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Analysis of alteration of gut microbial population under the exposure of graded hyperbaric pressures: Application of metagenomic approach

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Gastroenterological disorders are very common at hyperbaric conditions. The present study was conducted to find out the impact of gut flora on the gastrointestinal disorders created at such environmental circumstances. For this, male albino rat were exposed to graded hyperbaric pressures (915 and 1277 mmHg) and large intestinal content was examined for microbial composition using culture based and PCR-DGGE tools. After 30 day exposure, total aerobes (38.54 and 375.57 folds, 1.35 and 1.58 gdi) and *E. coli* (126.05 and 873.23 folds, 1.31 and 1.44 gdi) were increased whereas total anaerobes (7.01×10^4 and 8.84×10^3 folds, -1.56 and -1.39 gdi), *Enterobacter spp.* (-2.45 and -1.00 gdi) and *Clostridium perfringens* (12.88 and 54.16 folds, -1.38 and -1.75 gdi) were decreased significantly in respect to control after exposure of simulated hyperbaric pressures like at 915 and 1277 mmHg, respectively. Metagenomics study revealed an overall reduction in total microbial profile was noted than control at higher level hyperbaric pressure, i.e., 1277 mmHg air pressure for highest duration of exposure. Though, some new bands also appeared which indicated the expansion of dormant or new microbiota, Variation in the numbers of these newly dominated bacteria was correlated to dose and duration of hyperbaric treatment. The histological results clearly indicated that hyperbaric environment induced severe inflammation in the mucosal and submucosal layer of large intestine. Thus, the result suggest that hyperbaric pressure is an important exogenous factor that strongly modulated the intestinal morphology and microbial ecology, and induced several gastrointestinal ailments during hyperbarism.

Keywords: Hyperbaric pressure, Intestinal microflora, Metagenomics, PCR-DGGE

The gastrointestinal (GI) tract of human and other mammals are colonized by myriads of microbes in a very intricate manner[1,2]. Bacteria are the predominant inhabitant in the alimentary tract and they are called as indigenous microflora or popularly designated as gut microflora[3]. The nature of interaction between host and microbes is mainly symbiotic and dynamic, which have profound advantageous effect on human health[4-6]. The symbionts are metabolically very active and their collective activity is like a virtual organ[7]. They performs several beneficial role to the host, like breakdown of undigested food, metabolism of drugs, synthesis of vitamins, prevent the establishment of pathogens, induce host immunity, stimulate intestinal maturation, etc[8-10]. Recent research revealed an intricate relationship between gut flora and central nervous system (CNS), which eventually control the overall functioning of the host[11-13]. Their colonization on the gastrointestinal epithelial lining is disturbed by numerous host induced[14,15] and exogenous factors like food habits, antimicrobial agents[16], peristalsis disorders, life style stress, drugs and steroids, contaminants, geographical location, etc[17-19].

Hyperbaric atmosphere posses extreme stresses and is characterized by an elevated level of atmospheric pressure, CO poisoning, raised temperature, etc. During deep sea driving, digging tunnels beneath the river or mine, passengers in submarine, etc., individuals are exposed to hyperbaric atmosphere [an increase of 2.0 kilopascal (kPa) air pressure per 500 ft drop below sea level; with a sea level pressure of 101.3 kPa][8,20]. This induces several physiological problems like autism, cerebral palsy, gas embolism, multiple sclerosis, etc. Besides, some gastric disorders are also developed by the symptoms of indigestion, acid and gas (flatus) formation, diarrhoea, acute or chronic inflammatory bowel disease, psoriasis, etc[21-24]. The GI complication are mostly associated with the ecological disturbances of GI microflora as they contribute in nearly all aspects of gastrointestinal deeds[8,9]. But, there is still no detailed record that can

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correlate between the variations in composition of indigenous microflora with atmospheric pressure.

In the present study, the population of cultivation independent microbes has been investigated along with the quantitative variation of some common bacteria of large intestine like total aerobes and prominent anaerobes, some indicator strain like *Escherichia coli*, *Enterobacter spp.* (other than *E. coli*), *Bifidobacterium spp., Lactobacillus spp.* and *Clostridium perfringens* have been studied during exposure of graded hyperbaric atmospheric pressure for different duration on to the experimental model animal. Besides, the patho-physiological changes in the intestinal surfaces have also been examined at these changed environmental conditions.

Materials and Methods

Ethical clearance—This study was conducted with ethical approval from the Vidyasagar University Ethics Committee. Animals were reared and fed according to suggested rules of the committee.

Animals and diet—Healthy male albino rats with an average body weight of 115±7 g used in the present study were housed in metal-made cages (34 × 28 × 19 cm^3). All animals had access to autoclaved rat feed (containing carbohydrates, 74.05%; proteins, 10.38%; fibre, 2.20%; iron, 56 ppm; calcium, 400 ppm and sodium, 500 ppm) and sterile water *ad libitum*. The animals were kept in 12 h day/night cycle and maintained without interrupting their normal activity.

Sample size and experimental set up—A set of 30 healthy rats was subjected to two different simulated hyperbaromatric pressures (915 and 1277 mmHg) for 30 days at the rate of 6 h duration daily. Each set contained 15 rats and after every 10 days interval, 5 rats of both control and pressure treated group were sacrificed. Control animals (set of 15 rats) were also maintained side by side with adequate supplementation of food and water. After scarifice, particular portions of large intestine were dissected aseptically. Intestinal segments were suspended in sterilized phosphate-buffered saline (PBS; *p*H 7.0 and 9 g l^{-1} NaCl) and homogenized thoroughly using glass homogenizer for 5 min. The content was then centrifuged (1000 g for 5 min) and the clear supernatant was used for microbial analysis.

Analytical measurements and Growth Direction Index (GDI)—The quantities of prominent cultivable indicator groups of large intestinal bacteria were enumerated (on the basis of Colony Forming Unit) by using selective media following the standard protocol of Hi-Media Manual (www.himedialabs.com). Serial decimal dilutions with peptone water (for aerobic) and anaerobic dilution solution (anaerobic diluents with degassed distilled water) were made, and 0.1 mL of the diluted colonic digestal homogenate was poured on different culture media per duplicate. The total aerobic and anaerobic bacteria were enumerated by standard pour-plate technique in single strength trypticase soya agar (TSA, Hi-Media, India) and pre-reduced Wilkins Chalgren agar (WCA) with GN spore anaerobic supplement and non spore anaerobic supplement (alternative) (Hi-Media, India), respectively. For anaerobic culturing, inside oxygen-containing air was replaced catalytically with 80% N_2, 10% of both CO_2 and H_2 gas (v/v). Enumeration of *E. coli* and *Enterobacter spp.* (other than *E. coli*) was carried out using selective media like MacConkey and Levine EMB agar respectively after 24 h aerobic incubation at 37 °C. *Bifidobacterium spp.*, *Lactobacillus spp.* and *Clostridium perfringens* were enumerated by 24-72 h anaerobic incubation at 37 °C using selective media like bifidobacterium agar, Rogosa SL agar and pre-reduced perfringens agar base (with perfringens supplement - I and II) (Hi-Media, India) respectively[8].

The microbial population was expressed in colony forming unit (cfu) and the growth direction index (gdi) was calculated using the formulae previously described by Maity *et al*.[24].

Medium standardization—Medium selectivity was tested by standard reference strains like *E. coli* ATCC 35401 (for MacConkey agar medium), *Enterobacter cloacae* ATCC 35549 (for EMB agar), *Bifidobacterium bifidum* ATCC 15696, *Bifidobacterium breve* ATCC 15701, *Bifidobacterium adolescentis* ATCC 15703 (for bifidobacterium agar), *Clostridium perfringens* ATCC 12918 (for Perfringens agar base), *Lactobacillus acidophilus* ATCC 832, *Lactobacillus sp*. ATCC 11146 (for Rogosa SL agar).

Extraction of bacterial DNA from large intestinal digesta—Colonic digestal samples (1.0 g) were mixed with 2 mL of chilled Tris-EDTA buffer [contained glucose, 50 mM; Tris – Cl, 25 mM (*p*H 8.0) and EDTA, 10 mM]. DNA was extracted using the Bioline genomic DNA isolation Kit (Bioline, India) following user's instructions and stored in 30 µL autoclaved water at -20 °C until use. DNA Purity was checked by agarose gel electrophoresis following

$A_{260/280}$ ratio and finally the DNA concentration was adjusted to 50 - 70 mg mL^{-1}.

PCR amplification of V3 region of 16S rDNA, DGGE and its analysis—The flanked conserved sequences of the variable V3 region of the 16S r DNA were amplified by GC-clamped universal primer and used for DGGE analysis (341F, 5′CCTACGGGAGGCAGCAGCCG3′ and 907R, 5′TCAATTCMTTTGAGTTT3′ and GC clamp, CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGG CACGGGGGG 3′ end of the forward primer). The reactions programme was described previously by Muyzer *et al*[25]. Amplicon of approximately 200 bp was further resolved in 6% acrylamide gels with a denaturing gradient of 30–55% [with the gradient of 100% urea and 40% (v/v) formamide increasing in the direction of electrophoresis] using DCode, Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). The electrophoresis was conducted at 150 V at 60 °C for about 4 h. Gels were stained with ethidium bromide and viewed under UV transillumination (Bio-Rad, Hercules, CA, USA). Gel image was captured in Bio-print Mega–100/20.M (Vilber Lourmat, German) and the relative analysis was made by Bio-1D software (Vilber Lourmat, German). Cluster analysis was performed by unweighted pair group method with arithmetic mean (UPGMA) algorithm based on the Jaccard (Tanimoto) correlation coefficient[26].

Histology of large intestinal segments—Large intestinal segments were initially washed by cold formol solution and dipped in Carnoys fixative for 60 min. Specimens were dehydrated in progressive ethanol gradient, sliced into thin tissue sections (3-5 µm), stained by Hematoxylin and Eeosine (H&E) stain using Mayer's progressive method[27]. Stained tissue sections were observed under the polarized microscope (Olympus BX51-P). The number of villus, villus length, number of crypt cells, crypt cell diameter, etc in a particular area was counted according to standard protocol[28].

Scanning electron microscopy—Large intestinal parts were cleaned using brief formol saline flushing and immediately dipped into the Carnoy's fixative for 10 min. Then tissue section was dipped into the para-glutaraldehyde (2.5%, v/v) for 30 min. Next, the fixed tissue was dehydrated with progressive alcohol gradient (in ascending order i.e., in 30, 50, 70, 90% v/v and in absolute each for 5 min). The SEM analysis was done after gold coating of dehydrated intestinal segments using a sputter coater (Edward S 150, UK). Further, the coated tissue sections were examined under scanning electron microscope (FEI Quanta - 200 MK2) at room temperature. The acceleration voltage was 6 kV with the secondary electron image as a detector[29].

Statistical analysis—Collected data are presented as the arithmetic mean of five replicates (mean±SE). The variations in microbial count were examined by one-way ANOVA (Kruskal–Wallis). Significant variation between mean was accepted at the level of 5% and 1% (i.e. $P<0.05$ and <0.001) and was measured using Sigmastat 11.0 (USA) statistical software.

Results

Alteration of microflora in large intestine at graded hyperbaric pressures—After exposure to graded hyperbaric pressures onto the experimental model animal, it was found that total aerobes and *E. coli* were increased, whereas, total anaerobes and *Clostridium perfringens* were decreased significantly in duration dependent manner. Quantity of total aerobic bacteria was increased upto 38.54 folds [1.01×10^6 cfu g^{-1} of intestinal content at 915 mmHg] and 375.57 folds [9.84×10^6 cfu g^{-1} of intestinal content at 1277 mmHg] in respect to their control (2.62×10^4 cfu g^{-1} of intestinal content at 760 mmHg) after 30 days exposure ($P<0.05$) (Fig. 1a). The growth direction index of selected microbes indicated that they had occupied and expanded in a conservative manner in the large intestinal micro-ecosystem at varied pressures (1.47, 1.65, 1.35 at 915 mmHg and 1.40, 1.56, 1.58 at 1277 mmHg at day 10, 20 and 30 respectively) (Fig. 2a and b).

Anaerobes are the most dominant organism in large intestine. After exposure at 915 and 1277 mmHg for 30 days, the count of anaerobes was reduced up to 7.01×10^4 folds (1.07×10^9 cfu g^{-1}) and 8.84×10^3 folds (8.49×10^9 cfu g^{-1}) respectively in respect to their control counts (7.51×10^{13} cfu g^{-1} wet weight) (Fig. 1b). During graded hyperbaric pressure exposure, the diminution of total anaerobic populations were also reflected by its negative direction of gdi (-1.07, -1.11, -1.56 and -1.10, -1.24, -1.39 at 915 and 1277 mmHg after 10, 20 and 30 days respectively) (Fig. 2a and b). The count of *E. coli* was increased concomitantly with the enhancement of magnitude of air pressures. Approximately, 126.05 and 873.23 times increment was recorded after 915

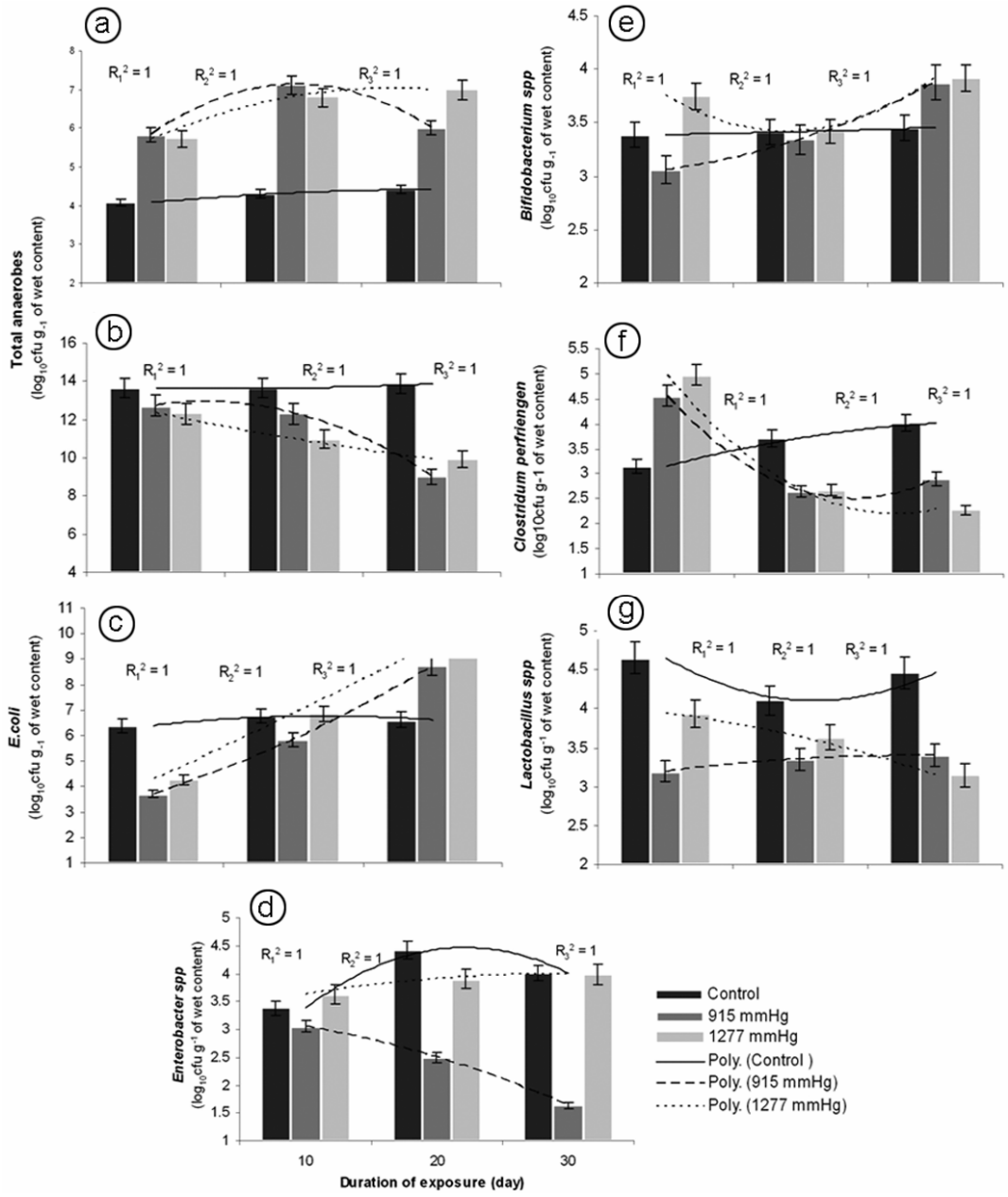


Fig. 1—Alteration in the population density of total aerobes (a), total anaerobes (b), *E. coli* (c), *Enterobacter spp.* (d), *Bifidobacterium spp.* (e), *Clostridium perfringens* (f) and *Lactobacillus spp.* (g) in the large intestine of rats during the whole animal exposure at 915 and 1277 mmHg air pressure for different day duration (10, 20 and 30 days).

and 1277 mmHg air pressure respectively from the normal counts (P<0.05) (Fig. 1c). *E. coli* had the gdi of -1.72, -1.15, 1.31 and -1.49, 1.01, 1.44 after 10, 20 and 30 days of exposure at 915 and 1277 mmHg air pressure (Fig. 2a and b). *Enterobacter spp.* (other than *E. coli*) was reduced significantly from their normal count (P<0.05) (Fig. 1d) with gdi of -1.10, -1.77, -2.45 and 1.07, -1.13, -1.00 (Fig. 2a and b). The count of *Bifidobacterium spp.* was not significantly altered with the enhancement of air pressure or in the duration of exposures (Fig. 1e). The gdi was changed as -1.10, -1.02, 1.12 and 1.10, 1.00, 1.13 after 10, 20 and 30 days at 915 and 1277 mmHg (Fig. 2). The count of *Lactobacillus spp.* was decreased (Fig. 1g) from their normal count (P<0.05). The gdi was as -1.45, -1.22, -1.31 and -1.18, -1.13, -1.41 respectively at 915 and 1277 mmHg after 10, 20 and 30 days (Fig. 2a and b).

Clostridium perfringens was not completely eliminated during *in vivo* study, it was only decreased

by 12.88 and 54.16 folds after 30 days of hyperbaric exposure respectively at 915 and 1277 mmHg (Fig. 1f). Growth direction index of *C. perfringens* was as follows 1.44, -1.4, -1.38 and 1.57, -1.39, -1.75 after 10, 20 and 30 days at 915 and 1277 mmHg air pressure (Fig. 2).

Diversity of PCR amplicons: DGGE analysis—During *in vivo* hyperbaric treatment, microbial profile in large intestinal samples was remarkably changed than its respective control. The hyperbaric pressure treated large intestinal samples yielded only 66 and 59 amplicon respectively at 915 and 1277 mmHg pressures. Considerable similarity level was shared among the control microbial profile (1.0% UPGMA). Comparatively, a higher degree of divergence was found among the 915 mmHg-10th day, -20th day and -30th day samples (with 64, 67 and 55% similarity respectively). The maximum dissimilarity was observed between the collected samples of 915 mmHg-30th day and 1277 mmHg-30th day (Fig. 3). The result inferred a reduction in total microbial profile at higher level hyperbaric pressure, i.e., 1277 mmHg air pressure for highest duration of exposure, i.e., 30 days (Fig. 3).

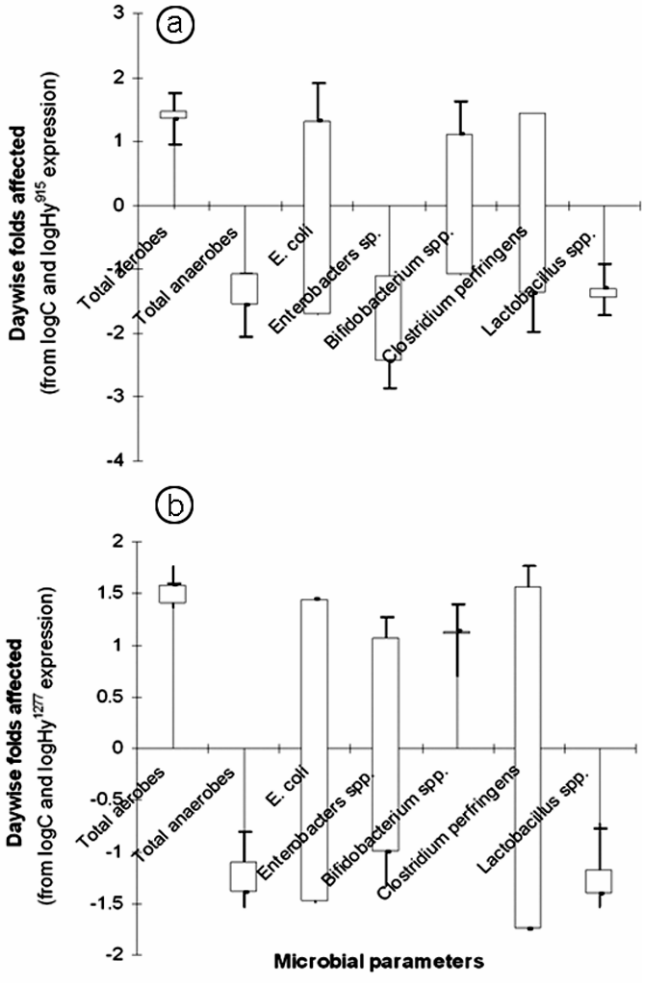


Fig. 2—Changes of growth direction index (gdi) of different bacteria in large intestinal contents during whole animal exposure of 915 mmHg (a) and 1277 mmHg (b) air pressure for different time duration (10, 20 and 30 days). The pattern of expression towards positive site (along with Y-axis) indicating growth expansion and towards negative direction indicating growth contraction.

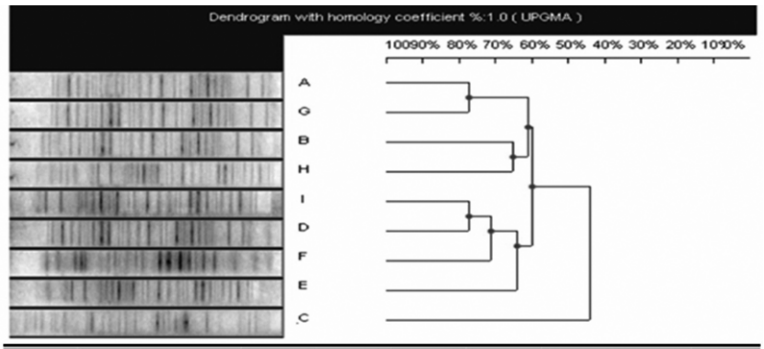
Histological analysis of large intestine—During hyperbaric pressure exposures, the villus height of large intestine of rat was decreased up to 1.23 and 1.39 folds at 915 and 1277 mmHg atmospheric pressure respectively in respect to control (220.56 µm) after 30 days. The crypt number of both small and large intestine was reduced massively than the normobaric groups.

Crypt depth in large intestine was decreased upto 1.43 and 2.15 folds. In contrast, lamina propia was widened up to 1.70, 2.51 folds than the control group (4.22 µm) respectively after 915 and 1277 mmHg air pressure treatment. Mucosal length of large intestine was shortened up to 1.15 and 1.36 folds than control groups after 30 days at 915 and 1277 mmHg air pressure treatments. Whereas, sub-mucosal length was increased up to 1.91 and 2.11 folds after 30 days of hyperbaric treatment respectively at 915 and 1277 mmHg (Table 1 and Fig. 4).

Scanning electron microscopy of epithelial surfaces—An arranged layer of microbial mat (biofilm) compiled with the broken food materials was found on the epithelial brush border in large intestine of control rat groups. The most striking observation that many injuries with atrophic villi and dominant inflammatory holes on mucosal surface were observed was noted after 1277 mmHg air pressure treatment (Fig. 5).

Discussion

Human at hyperbaric condition (depth of 15 m from the sea level) can no longer breathe normal air due to the possibility of oxygen toxicity. Other problems such as bone necrosis, inert gas narcosis, high pressure nervous syndrome, carbon dioxide poisoning, thermal problems and chronic ear infections increase the risk of living in ambient pressure conditions[30]. Besides these physiological problems, several gastrointestinal problems are also known to be happen. In hyperbaric condition, the changes in gastrointestinal microbiota are owing to



	Lane A	Lane B	Lane C	Lane D	Lane E	Lane F	Lane G	Lane H	Lane I
Lane A	100								
Lane B	63	100							
Lane C	61	73	100						
Lane D	64	66	77	100					
Lane E	61	60	64	64	100				
Lane F	56	55	71	60	67	100			
Lane G	77	55	66	66	63	55	100		
Lane H	60	65	63	60	48	56	68	100	
Lane I	44	54	56	64	56	42	50	52	100

Fig. 3—DGGE profiles of V3-V5 amplicons of the microflora in the large intestinal samples from weaned rats after the *in vivo* treatment of different hyperbaric pressures (915 and 1277 mmHg) for 10, 20 and 30 days exposures. The combined table contains the similarity indices of the DGGE profiles. A group of 5 samples from each treatment group were pooled into a single sample. Lane A, B and C = colonic digesta at normobaric pressure (760 mmHg) 10, 20, 30 days; Lane D, E and F = digesta at 915 mmHg hyperbaric pressure after 10, 20 and 30 days; Lane G, H and I = digesta at 1277 mmHg hyperbaric pressures after 10, 20 and 30 days.

Table 1—Changes in histological parameters of small and large intestine of rat under hyperbaric pressures after 30 days exposure [Values are mean ± SE]

Pressure groups	Villus length (µm)	No of Crypt	Crypt depth (µm)	Wide of lamina propia (µm)	Length of mucosa (µm)	Sub mucosa (µm)
Control	220.56±23.8	26±2	12.65±3.8	4.22±0.75	293.95±27.3	8.20±1.45
915 mmHg	179.01±23.8	17±4	8.79±2.7	7.21±1.49	254.83±28.7	15.63±3.69
1277 mmHg	158.82±27.3	11±2	5.86±2.1	10.62±1.95	215.65±32.5	17.34±4.15

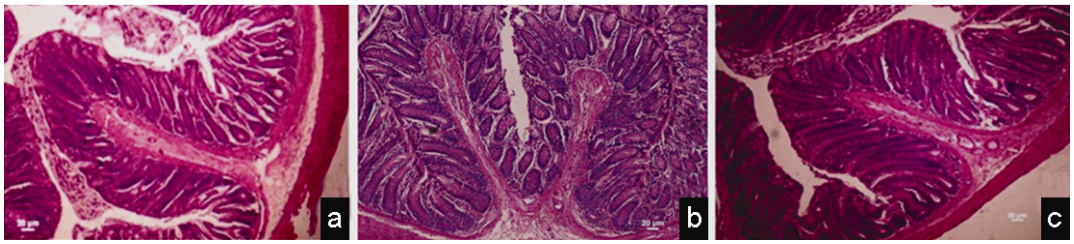


Fig. 4—Histological changes of large intestine of rat exposed to simulated hyperbaric [915 (b) and 1277 (c) mmHg] pressures and compared with the control (normobaric pressure, a). Polarized microscopic view of H and E stained tissue slices.

altered blood PO_2, intestinal motility, oral infusion of air at high pressure, endocrinal response, etc[31]. In the present study, animals were exposed to two different simulated hyperbaric environments for different time durations and the quantity of some cultivable indicator intestinal flora were estimated that can give some clue about the gastrointestinal disorders in this extreme environment. It has been well established that the large intestine of rat is normally harboured by total aerobes, facultative anaerobes (*E. coli* and *Enterobacter spp.*) and total anaerobes by a ratio of $1:2.01\times10^6:2.86\times10^9$ with some fluctuation over the experimental duration. This ratio was changed to $1:1.35\times10^6:8.50\times10^2$ after 30 days of exposure to 1277 mmHg air pressure. The results clearly showed that the population of facultative anaerobes were not remarkably changed, whereas, total anaerobes population was reduced by about 9.03×10^3 folds ($P<0.05$).

Expansion of *E. coli* was very sound due to its complex adaptation character in broad and extreme environment as reflected in literature. The lipid bilayer may play crucial role in its homeoviscous adaptive behaviour. Lipids in biological membranes

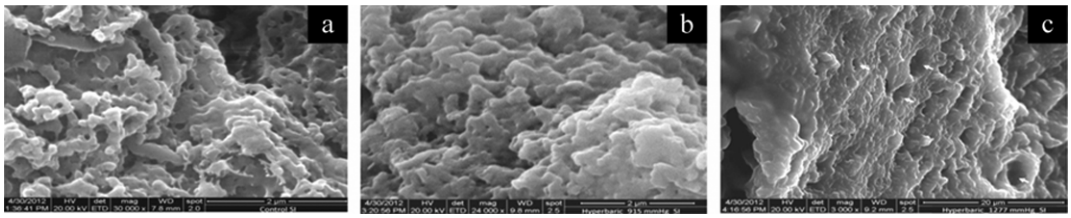


Fig. 5—Scanning electron micrograph of large intestinal surfaces of rat, showing microbes adhesion to the mucosal layer at normobaric (760 mmHg) (a), shrinkage and atrophy in mucosal arranged structures at 915 mmHg (b) and inflammatory sores in regular microbial mat at 1277 mmHg (c).

are in the fluid (liquid crystalline) phase, which allows fast lateral movement of molecules[32]. As pressure upshift or temperature downshift, phospholipid bilayers induces a sharp phase transition, the liquid crystalline phase turns into the gel phase due to removal of water[33]. Comparatively, the population of *Enterobacter spp.* showed a different trend, i.e., increased at lower hyperbaric pressure (915 mmHg) and vice versa at higher hyperbaric pressure (1277 mmHg). This complex paradoxical response is the output of co-signalling response among other community member of gut microbiome. Possibly, the main counter competitor is *E. coli*, predominant member of *Enterobacteriaceae* which express outstanding adaptive behaviour at altered redox environment. The amplified population of *E. coli* may trim down the other members of *Enterobacteriaceae* for nutrient competition. However, the overall growth response of *Enterobacter spp.* was negative which was imitated by the results of growth direction index.

The growth of total aerobes was increased after both the hyperbaric treatment viz. at 915 and 1277 mmHg air pressure. The specific clue in this regard is unknown. This sort of alteration of total aerobes was earlier reported by Prapaiwong *et al.*[34] during high pressure storage of raw oysters. They found that the total aerobic bacterial count (TABC) in high pressure (HP) treated oysters reached 10^8 cfu/g at 14 days of storage.

The proliferation of *Clostridium perfringens* was arrested remarkably during hyperbaric exposure. Some reports indicated that at hyperbaric hyperoxia, their antibacterial and anti-toxin production ability is particularly blocked[35,36]. Population of *Lactobacillus spp.* was reduced 1.31 and 1.16 ($\log_{10}$) folds respectively at 915 and 1277 mmHg air pressure after 30 days exposure than their control level ($\log_{10}$4.46). Their ATP generating system F_0F_1 ATPase is very sensitive to air pressure as reported by Wouters *et al.*[37]. The inactivation of membrane-bound enzymes is also reported to sensitive air pressure-mediated sublethal injury.

Elucidation of the complex microbial diversity by molecular techniques has been executed recently to reveal important interrelationship among microbes with their hosts, physiological and environmental parameters. A unique gut microbiota composition in healthy rat has been reported previously[38]. Dice cluster analysis of DGGE profiles reflected the predominant bacteria in the large intestinal digesta and therefore, expected to detect major changes induced by the different atomospheric pressures. Though, some new bands were appeared after hyperbaric pressure treatment, an overall reduction in total microbial profile was noted at higher level hyperbaric pressure, i.e., 1277 mmHg air pressure for highest duration of exposure, i.e., 30 days. In this environment, the higher pO_2 saturation and reactive oxygen species (ROS) may be responsible for the elimination of the most anaerobic populations and ultimately loss of species diversity in the specified sample.

Histological studies of large intestine revealed the decrease in villus height, crypt depth and mucosal length with the widened lamina propia and increased length of sub-mucosa. Hyperbaric pressurization favours the excess oxygenation at tissue level. This clearly demonstrated that variation of atmospheric pressure greatly affected the structural organization of mucosal barrier of the intestine, which is actually the 'sleeping bed' of vast number of microflora. The severe inflammation in submucosal layer may facilitate the necrosis and atrophy of mucosal layer[39]. There is also evidence that lipo-polysaccharides (LPS) of Gram negative bacteria at these situations, can induce the proinflammatory and anti-inflammatory responses, which may be the cause of activation and infiltration of lymphocytes in submucosa and lamina propria[29,39,40]. The trend of histological damages of intestinal epithelia can be

significantly correlated with the alteration of gastrointestinal microflora during hyperbaric stresses (Fig. 4).

Scanning electron micrograph of the large intestinal surface revealed many injuries on mucosal surface with atrophic villi and dominant inflammatory holes (Fig. 5). This observation indicated that alteration of atmospheric pressure causes severe damage on the mucosal barrier on intestine which ultimately resulted in disintegration in the host-microbes relationship. Several clues may be took forward for this kind of pathological damages on intestinal mucosal layers like severe inflammatory responses, acidosis or alkalosis and overgrowth of some pathogenic microbes in these extreme environment. The overgrowth of gram-negative bacteria, the lipo-polysachcharide (LPS) of their membrane may induce the local inflammation and may be the cause of impairment of gut barrier functions[29]. This sort of alteration of barrier function can facilitate the translocation of autochthonous microbes into the systematics.

Conclusion

The present study strengthens the hypothesis that the environmental pressure could play significant role in alteration of microbial population and initiate intestinal inflammation that fracture the microbial patrimony inside the gut. Hence, the extreme environmental conditions can be considered as homeostasis breaker that commences the overall 'dysbiosis' in the host's physiology.

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