoxia versus hypoxia) and ***$p < 0.001$ (normoxia versus hypoxia).

	Group 1 (BNP ≤15) n = 25		Group 2 (BNP > 15) n = 10	
	Normoxia	Hypoxia	Normoxia	Hypoxia
SBP (mmHg)	122 ± 8	124 ± 12	119. ± 13	121 ± 11
DBP (mmHg)	76 ± 6	80 ± 8*	76 ± 8	81 ± 6**
HR (beats/min)	72 ± 8	73 ± 8	66 ± 8	65 ± 7*
SpO_2 (%)	98 ± 1	91 ± 2***	99. ± 1	91 ± 2***
Ppa (mmHg)	14.98 ± 4.76	17.18 ± 6.22**	16.59 ± 4.39	20 ± 5.96**
Q (l/min)	4.31 ± 1.11	4.43 ± 1.03	4.08 ± 0.84	4.14 ± 0.91
SV (ml)	59.98 ± 12.16	61.15 ± 13.03	61.68 ± 9.41	62.97 ± 10.26
BNP (pg/ml)	15 ± 0	15 ± 0	26 ± 8.92[a1]	33.5 ± 11.74**[a2]
MAP (mmHg)	91.57 ± 4.89	94.6 ± 8.95	90.28 ± 9.57	93.97 ± 7.14**
FeNO (ppb)	20.84 ± 10.92	27.68 ± 14.57*	26.24 ± 18.74	29.61 ± 15.83

between the two groups. There was no significant difference in forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), FEV1/FVC, total lung capacity (TLC), functional residual capacity (FRC), pulmonary diffusion capacity for carbon monoxide (DLCO) and alveolar volume (VA), DLCO/VA between two groups.

3.2. Effect of acute hypoxia on hemodynamics, FeNO

Table 2 shows no baseline difference in hemodynamic parameters in between two groups like systolic blood pressure SBP ($p = 0.34$), diastolic blood pressure DBP ($p = 0.93$), heart rate HR ($p = 0.07$) cardiac output Q ($p = 0.55$), stroke volume SV ($p = 0.59$), mean pulmonary artery pressure (Ppa) ($p = 0.35$) and peripheral oxygen saturation SpO_2 ($p = 0.41$). Hypoxia exposure led to a significant rise in DBP ($p = 0.013$) and Ppa ($p = 0.01$) in group 1 while there was a significant rise in DBP ($p = 0.002$), mean arterial pressure (MAP) ($p = 0.009$) and Ppa ($p = 0.004$) in group 2. Both groups show a significant decrease in SpO_2 after hypoxia exposure ($p < 0.001$).

Forced exhaled Nitric oxide (FeNO) levels were significantly increased ($p = 0.015$) after hypoxia exposure only in Group 1 individuals.

3.3. Effect of hypoxia on BNP levels

As shown in Fig. 1 when healthy individuals of both groups were administered acute hypoxia an exaggerated increase in BNP levels (26 vs 33.5 pg/ml, $p = 0.002$) was found in group 2 only. Elevated BNP levels were also found in HAPE patients (74.54 ± 32.98 pg/ml) at high altitude. While Group 1 individuals and control subjects at high altitude had BNP levels 15 and 15.15 ± 0.49 pg/ml respectively.

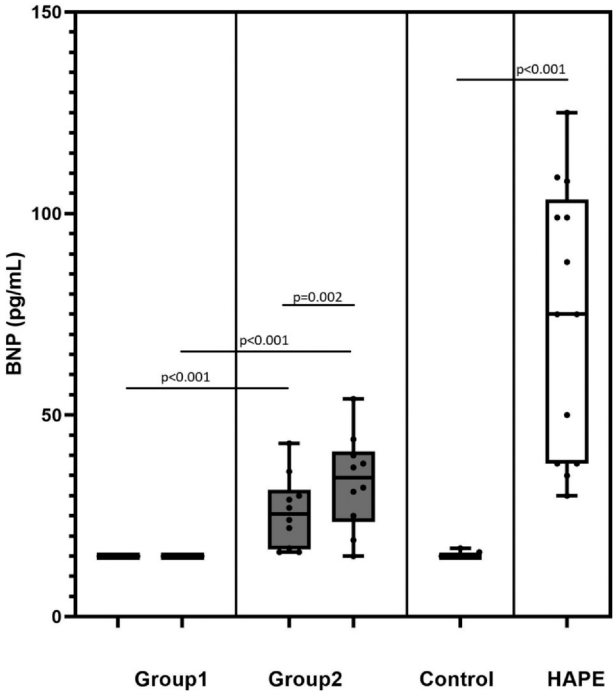

Fig. 1. Figure showing the comparison between Group 1 and Group 2 individuals of brain natriuretic peptide (BNP) levels in normoxia and hypoxia. Group 1 showed no significant change while in Group 2 BNP levels changed significantly in all individuals after hypoxia exposure. BNP levels of Control and HAPE patients at high altitude were shown with BNP (15.15 ± 0.49 pg/ml vs 74.54 ± 32.98 pg/ml).

3.4. Blood gas and biochemical parameters

Table 3 shows, there were no difference in blood gas pH ($p = 0.06$), PCO_2 ($p = 0.08$), PO_2($p = 0.25$), TCO_2($p = 0.35$), HCO_3($p = 0.71$) and sO_2($p = 0.17$) levels between two groups except lactate found to be significantly higher ($p = 0.04$) at baseline in group 2. Similarly, no baseline difference in blood Glucose (Glu, $p = 0.98$), Hematocrit (Hct %, $p = 0.89$) and Hemoglobin (Hb, $p = 0.88$) were found between the two groups.

In order to determine the hypoxia response, we measured the pH and anion gap. Group 1 alone showed a significant increase in pH and a fall in anion gap post hypoxia. Then, to determine the effect of reduced oxidative phosphorylation contributing to hypoxia susceptibility PO_2 and GLUT1 was measured. Post hypoxia fall in PO_2 and rise in GLUT 1 along with baseline high lactate levels in group 2 suggests evidence of chronic hypoxia in these individuals.

Further, thyroid functions were measured to determine evidence of downstream adaptive changes to chronic hypoxia. Hypoxia exposure results in the rise of T3 and TSH levels, while a fall in T4 levels, and left shift of *P*50 in group 2 suggests changes due to chronic hypoxia present in the system.

The blood Hb fractions showed that only HbA1c levels were significantly high ($p = 0.03$) in Group 2 as shown in Table 4. While both groups didn't show any difference in other Hb fractions and were comparable.

3.5. Effect on fluid compartments

Table 5 shows the effect of acute hypoxia on the fluid compartment.

Table 3
Biochemical response to acute hypoxia in Group 1 and Group 2 subjects. Values are presented as Mean ± SD. a1: $p < 0.05$ (Group 1 versus Group 2 under normoxia). a2: $p < 0.05$ (Group 1 versus Group 2 under hypoxia), $*p < 0.05$ (normoxia versus hypoxia) and $**p < 0.05$ (normoxia versus hypoxia).

	Group 1 (BNP ≤15) n = 25		Group 2 (BNP > 15) n = 10	
	Normoxia	Hypoxia	Normoxia	Hypoxia
Ph	7.33 ± 0.03	7.35 ± 0.03*	7.3 ± 0.05	7.32 ± 0.03
PO_2 (mmHg)	21.92 ± 6.88	18.17 ± 3.74**	19.2 ± 4.16	17.4 ± 3.41
PCO_2 (mmHg)	54.44 ± 4.93	52.67 ± 6.09	58.64 ± 8.98	54.97 ± 5.30
HCO_3 (mmol/l)	28.57 ± 1.47	28.77 ± 1.76	28.79 ± 1.84	28.45 ± 1.30
sO_2 %	31.88 ± 15.32	24.09 ± 8.10**	24.6 ± 8.36	22.3 ± 7.83
Lactate (mmol/l)	1.40 ± 0.46	1.15 ± 0.62	1.92 ± 0.94[a1]	1.66 ± 0.75
Anion gap (mmol/l)	17.8 ± 1.85	17.25 ± 1.86*	17.8 ± 2.1	17.3 ± 2.31
Hematocrit (Hct) (PCV)	44.5 ± 3.7	44.3 ± 3.8	44.3 ± 3.8	44.5 ± 4.1
Hemoglobin (Hgb) (g/dl)	15.12 ± 1.25	15.06 ± 1.26	15.05 ± 1.28	15.13 ± 1.41
TCO_2 (mmol/l)	29.8 ± 1.9	30.1 ± 2.2	30.5 ± 2.2	29.9 ± 1.4
GLU (mg/dl)	96.8 ± 15.3	90.7 ± 7.9	96.7 ± 12.9	92.1 ± 7.6
p50 (mmHg)	26.33 ± 0.94	26.14 ± 0.88	25.98 ± 1.03	25.39 ± 1.15[a2]
T3 (ng/ml)	1.24 ± 0.24	1.25 ± 0.31	1.09 ± 0.17	1.10 ± 0.18
T4 (ug/dl)	7.04 ± 1.03	6.93 ± 1.12	7.27 ± 1.42	6.43 ± 1.29*
TSH (μIU/ml)	1.93 ± 2.07	1.75 ± 1.75	1.65 ± 0.55	1.89 ± 0.59*
GLUT1 (ng/ml)	1.98 ± 0.83	1.95 ± 0.85	2.34 ± 0.86	2.86 ± 0.97**[a2]

Table 4
Hb fraction in Group 1 and Group 2 subjects. Values are presented as Mean ± SD. $*p < 0.05$ is considered significant (Group 1 versus Group 2 under normoxia).

	Group 1 (BNP ≤15) (n = 10)	Group 2 (BNP > 15) (n = 5)	p value
HbA1C %	5.5 ± 0.4	6.0 ± 0.4*	0.03
HbA2 %	3.11 ± 0.28	2.84 ± 0.33	0.06
Total HbA %	92.1 ± 0.9	92.4 ± 0.5	0.54

Table 5
Body fluid compartment response to acute hypoxia in Group 1 and Group 2 subjects. Values are presented as Mean ± SD. a1: $p < 0.05$ (Group 1 versus Group 2 under normoxia). a2: $p < 0.05$ (Group 1 versus Group 2 under hypoxia), $***p < 0.001$ (normoxia versus hypoxia).

	Group 1 (BNP ≤15) n = 25		Group 2 (BNP > 15) n = 10	
	Normoxia	Hypoxia	Normoxia	Hypoxia
TBW%	55.32 ± 4.89	53.64 ± 5.10***	56.9 ± 7.55	56.83 ± 9.46
ECW%	42.50 ± 3.45	41.02 ± 3.61***	44.18 ± 5.37	43.17 ± 6.24
ICW%	57.49 ± 3.45	58.97 ± 3.61***	55.78 ± 5.47	56.83 ± 6.24

At baseline levels, there were no significant changes in body fluid compartments between two groups but after hypoxia exposure, there was a significant decrease ($p < 0.001$) in total body water, TBW%, extracellular water, ECW% but significant rise ($p < 0.001$) in Intracellular water, ICW% only in Group 1.

3.6. Correlation between HbA1c and BNP

To determine the usefulness of BNP in predicting prediabetic microangiopathy we looked at the degree of correlation between BNP value and HbA1c. Correlation of BNP and HbA1c levels was calculated using Spearman's rho and found to be positively correlated with $r = 0.49$ ($p = 0.04$). Also, BNP level of these individuals was found to be negatively correlated with change in pO_2 during hypoxia exposure denoted as delta pO_2 with $r = -0.52$, ($p = 0.04$). Then for making ROC, we grouped individuals for their baseline HbA1c levels in non-diabetic who had HbA1c < 5.7 ($n = 10$) and pre-diabetic with HbA1c 5.7–6.4 ($n = 5$) using BNP as predictor for microangiopathy. Receiver operator characteristic (ROC) curve showed BNP level of 27 pg/ml is found to be 100% sensitive and specific, AUC 0.78 (95%CI- 0.53-1.00), p value > 0.05 (Fig. 2).

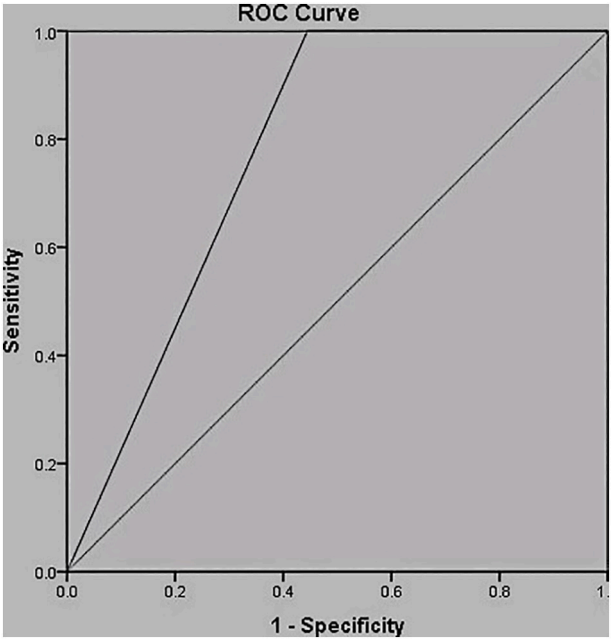

Fig. 2. ROC curve plotted between non-diabetic and pre diabetic individuals based on BNP levels indicating the AUC 0.778, $p > 0.05$.

3.7. High altitude study

At high altitude control subjects showed significantly lower heart rate as compared to admitted HAPE-Patients, all other anthropometric variable did not show any significant difference between two groups (Table 6).

4. Discussion

Exaggerated increase in pulmonary vascular response (PVR) to acute hypoxia has been considered as a gold standard screening test to identify individuals susceptible to HAPE [25]. However, some studies showed that increased PVR to hypoxia can't be a surrogate marker for HAPE susceptibility [14,26]. Therefore, it is important to have a

Table 6
Anthropometry and physiological parameters in controls and HAPE-patients at high altitude. Values are presented as Mean ± SD. $p < 0.05$ is considered as significant.

Parameters	Control (n = 15)	HAPE-patient (n = 9)	*p* value
Female	6	2	
Age (yr)	37 ± 13	41 ± 17	0.46
Height (cm)	164 ± 8	166 ± 7	0.59
Weight (kg)	68 ± 14	71 ± 09	0.49
SBP (mmHg)	129 ± 10	128 ± 31	0.98
DBP (mmHg)	88 ± 06	75 ± 13	0.26
HR (beats/min)	86 ± 13	101 ± 19	0.03
SpO_2 (%)	92 ± 2	72 ± 7	< 0.001

reliable and user-friendly screening test to identify hypoxia susceptible individuals among a healthy population. Our previous study has shown that elevated baseline BNP levels can predict HAPE susceptibility (AUC 0.86) [8]. Here, we show that endothelial dysfunction contributes to hypoxia susceptibility and BNP ≤ 15 pg/ml at sea level indicative of hypoxia resistance [8,11,14]. The present study is in accordance with a previous study where patients with severe AMS at 5100 m have BNP value significantly high compared to control (58.4 ± 18.7vs 22.7 ± 8.6 pg/ml) and greater baseline levels [9]. To the best of our knowledge, this is the first study where endothelial functions were evaluated under acute hypoxia challenge and correlated with BNP levels as endothelial dysfunction has an implication on HA maladies like AMS, HAPE and HACE [16]. However, the present study has a limitation that the validation of individuals found hypoxia susceptible at sea level could not be done under actual high-altitude conditions. Instead, BNP was measured in another subgroup consisting of tourists who visited high altitude and get hospitalized for HAPE and compared with controls who didn't develop HAPE at the same altitude. The merit of the study was the inclusion of young healthy individuals to evaluate endothelial functions since the BNP level increases with age. The present study is similar to previous studies on hypoxia susceptibility with the difference that hypoxia challenge with FiO_2 0.15 was given, which was different from previous hypoxia exposure (FiO_2 0.12) given during acute hypoxia test [25,27]. Since the main focus of the study was whether BNP can predict hypoxia susceptibility at threshold FiO_2 0.15 where acclimatization mechanisms become prominent as PaO_2 become less than 60 mmHg.

The physiological response of the cell to acute hypoxia is the generation of NO facilitating mitochondrial biogenesis as the first line of defence. Hypoxia susceptibility has been related to reduced mitochondrial biogenesis evidenced in HAPE-S [28]. The fraction of exhaled nitric oxide (FeNO) measures pulmonary endothelial NO levels and regulates the homeostasis in endothelium showed no increase in Group 2 post hypoxia similar to HAPE-S [16]. On the contrary, studies showed females having less hypoxia susceptibility related to relatively more NO production due to a direct action of estrogen on vascular endothelium [29,30]. We found evidence of reduced oxidative phosphorylation in Group 2 as GLUT1 level was high during hypoxia, moreover, elevated baseline lactate and no significant fall in PO_2 on acute hypoxia is in contrast to Group 1. Increased GLUT1 is a cellular response in adaptation to chronic hypoxia [31,32]. Elevated TSH during hypoxia was linked with an increased sympathetic activity associated with chronic hypoxia [2,33]. Rise of MBP in Group 2 on hypoxia exposure is similar to the response observed in our previous study on HAPE-S and could be due to reactive oxygen species (ROS) mediated reduction in mitochondrial biogenesis in endothelium resulting in the development of vascular remodeling due to chronic hypoxia [8,34,35]. Exaggerated rise of BNP post hypoxia in Group 2 can be due to the stabilization of HIF-1α similar to HAPE-S [2,36]. Blood vessels dilate in response to acute hypoxia in Group 1 with intact endothelial functions show a decrease in extracellular volume and an increase in intracellular volume. The fluid shift from extracellular to the intracellular compartment is part of the acclimatization process as AMS generally occurs in individuals who pass less urine during initial days of induction to high altitude [9,37]. The right shift in P50 during hypoxia in group 1 indicates better oxygen delivery to peripheral tissue [38,39] suggesting normal endothelial function.

We did not find any significant differences in lung function or hemoglobin A (HbA) fraction that would explain the elevated hypoxic response in group 2 unlike our previous study showing subclinical pulmonary dysfunction and abnormal HbA fraction contributes to HAPE susceptibility [2,11]. Interestingly, increased hypoxia response can be linked to microangiopathy associated with prediabetic disorder HbA1c (6 ± 0.44%) levels in group 2, while baseline physiological parameters were comparable between the two groups. Prediabetic disorder primes microvasculature at a subclinical level which later can manifest as retinopathy, nephropathy, and neuropathy. Microangiopathy characterized by reduced perfusion of peripheral tissue due to increased vascular tone occurring in prediabetics [40], can be linked to hypoxia susceptibility as previous studies have shown that chronic hypoxia increases the sensitivity of vasculature to acute hypoxia due to vascular remodeling in HAPE-S individuals [41]. Microvasculature when challenges with acute hypoxia in Group 2 showed an exaggerated increase in BNP levels suggesting endothelial dysfunction sufficient enough to reduce tissue perfusion further enhancing hypoxia leading to rise in GLUT1 level. A previous study showed BNP (median value of 29.5 pg/ml) can predict endothelial functions when assessed by endothelium-dependent vasodilation [6]. BNP has an advantage over HBA1c due to its limited capability to identify individuals who require fast track lifestyle modification to ameliorate effects of microangiopathy since its levels depend on differences in glycation or red blood cell survival due to ethnic, racial and gender differences [42]. Therefore, when we prepared ROC using BNP values grouped based on HbA1c we find AUC 0.78 with $p > 0.05$, non-significant. However, BNP showed negative correlation with oxygen consumption $r = -0.52$ with p value of 0.04 while HbA1c also showed negative correlation with oxygen consumption $r = -0.34$ but p value is 0.19 suggesting BNP a better predictor for endothelial dysfunction.

Baseline chronic hypoxia-mediated subclinical pulmonary hypertension predisposes to HAPE susceptibility since nifedipine helps in reducing the prevalence of HAPE from 70% to 10% in HAPE-S on a rapid ascent to HA [2,43]. Also, the level of BNP correlates with the severity of hypoxia, since, lower (> 15 pg/ml) and higher (52.39 ± 32.9 pg/ml) basal values of BNP were associated with mild (AMS) and severe (HAPE) [8] form of HA disorders respectively.

Exposure to HA above 2500 m triggers acclimatization mechanisms mediated by HIF 1α which includes erythropoiesis, increased ventilation, increase in capillary density, etc. in order to normalize blood oxygen, i.e. increase of blood oxygen content to acclimatize at a particular altitude [44]. HIF-1α acts on the promoter site of pro-BNP to increase the production of BNP [45,46]. BNP causes pulmonary artery dilation and natriuresis also contributing to increased blood oxygen content at HA. Baseline rise in BNP (> 15 pg/ml) due to endothelial dysfunction could be primary or secondary to systemic disorders (cardio-respiratory, metabolic etc.) indicates hypoxia susceptibility. However, in hypoxia susceptible there is a failure of a fluid shift from intercellular and intravascular to intracellular compartment due to endothelial dysfunction. Exaggerated increased sympathetic activity [3] along with reduced vascular compliance in hypoxia sensitive individuals may results in congested cerebral blood vessels on induction to high altitude and can manifest as symptoms due to cerebral tissue compression like headache, nausea and vomiting are features of AMS and generally clears off by acetazolamide [47]. In severe cases, vascular remodeling of cerebral veins can produce hypoxia-mediated venous constriction leading to the development of high altitude cerebral edema (HACE). HACE is a severe form of AMS characterized by encephalopathy which requires descent to low altitude and mannitol. Similarly,

remodeling of pulmonary arterioles and veineoles due to endothelial dysfunction in HAPE-S may result in a simultaneous increase in Ppa and pulmonary capillary wedge pressure in those who developed HAPE at high altitude [48]. Also, various risk factors for HAPE like reduced forced vital capacity [11], patent foramen ovale [49] and reduced Hb A fraction [2] are associated with decreased blood oxygen levels producing hypoxia susceptibility.

Pulmonary endothelial dysfunction has also been implicated in acute lung injury due to COVID-19 as ACE2 is highly expressed in human lung tissue [50,51]. Binding of COVID-19 with ACE2 result in exhaustion of ACE2 thus inhibiting the ACE2/Ang(1–7)/Mas receptor pathway leading to exacerbation of acute severe pneumonia [52–54]. Experiments on mice also have shown that the expression of ACE2 in lung tissues was significantly downregulated after SARS-CoV infection leading to increased pulmonary vascular permeability, pulmonary edema, and development of ARDS [53,55]. Comorbidity has been associated with stabilization of HIF1α, a key transcription factor that upregulates ACE protein expression and inhibits ACE2 expression further accelerating inflammatory damage due to COVID-19 [56]. ACE enhances the proliferation and migration of pulmonary artery smooth muscle cells which contribute to the pathogenesis of hypoxic pulmonary hypertension. Further evidence of hypoxic pulmonary hypertension has been found in COVID-19 patients who do not survive and showed a progressive rise in NT proBNP and a rise in Troponin T (TnT) during hospital admission [57]. In contrast, those who survived and responded to treatment showed no progressive rise in NT proBNP and TnT levels during hospitalization. Also, retrospective studies have shown that COVID-19 patients who received calcium channel blockers (pulmonary vasodilators) had lesser number of intubation and better prognosis [58]. BNP is a known marker of vascular and myocardial remodeling associated with the HIF1α signaling pathway in various chronic disease conditions where stabilized HIF1α acts on the promotor site of the BNP gene [45,46]. Baseline elevated HIF1α and BNP levels (> 15 pg/ml) were also found in subclinical comorbid states like prediabetes and subclinical pulmonary dysfunction in the present and previous studies [8,9,11] and have shown a predisposition to acute mountain sickness and high altitude pulmonary edema due to development of hypoxic pulmonary hypertension [8,12]. Similarly, COVID-19 patients with high baseline BNP levels > 100 pg/ml at admission were associated with a poor prognosis due to the development of severe hypoxic pulmonary hypertension in comparison to those with lower initial BNP levels suggesting pre-existing vascular remodeling predispose to hypoxic injury [23]. The higher degree of pulmonary vascular remodeling has been associated with severe pulmonary hemodynamic derangement in response to hypoxia, therefore, depending on the degree of pre-existing vascular remodeling, patient with positive SARS COV2 may present from asymptomatic, silent hypoxia (mild) to ARDS (severe) disease. The development of an effective vaccine against SARS COV2 is one logical approach to fight the COVID-19 pandemic, the other could be to target HIF1 α. Although stabilization of HIF1α could display protection during acute clinical conditions, however, targeting the HIF1 α signaling pathway could hold promising in the effective management of disease due to COVID-19. Therefore, prophylactic inhibition of HIF1 α mediated pulmonary hypertension in comorbid populations by cyclosporin-A might reduce morbidity and mortality due to COVID-19 [59].

The present study on HAPE patients showing elevated BNP (74.54 ± 32.98 pg/ml at 3200 m) measured within first three days of HA induction is in accordance to the previous study on mountaineers having BNP (40.7 pg/ml at 5642 m) [12] who suffered subclinical pulmonary edema with prophylactic acetazolamide. Another study on the military population showed severe AMS having BNP (58.4 pg/ml at 5150 m) on the ninth day of HA induction [9]. Higher BNP in the present study on HAPE patients at comparable low altitude (3200 m) probably indicates individual susceptibility to hypoxia and lack of proper acclimatization in contrast to army and mountaineer population.

5. Conclusion

BNP rise with altitude is in proportion to oxygen availability and can be used to determine acclimatization status. It is intriguing that BNP levels at sea level and at various altitudes can be used to devise preventive strategies against HA maladies. The present study indicates that elevated BNP > 15 pg/ml indicates endothelial dysfunction contributing to hypoxia susceptibility, while BNP ≤15 pg/ml at sea level is suggestive of hypoxia resistance. This is an early and useful step towards personal protection before going to HA.

CRediT authorship contribution statement

RKG has set up the study concept and design, data acquisition, ECHO analysis, interpretation and drafting and reviewing of manuscript. RK performed data acquisition, biochemical analysis, HPLC analysis, P50 calculation, body composition, statistical analysis, preparation of figures and preparation of manuscript. PKR performed data acquisition. VB & AY was responsible for data acquisition and preparation of manuscript. PV, PC and SS performed biochemical estimation and analysis. AB, TPB and ACB have set up normobaric hypoxia protocol and data acquisition. DD performed body composition analysis. SBS and BK were responsible for drafting of manuscript, supervision and coordination of study process. All the authors read and approved the manuscript.

Declaration of competing interest

Authors declare no conflict of interest.

Acknowledgment

We thank all volunteers who participated in this study. We thank Sachin Kumar and Ashok Kumar for their assistance during hypoxia exposure and blood sampling.

The study was supported by grants from the Defence research development organization (DRDO), Ministry of Defence, India. Fellowship supported by the Department of Biotechnology (DBT), India.

The authors declare that there is no conflict of interest.

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