[Downloaded free from http://www.sjkdt.org on Monday, November 2, 2020, IP: 202.142.96.89]

Saudi J Kidney Dis Transpl 2018;29(4):879-888
© 2018 Saudi Center for Organ Transplantation

Saudi Journal of Kidney Diseases and Transplantation

Original Article

Hypoxia: A Cause of Acute Renal Failure and Alteration of Gastrointestinal Microbial Ecology

Animesh Samanta[1], Arpita Patra[1], Shreya Mandal[1], Suchismita Roy[1], Koushik Das[1], Sanjay Kar[2], Dilip Kumar Nandi[1]

[1]Department of Microbiology, Nutrition, and Human Physiology, Raja N. L. Khan Women's College, [2]Department of Botany, Midnapur College, Midnapore, West Bengal, India

ABSTRACT. Oxygen is very important to the existence of life. Oxygen deficiency, defined as hypoxia, elicits adaptive responses in cells and tissues. Lower oxygen concentration can cause the alteration of renal function, affects the maintenance of a balance of the body fluids, electrolytes, pH, and blood pressure homeostasis. Impaired fluid regulation could, in addition, contribute to the precipitation of pulmonary edema and exacerbate hypoxemia which may accelerate the progression of chronic kidney disease. In this context, the present study attempted to evaluate the association of renal injury and oxidative stress at different atmospheric pressures (1829, 3657, and 5486 m). Limited fecal analysis of experimental animals was also done to evaluate the impact of hypobaric hypoxia on the composition of dominant gastrointestinal microbiota. The study was performed on 24 male Wister strain rats and divided into four groups (C, HA-I, HA-II, and HA-III), and exposure was carried out for seven days period. In hypoxic exposure rats, plasma urea, creatinine, electrolytes and malonaldehyde level elevated and catalase and superoxide dismutase level diminished significantly compared to the controls. Increase in blood uremia profile, toxicity markers, and lipid peroxidation marker enzymes indicated that hypoxia causes renal failure. Histological structures of the kidney of group HA-II and HA-III animals showed severe disorganization of glomerulus and dilation of renal tubules. These results indicate nephrotoxicity or acute renal failure can occur at hypobaric hypoxia and it also affected the gut microbial population. This alteration was observed significantly above 3000 m.

Correspondence to:

Dr. Dilip Kumar Nandi,
Department of Microbiology, Nutrition, and Human Physiology, Raja N. L. Khan Women's College, Midnapore, Pin-721102, West Bengal, India.
E-mail: dilipnandi2004@yahoo.co.in

Introduction

Oxygen is very important to the endurance of mammalian cells and tissues. Deficiency of adequate oxygen supply, defined as hypoxia or hypoxemia (lower concentration of O_2 in the blood) which develop from varieties of physiological difficulties including changes of body

[Downloaded free from http://www.sjkdt.org on Monday, November 2, 2020, IP: 202.142.96.89]

weight and hematological changes.[1] Gastrointestinal (GI) disorders and acute mountain sickness are collectively called altitude related sickness.[2] This is due to low level of oxygen in the air, improper exchange of gases in the lungs (ventilation, diffusion, and perfusion), not enough hemoglobin for oxygen transfer and improper function of cardiovascular system.[3] Researchers revealed that these causes are not only critical in cardiopulmonary conditions but also in the regulation of the microenvironment of every cell.[4] The oxygen delivery in the body and the kidneys are clinically important ones in both health and disease.[5]

Kidney shows an amazing difference between blood supply and oxygenation. Even though it has high blood flow and oxygen delivery, oxygen tensions in the kidney are comparatively low, in particular in the renal medulla (average PO_2 ≤10 mm Hg) compared to renal cortex (average PO_2 of approximately 30 mm Hg). The reason is the parallel arrangement of arterial and venous preglomerular and postglomerular vessels, which allows oxygen to pass from arterioles into the postcapillary venous system via shunt diffusion. During renal filtration, most tubular segments have a very inadequate ability for anaerobic energy generation, thus are reliant on oxygen to continue active transtubular reabsorption of solutes, in particular, sodium. Erythropoietin (EPO), responsible for hematopoiesis gives a signal from the renal cortex of the kidney. The synthesis or induction of EPO in peritubular fibroblasts of the renal cortex of kidney is regulated by a negative feedback loop responses to renal tissue hypoxia.[6,7] So, the limitations of oxygen supply in renal tissue render the kidney susceptible for hypoxia and may lead to the pathogenesis of acute renal injury.

Literature reveals that the hypobaric-hypoxic stress is solely dependent on the rise in altitude from sea level. An increase of 1 km above sea level drops 10 kPa air pressure; with a sea level air pressure of 101.3 kPa (at 15°C and 0% humidity).[8] It has been well known that individuals, in hypobaric atmospheric pressure >1493 m and extreme high altitudes >5486–6096 m suffer from several pathophysiological disorders. These may lead to modulation of the GI microbial community.[9] An imbalance of GI microbiome ecology ultimately affects the gut physiological homeostasis associated with several GI complications.[10] In this context, the present study attempts to evaluate the association of renal injury and hypoxic stress at different atmospheric pressures and the relation to microbial alterations. Fecal analysis of experimental animals was also done to evaluate the impact of hypobaric hypoxia on the composition of dominant GI microbiota.

Materials and Methods

Selection of animals and diet

The study was conducted with twenty four healthy, adult, male albino rats of Wistar strain (Supplied from Ghosh animal, Animal foods and animal cages Supplier, Kolkata – 700 054) having the body weight of 108.47 ± 1.53 g. They were acclimatized in the laboratory condition for two weeks prior to conducting experiment. Animals were housed at room temperature (20°C ± 3°C) with 12–12 h dark-light conditions with humidity of 60% ± 10%. Normal diet was used for their feed and purified water was used for drinking ad libitum at regular intervals. The guidelines of the National Institute of Health, USA were followed for taking care of the experimental animals, throughout the experiment.[11] Animal experiments were permitted by the Ministry of Environment, Government of India under registration no. 1905/PO/Re/S/ 2016/CPCSEA issued by Committee for the Purpose of Control and Supervision of Experiments on Animals and approved by the Institutional Animal Ethic Committee of Department of Nutrition, Raja N. L. Khan Women's College, Midnapore, West Bengal, India. All animals used in these experiments were free from any type of infection.

Grouping of animals and experimental procedure

Animals were selected randomly and divided into four groups having six animals each. Group C served as control and was exposed to

[Downloaded free from http://www.sjkdt.org on Monday, November 2, 2020, IP: 202.142.96.89]

Table 1. Exposure of animals (group) to different barometric pressure.

Groups			
Control (C)	**Moderate altitude exposure (HA-I)**	**High altitude exposure (HA-II)**	**Extreme high altitude exposure (HA-III)**
Sea level (<500 m)	6000 feet/1829 m	12,000 feet/3657 m	18,000 feet/5486 m
Barometric pressure: 14.7 psia	Barometric pressure: 11.8 psia	Barometric pressure: 9.3 psia	Barometric pressure: 7.3 psia

normal room air (normoxia). Group HA-I, HA-II and HA-III were exposed to different barometric pressures, as shown in Table 1. Exposure was carried out in a decompression chamber (Instrumentation India, India) for 7 days following the protocol of Maity et al.[12]

Collection of blood, kidney, and liver from the rat

After seven days, animals were sacrificed by mild chloroform anesthesia.[13] Blood was collected into heparin-coated sample bottles for analyzed hematological parameters like measurement of red blood cell (RBC), white blood cell (WBC) present in the blood. The blood cells were counted by hemocytometer and hemoglobin (Hb) by standard kit method (Merck, Japan). Plasma was separated by centrifugation at 3000 rpm for 20 min and preserved at −20°C. Livers and kidneys were immediately dissected out, washed and stored in ice cold normal saline (0.9% NaCl) for different biochemical examination.

Blood uremia profile

Biochemical estimation of blood urea

Urea level of plasma was measured by commercially available standard Blood Urea Kit (Merck, Japan) and detected by Semiauto-analyser (Merck, Japan) according to the method of Burtis et al[14] with slight modification.

Biochemical estimation of blood creatinine

Creatinine (Cr) level of separated plasma fraction was determined by standard blood Cr kit (Merck, Japan) following the standard protocol for photometric determination of Cr based on Jaffe kinetic method.[14]

Antioxidant enzymes

Biochemical assay of catalase activity

Catalase (CAT) activity was measured through biochemically. Briefly, 0.5 mL of hydrogen peroxide (H_2O_2) and 2 mL of phosphate buffer (PBS) were mixed in a spectrophotometric cuvette, and tissue supernatants and plasma were added to it at a volume of 20 µL. The spectrophotometric readings were noted after every 30 the second interval at 240 nm.[15]

Biochemical assay of superoxide dismutase

Kidneys were homogenized in ice-cold 100 mM Tris-cacodylate buffer (LOBA Chem, India) to give a tissue concentration of 50 mg/mL centrifuged at 10,000 g for 20 min at 4°C and also the whole blood was centrifuged, and plasma fraction was separated. The superoxide dismutase (SOD) activity of kidney tissue and plasma will be estimated by measuring the percentage of inhibition of the pyrogallol auto-oxidation by SOD using spectrophotometer at 420 nm and values were expressed as mmol of H_2O_2 consumption/dL of plasma/min.[15]

Estimation of lipid peroxidation from the levels of malondialdehyde (MDA)

The kidneys and livers were homogenized separately at the tissue concentration of 50 mg/mL in 0.1 M of ice-cold PBS (pH-7.4). The homogenates were centrifuged at 10,000 g at 4°C for 5 min individually. After centrifugation, the supernatants were separated used for the estimation of MDA. The whole blood was centrifuged, and the plasma fraction was separated. The MDA in samples were estimated

[Downloaded free from http://www.sjkdt.org on Monday, November 2, 2020, IP: 202.142.96.89]

using the extinction coefficient and expressed in the unit of nM/mg of tissue and nM/mL of plasma.[16]

DNA fragmentation assay

The extent of DNA fragmentation in the kidney tissue was determined by the method as described by Lin et al.[17] Briefly, kidney tissue homogenates were treated with 100 mM Tris buffer, pH 8.0, 1 mM EDTA and 0.5% triton X-100 followed by centrifugation at 10,000 g at 4°C for 5 min. The supernatant was transferred carefully in a tube, and then 1 mL of 25% TCA was added to it. The mixture was gently vortex vigorously and incubated overnight at 4°C in refrigerator.

Histological studies

Kidneys from the normal and experimental rats were fixed with 10% buffered Bouin's reagent and were processed for paraffin sectioning. Paraffin-embedded specimens were cut into section followed by staining with hematoxylin-eosin stain for light microscopic examination. All section of kidney samples was examined for characteristic histological changes including tubular necrosis or derangement of renal tissue.[17]

Limited analysis of fecal

Limited analysis of fecal microbiota was conducted from all experimental animal groups. Fresh fecal samples were collected from individual animals before and after seven days of the experiment. Samples were re-suspended in PBS saline at an approximate concentration of 0.03 g/mL. Serial dilutions were prepared, and 0.1 mL aliquots from dilutions of 10^{-6} and 10^{-7} were cultured on single-strength trypticase soya agar (TSA, Himedia, India) and reduced Wilkins Chalgren agar (WCA, Micromaster, India) for enumerating of total aerobic and anaerobic fecal bacteria, respectively. Man, Rogosa, Sharpe (MRS) (HiMedia, India) agar was used to obtain lactic acid bacterial population, thiosulfate-citrate-bile-sucrose agar was used for *Vibrio* sp., and MaConkey agar for enumeration of total enteric bacteria. *Salmonella* sp. and *Shigella* sp. were isolated by using xylose lysine deoxycholate agar. All experimental Petri plates were incubated at 37°C for 48 h.[18,19]

Growth direction index (GDI)

Colony-forming units (cfu) represent the actual number of bacteria present in the fecal sample. These cfu values were converted to their logarithmic value and tallied with the corresponding experimental set of specified conditions. When the log value of control cfu is higher than the log value of test cfu, then GDI is designated as negative, and the reverse event is designated as GDI positive. GDI gives the expansion or contraction of bacterial populations in a particular biosystem.[12,20]

Statistical Analysis

Analysis of variance followed by a multiple two-tailed *t*-test were performed and this along with Bonferroni's modification was used for statistical analysis of the collected data. Differences were considered statistically significant when $P < 0.05$.

Result

Somatic index and hematological parameter

After seven days of the experiment, the body weight increased in group C, HA-I and decreased in the group HA-II, HA-III in comparison with their initial body weight. In HA-I animals, the percentage of increase in body weight was dramatically less than the Group C. On the other hand, it decreased in the group HA-II and group HA-III, due to hypobaric induced oxidative stress (Table 2). Although the weight of kidney and liver in the Group HA-I, HA-II and HA-III were also slightly decreased. Total RBC, total WBC, and hemoglobin level were significantly increased in the blood of HA-I, HA-II and HA-III animals group, comparison to Group C. However, expansion in HA-II and HA-III group is much higher with respect to group HA-I (Table 3).

Estimation of uremia profile

The blood urea and Cr levels were significantly

[Downloaded free from http://www.sjkdt.org on Monday, November 2, 2020, IP: 202.142.96.89]

Table 2. Changes in body weight, kidney, and liver somatic index of rats at a different altitude.

Groups	Initial body weight (g)	Final body weight (g)	Elevation/ diminution in body growth (g%)	Kidney somatic index	Liver somatic index
C	107.1 ± 4.52[a]	125.6 ± 4.76[a]	+17.4	0.81 ± 0.01[a]	2.28 ± 0.07[a]
HA-I	110.5 ± 3.87[a]	112.7 ± 3.27[b]	+2.09	0.80 ± 0.01[a]	2.26 ± 0.04[a]
HA-II	107.4 ± 3.86[a]	95.2 ± 4.34[c]	−11.9	0.76 ± 0.03[b]	2.21 ± 0.08[a]
HA-III	108.8 ± 1.87[a]	94.6 ± 1.89[c]	−12.9	0.71 ± 0.02[c]	2.02 ± 0.12[b]

Data are expressed as mean ± SE (n = 6). ANOVA followed by multiple two-tailed t-test and data with different superscripts (a, b) in a specific vertical column differ from each other significantly (P <0.05). SE: Standard error, ANOVA: Analysis of variance.

Table 3. Effect of different atmospheric pressure on hematological parameters (red blood cell , white blood cell , and hemoglobin) of male rats.

Groups	RBC (cumm×1,000,000)	WBC/cumm×1000	Hemoglobin [g %]
C	6.69 ± 0.37[a]	7.62 ± 0.35[a]	8.35 ± 0.24[a]
HA-I	8.16 ± 0.43[b]	9.22 ± 0.51[b]	10.24 ± 0.29[b]
HA-II	11.59 ± 0.59[c]	13.43 ± 0.52[c]	12.96 ± 0.57[c]
HA-III	13.07 ± 0.27[d]	14.28 ± 0.45[c]	14.06 ± 0.29[d]

Data are expressed as mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, and d) in a specific vertical column differ from each other significantly (P <0.05). RBC: Red blood cell; WBC: White blood cell, SE: Standard error, ANOVA: Analysis of variance.

increased in Group HA-I, HA-II and HA-III animals (hypobaric-hypoxic exposure animals) compared to group C. However the increase of urea, Cr was significantly higher in HA-II and HA-III in comparing with the group HA-I (Table 4). From the study, it was observed that sodium level was significantly decreased in hypobaric-hypoxic exposure animals (Table 4).

Estimation of antioxidant enzymes (CAT and SOD)

After exposure of low atmospheric pressure

Table 4. Changes of uremia profile and oxidative stress markers of rats at a different altitude.

Parameters	Groups			
	C	HA-I	HA-II	HA-III
BUN (mg/dL)	22.55 ± 0.57[a]	27.72 ± 0.85[b]	38.49 ± 1.31[c]	41.16 ± 0.66[d]
Creatinine (mg/dL)	0.53 ± 0.03[a]	0.64 ± 0.02[b]	0.94 ± 0.08[c]	1.04 ± 0.10[d]
Sodium (mmol/L)	160.03 ± 1.73[a]	155.38 ± 2.32[b]	143.35 ± 0.72[c]	141.35 ± 2.05[c]
Potassium (mmol/L)	6.27 ± 0.36[a]	6.61 ± 0.42[a]	7.09 ± 0.39[b]	8.92 ± 0.39[c]
Chlorine (mmol/L)	104.35 ± 1.52[a]	111.01 ± 2.76[b]	112.3 ± 2.74[b]	115.64 ± 1.36[b]
Blood CAT (mmol/mL/min)	0.79 ± 0.02[a]	0.47± 0.02[b]	0.41 ± 0.04[c]	0.32 ± 0.03[d]
Kidney CAT (mmol/mL/min)	0.59 ± 0.07[a]	0.35 ± 0.04[b]	0.28 ± 0.04[c]	0.27 ± 0.03[c]
Liver CAT (mmol/mL/min)	0.69 ± 0.03[a]	0.50 ± 0.02[b]	0.36 ± 0.03[c]	0.26 ± 0.03[d]
Blood SOD (mmol/mL/min	1.09 ± 0.06[a]	0.80 ± 0.08[b]	0.52 ± 0.08[c]	0.39 ± 0.05[d]
Kidney SOD (mmol/mg/min)	1.9 ± 0.47[a]	0.99 ± 0.19[b]	0.94 ± 0.35[b]	0.53 ± 0.22[c]
Liver SOD (mmol/mg/min)	0.93 ± 0.17[a]	0.76 ± 0.08[b]	0.49 ± 0.08[c]	0.37 ± 0.04[d]
Blood MDA (nM/mL)	31.18 ± 1.70[a]	47.71 ± 2.32[b]	55.9 ± 1.31[c]	62.2 ± 1.92[d]
Kidney MDA (nM/mL)	50.61 ± 2.13[a]	62.94 ± 2.20[b]	89.05 ± 1.84[c]	87.34 ± 2.18[c]
Liver MDA (nM/mL)	41.18 ± 1.95[a]	53.38 ± 0.74[b]	66.44 ± 1.02[c]	69.03 ± 3.68[c]

Estimation of uremia profile and oxidative effect in blood, kidney and liver of experimental male albino rats at three different high altitudes. Data are expressed as mean ± SE (n = 6). ANOVA followed by multiple two-tailed t-test (Dunkan's multiple test) and data with different superscripts (a, b, c, d and e) in a specific horizontal row differ from each other significantly with respect to control (P <0.05). SE: Standard error, ANOVA: Analysis of variance

[Downloaded free from http://www.sjkdt.org on Monday, November 2, 2020, IP: 202.142.96.89]

(Group HA-I, HA-II, and HA-III animals), the antioxidant enzymes activity in blood plasma, kidney and liver were decreased significantly, in all experimental groups in compared to group C (control). At the end of the experiment, the activities of those enzymes in above mentioned tissues had decreased significantly in Group HA-II and HA-III. The rate of reduction of CAT and SOD is respectively more in kidney and blood of the group HA-III at the end of experiment (Table 4).

Quantification of lipid per oxidation by malonaldehyde level

Quantities of MDA altered significantly in blood plasma, kidney, and liver among all test groups (P <0.05). After seven days of low atmospheric pressure blood, kidney, and liver parameters were elevated in the Group HA-I, HA-II, and HA-III (Table 4).

DNA fragmentation assay

DNA fragmentation analyses were performed for further verification of hypobaric hypoxic induced renal damage and it showed that random fragmentation of genomic DNA of kidney tissues along with subsequent formation of a DNA smear on agarose gel without ladder formation (Figure 1).

Histological assessment

Histological assessments of kidney segments of control and experimental animals are presented in Figure 2. Kidney sections of hypobaric hypoxic unexposed animals (group C) and exposed Group HA-I showed well organized kidney tissue shown in Figure 2a and b, respectively. On the other hand, kidney sections of hypobaric hypoxic exposed animal of Group HA-II and group HA-III showed the moderate and massive glomerular and renal tubular damage by inflamed and necrotic epithelial cells shown in Figure 2c and d, respectively.

Limited analysis of fecal matter

The population of total aerobes was 6.13 $\log_{10}$ CFU/g in the fecal matter of C group (without exposure animals). However, it was reduced drastically in HA-II and HA-III groups

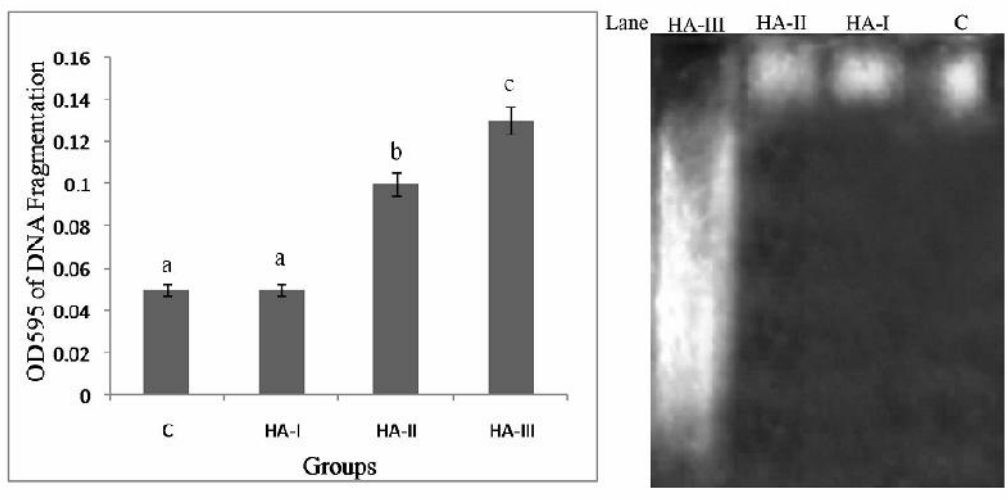

Figure 1. DNA fragmentation assay. (a) Effect of hypoxia on the extent of DNA, (C) Normal control rats, HA-I (6000 feet/1829 m), HA-II (12,000 feet/3657 m) and HA-III (18,000 feet/5486 m) experimental rats. Each column represents mean ± SE (n = 6). ANOVA followed by multiple two-tail t-test and data with differentsuperscripts (a, b, c, d) in a specific vertical column differ from each other significantly (P <0.05). (b) DNA isolated from experimental kidney tissues was loaded on 1% (w/v) agarose gels. (C) Normal control rats, HA-I, HA-II and HA-III high altitude exposure treated kidney DNA.

[Downloaded free from http://www.sjkdt.org on Monday, November 2, 2020, IP: 202.142.96.89]

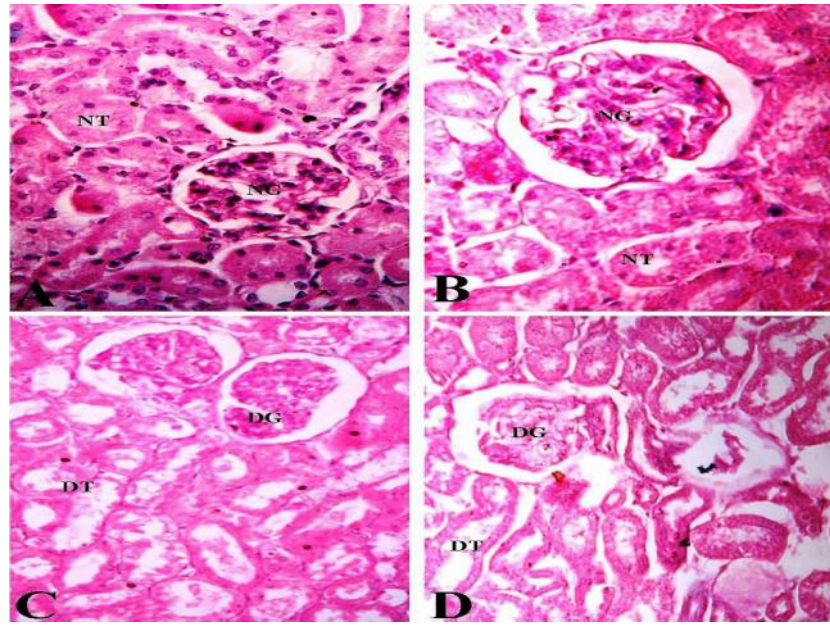

Figure 2. Representation of light microscopy of kidney tissue section H and E ×100. (a) Histology of kidney tissue in group C (Control), (b) Histology of kidney tissue in group HA-I (6000 feet/1829 m), (c) Histology of kidney tissue in group HA-II (12,000 feet/3657 m), (d) Histology of kidney tissue in group HA-III (18,000 feet/5486 m)

NG: Normal glomerulus, NT: Normal renal tubules with intact well organized cellular boundary that are not affected due to the lower atmospheric pressure, DG: Damaged glomeruli and DT: Damaged tubules are dilated with loss of cellular boundary.

(P <0.05) by 4.77 and 2.94 with final GDI of −2.08 ($\log_{10}$C/$\log_{10}$HA-III$_7$). The quantity of total anaerobes increased [9.1–11.16 ($\log_{10}$ CFU/g)] with GDI of +1.22. The ratio (ratio of $\log_{10}$CFU/g of total aerobe and anaerobic population was altered from 1:1.48 (control) to 1:1.44 (HA-I); 1:1.48 (control) to 1:2.48 (HA-II) and 1:1.48 (control) to 1:3.79 (HA-III), respectively. The *Escherichia coli* content was expended with positive GDI in hypoxic condition. The quantity of strict anaerobes such as *Bacteroides* sp. and *Bifidobacterium* sp. was increased with GDI of +1.23 and +1.42 after the seven day of exposure in HA-III. Normally they were present (at base level) in fecal sample at a ratio of 1:1.03 (ratio of $\log_{10}$ CFU/g) that changed to 1:1.01 (HA-I), 1:1.13 (HA-II) and 1:1.19 (HA-III) respectively after seven days of hypoxic stress (Table 5). Total lactic acid bacterial population and selected pathogen like *Salmonella* sp. was also increased.

Discussion

Loss of body weight at hypobaric hypoxic condition had been described in several studies. In this study final body weight of the animals in HA-II and HA-III groups decreased significantly (P <0.05) compared to initial body weight. This alteration occurred probably due to higher metabolic rate, different energy output, loss of body water and several endocrine factors. The weight of kidney and liver in group HA-I, HA-II and HA-III were slightly decreased (Table 2). The reasons for these changes were mentioned in the previous work done by Benso et al[21] and Wall et al[22] In our study blood RBC, Hb, and WBC increased (Table 3). At hypobaric hypoxic condition partial pressure of oxygen (PO_2) is decreased which cause the excessive secretion of erythropoietin to carry out cellular function by increasing blood RBC, Hb, and WBC.[23] It was a great capacity for physiological adjustments

[Downloaded free from http://www.sjkdt.org on Monday, November 2, 2020, IP: 202.142.96.89]

Table 5. Changes of microbial load in faecal sample after in vitro treatment of graded hypobaric (11.8 psia, 9.3 psia and 7.3 psia) conditions alteration after 7 days with GDI.

Microbial parameter	Groups (Hypobaric hypoxic exposure for 7 days)				GDI ($log_{10}C/log_{10}HA_7$)
	C	HA-I	HA-II	HA-III	
Total aerobes	6.13 ± 0.50a	6.30 ± 0.24a	4.77 ± 0.43b	2.94 ± 0.46c	- 2.08
Total anaerobes	9.1 ± 0.05a	9.10 ± 0.07a	11.10 ± 0.05b	11.16 ± 0.07c	+ 1.22
Escherichia coli	6.9 ± 0.33a	6.91 ± 0.33a	8.00 ± 0.30b	8.95 ± 0.42c	+ 1.29
Bacteroidetes sp.	5.03 ± 0.37a	5.20 ± 0.15a	5.78 ± 0.24b	6.23 ± 0.08c	+ 1.23
Total lactic acid bacteria	6.05 ± 0.08a	6.14 ± 0.07a	7.17 ± 0.05b	7.35 ± 0.04c	+ 1.21
Bifidobacterium sp.	5.21 ± 0.49a	5.27 ± 0.58a	6.51 ± 0.35b	7.42 ± 0.59c	+ 1.42
Salmonella sp.	2.17 ± 0.08a	2.28 ± 0.17a	3.52 ± 0.16b	4.29 ± 0.56c	+ 1.97

Microbial population density was expressed (mean of log10 CFU/g ± SE). Letters (a, b, c, d) in a row are significantly different at P <0.05

to compensate for this reduced pressure gradient.

Blood urea and Cr supplementation levels were considered to be nephrotoxicity biochemical markers.[24] Hypobaric hypoxic exposure of rat (group HA-II and HA-III) resulted in a significant elevation of blood urea and Cr when compared with control rats (group C) (Table 4). In renal disease, the blood urea accumulates because the rate of blood urea production exceeds than the rate of clearance. Elevation of urea and Cr levels in the blood is taken as the index of nephrotoxicity. Cr, on the other hand, is mostly derived from endogenous sources by tissue Cr breakdown.[25] Thus blood urea concentration is may be a more reliable renal function predictor than plasma Cr.

Hypobaric-hypoxic related oxidative stress in blood, kidney and liver tissue was established in this study. Observation of the low activities of SOD and CAT, both important antioxidant enzymes, which were consistent with the other studies (Table 4). During kidney injury, superoxide radicals are generated at the site of damage. That modulates SOD and CAT. This ensuing in the loss of activity and accumulation of superoxide radicals, eventually leads to kidney damage.[26] MDA is an indicator of the degree of lipid peroxidation.[27] Significant increase in MDA levels was observed in plasma cell, kidney and liver tissue of rats exposed with hypoxic condition (HA-II and HA-III) compared to control (Table 4). This free radical mediated chain reaction causes damage the cell membranes.[27]

DNA fragmentation analyses were performed for additional confirmation of hypobaric induced renal injury. Hypobaric induced random fragmentation of genomic DNA along with subsequent formation of a DNA smear on agarose gel without ladder formation. DNA fragmentation assay suggested that hypobaric hypoxic environment induced renal cell damage through necrotic pathway with apoptosis (Figure 1). On the other hand, the hypobaric-hypoxic induced renal injure is reliable with acute tubular necrosis. In this study, the results of histopathological examination showed clear evidence of nephrotoxicity. Necrosis of kidney tissue is a key marker for detection of histopathochemical changes. Kidney histology of control group shows a normal morphology of renal parenchyma with normal tubular (NT) brush-borders and intact normal glomerulus (NG) and surrounding Bowman's capsule (Figure 2). Furthermore, the lower hypoxic exposure (HA-I and HA-II) does significantly alter the histopathology of kidney. However in the group HA-III, disorganized glomerulus (DG) was clearly seen due to glomerular compressing and partial endothelial damage in capsule. Proximal tubules were dilated with loss of cellular boundary and epithelial degeneration.

Fecal analysis showed that the total aerobes of fecal samples decreased (-GDI) in significant level during hypobaric hypoxic exposure

[Downloaded free from http://www.sjkdt.org on Monday, November 2, 2020, IP: 202.142.96.89]

of HA-II and HA-III group. Total anaerobes increased (+GDI) after seven days of HA exposure. It in a higher anaerobic state of intestinal epithelia and alterations of GI mucosal microenvironment were the major limiting factors for such group.[12] It was well known that the *E. coli* population was generally 10^2 times higher than total aerobes in feces. Total aerobes, facultative anaerobes (*E. coli*) and total anaerobes present in the ratio of $4.36:1:4.03 \times 10^5$. However, this may differ within species and even between individuals in the same species.[28] At lower level of oxygen, this ratio was changed to $1:2.94 \times 10^4:2.16 \times 10^7$. *E. coli* cell proliferation (10^6) was higher as it possessed elaborate genetic regulatory network for sensing oxygen.[19] Researcher revealed that 6 h immobilization stress initiates the increase of the concentration of *E. coli* in the proximal sections (the duodenum and the jejunum) of the digestive tract.[29] This rapid expansion of *E. coli* population may encourage the growth of other strict anaerobes like *Bacteroidetes* sp. *Bifidobacterium* sp. and *Lactobacillus* sp. and pathogenic *Salmonella* sp.[30] it does not eventually decrease that the growth of *Bacteroidetes* sp. and lactic acid bacteria were lower than other anaerobes.

Conclusion

It may be said that the lower pressure of atmospheric oxygen at high altitude (hypoxia) reduced the blood PO_2 that alter the physiological buffering system, as a result nephrotoxicity or acute renal failure occur. It also affects the gut microbial ecology.

Grants and Funding

Department of Microbiology and Department of Physiology, Raja N. L. Khan Women's College, Midnapore, West Bengal, India.

Conflicts of interest: None declared.

Date of manuscript receipt: *29 July 2017*.
Date of revised copy receipt: *12 October 2017*.
Date of final acceptance: *12 December 2017*.

References

1. de Paula P, Niebauer J. Effects of high altitude training on exercise capacity: Fact or myth. Sleep Breath 2012;16:233-9.
2. Wang SX, Wu WC. Effects of psychological stress on small intestinal motility and bacteria and mucosa in mice. World J Gastroenterol 2005;11:2016-21.
3. Eckardt KU, Bernhardt WM, Weidemann A, et al. Role of hypoxia in the pathogenesis of renal disease. Kidney Int Suppl 2005;99:S46-51.
4. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N Engl J Med 1982;307:652-9.
5. Pierson DJ. Respiratory considerations in the patient with renal failure. Respir Care 2006;51: 413-22.
6. Meinders AJ, Bosch FH, Meinders AE. Travelling to high altitudes: Do not increase fluid intake. Ned Tijdschr Geneeskd 2011;155: A3526.
7. Painschab MS, Malpartida GE, Dávila-Roman VG, et al. Association between serum concentrations of hypoxia inducible factor responsive proteins and excessive erythrocytosis in high altitude Peru. High Alt Med Biol 2015;16:26-33.
8. Hackett PH, Roach RC. High-altitude illness. N Engl J Med 2001;345:107-14.
9. Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. Nat Rev Gastroenterol Hepatol 2009;6:306-14.
10. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006; 124:837-48.
11. Olert ED, Cross BM, McWilliam AA. Guide to Care and Use of Experimental Animals. 2nd ed. In: Olert ED, McWilliam BM, editors. Ottawa: Canadian Council on Animal Care; 1993. p. 1-90.
12. Maity C, Adak A, Pathak TK, Pati BR, Chandra Mondal K. Study of the cultivable microflora of the large intestine of the rat under varied environmental hyperbaric pressures. J Microbiol Immunol Infect 2012;45:281-6.
13. Anupama V, Narmadha R, Gopal Krishnan VK,

[Downloaded free from http://www.sjkdt.org on Monday, November 2, 2020, IP: 202.142.96.89]

Devaki K. Enzymzymatic alteration in the vital organs of streptozotocin diabatic rats treated with aqueous extract of *Erythrina veriegata* bark. Int J Pharm Pharm Sci 2012;4:134–47.
14. Burtis CA, Ashwood ER, Tietz A. Textbook of Clinial Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999.
15. Das K, Samanta TT, Samanta P, Nandi DK. Effect of extract of Withania somnifera on dehydration-induced oxidative stress-related uremia in male rats. Saudi J Kidney Dis Transpl 2010;21:75-80.
16. Mandal A, Paul T, Roy S, Mandal S, Pradhan S, Mandal KC, Nandi D. Effect of newly isolated *Lactobacillus ingluviei* ADK10 from the intestinal tract of chicken. Indian J of Exp Biol 2013;51:174-80.
17. Roy S, Das K, Mandal S, et al. *Asparagus racemosus* Roots Ameliorates Acetaminophen Induced Hepatotoxicity in Rats: An Experimental, Biochemical and Histological Study. Int J Recent Sci Res 2014;5(6):1192-7.
18. Adak A, Maity C, Ghosh K, Pati BR, Mondal KC. Dynamics of predominant microbiota in the human gastrointestinal tract and change in luminal enzymes and immunoglobulin profile during highaltitude adaptation. Folia Microbiol 2013; 58:523-28.
19. Maity C, Rana S, Pati BR, Mondal KC. Effect of graded hyperbaric atmospheric pressure on the quantity and composition of faecal flora. Microb Ecol Health Dis 2009;21:104-8.
20. Benso A, Broglio F, Aimaretti G, et al. Endocrine and metabolic responses to extreme altitude and physical exercise in climbers. Eur J Endocrinol 2007;157:733-40.
21. Wall R, Ross RP, Shanahan F, et al. Metabolic activity of the enteric microbiota influences the fatty acid composition of murine and porcine liver and adipose tissues. Am J Clin Nutr 2009;89:1393-401.
22. Wickler SJ, Anderson TP. Hematological changes and athletic performance in horses in response to high altitude (3,800 m). Am J Physiol Regul Integr Comp Physiol 2000;279: R1176-81.
23. Pathan MM, Khan MA, Moregaonkar SD, Somkuwar AP, Gaikwad NZ. Amelioration of paracetamol induced nephrotoxicity by Maytenus emarginata in male rats. Int J Pharm Sci 2013;5:471-4.
24. Vanholder R, Smet de R. Pathophysiologic effect of uremic retention solutes. J Am Soc Nephrol 1999;10:1815-23.
25. Pande M, Flora SJ. Lead induced oxidative damage and its response to combined administration of alpha-lipoic acid and succimers in rats. Toxicology 2002;177:187-96.
26. Abraham P. Vitamin C may be beneficial in the prevention of paracetamol-induced renal damage. Clin Exp Nephrol 2005;9:24-30.
27. Zhang Y, Zhang L, Du M, et al. Antimicrobial activity against Shigella sonnei and probiotic properties of wild lactobacilli from fermented food. Microbiol Res 2011;167:27-31.
28. Holý O, Chmelař D. Oxygen tolerance in anaerobic pathogenic bacteria. Folia Microbiol (Praha) 2012;57:443-6.
29. Gritsenko VA, Brudastov IA, Zhurlov OS, Chertkobv KL. The properties of Escherichia isolated from the bodies of mice in bacterial translocation after immobilization stress. Zh Mikrobiol Epidemiol Immunobiol 2000;1:37-41.
30. Gombošová L, Lazúrová I, Zakuciová M, et al. Genes of intestinal Escherichia coli and their relation to the inflammatory activity in patients with ulcerative colitis and Crohn's disease. Folia Microbiol (Praha) 2011;56:367-72.