Antarctic Harsh Environment as Natural Stress Model: Impact on Salivary Immunoglobulins, Transforming Growth Factor- β and Cortisol Level

K. P. Mishra · A. P. Yadav · Lilly Ganju

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Abstract The Antarctic continent on the planet Earth is full of environmental extremes. It is considered as natural stress model. Therefore, the present study examined the effect of harsh environment on the certain salivary markers of 28th Indian Antarctic expeditioners. Thirty healthy men and women (median age 36 year; range 22–61 year) participated in this study. Parameters measured were salivary IgA (SIgA), IgM (SIgM), TGF- β and cortisol level at three different time points: (I) before leaving India on 26th October 2008 for base line level; (II) after 1 month on-board journey on 31st January 2009 in Southern Ocean and (III) after 1 month staying at Maitri, Antarctica on 3rd March 2009. Our observation indicated that Ship borne journey and Antarctic environment increased the SIgA levels while that of SIgM level was not altered on-board but decreased by staying for 1 month at Antarctica. No significant alteration was found in the TGF- β and cortisol level at any point of time. The present study concluded that ship borne journey and Antarctic environment may induce the SIgA level while SIgM level decreased in environmental extremes of Antarctica.

Keywords Cytokine · Immunoglobulins · Antarctica

Introduction

The oral immune system is part of an extensive and specialized compartment of mucosa associated lymphoid

tissues. Many of the stressors present in the Antarctic environment are known to affect the mucosal immune system [1, 2]. Saliva has tended to be the mucosal secretion of choice for most studies of the human mucosal immune system, due to ease of collection. Secretory IgA (sIgA) antibodies play the major role in effective specific immunity [3]. Immunoglobulin A (IgA) is the predominant antibody present in mucosal fluids and can be measured noninvasively in saliva. The salivary IgA protect the oral and nasal cavities from the pathogenic microorganisms [4]. Secretory IgM (sIgM) antibodies contribute to a lesser extent in the normal adult, but play a significant role in mucosal defence in the neonate and in IgA-deficiency states [5]. IgG plasma cells are in low numbers at mucosal sites but serum-derived and locally produced IgG antibodies are important in protection of the respiratory tracts [6, 7]. The sIgA level is thought to be affected by increases in circulating stress hormones such as catecholamines and cortisol. Cortisol has been shown to inhibit transepithelial transport of sIgA in rodents and might inhibit IgA synthesis by B cells in the submucosa [8, 9].

The Antarctic weather, strong winds and brutal storms have been synonymous with Antarctica since its discovery and continue to be a major challenge for modern explorers. So far, very little is known about the effects of Antarctic harsh environment on mucosal immune responses. Gleeson et al. [10] reported the effect of a year's isolation in Antarctica on the human mucosal immune system during the winter of 1992 at three Australian Antarctic stations Casey, Davis and Mawson. Saliva samples were collected from each expeditioner prior to their departure from Australia and during each month in Antarctica. The mean concentrations of IgA were higher at Mawson, and the mean concentrations of IgG were lower at Davis compared with the other stations. Francis et al. [11] were also reported the

K. P. Mishra (✉) · A. P. Yadav · L. Ganju
Immunomodulation Laboratory, Defence Institute of Physiology
and Allied Sciences, Lucknow Road, Timarpur, Delhi 110054,
India
e-mail: kpmptgi@rediffmail.com

monthly salivary immunoglobulins level in six Australian winter expeditions. The report suggests that the changes in salivary IgA and IgM concentrations over the 1-year period revealed a consistent pattern between expeditions. Salivary IgA levels were lower in March, April and May compared to other months of the year. Salivary IgM levels were lowest in the first 4 months of the year, with peak levels in June and July. SIgA levels in saliva decreased as a result of exposure to chronic stress [12].

Cytokines particularly TGF- β 1 profoundly affects mucosal and systemic immunity and inflammation [13]. So far the salivary TGF- β 1 level has not been reported in Antarctic harsh conditions. The present study was designed to study the effect of ship borne journey and Antarctic harsh environment on SIgA, SIgM and TGF-beta level in 28th Indian Antarctic summer expeditioners. Our observation indicated that Ship borne journey and Antarctic environment increased the SIgA levels while that of SIgM level was not altered on-board but decreased by staying for 1 month at Maitri, Antarctica. No significant alteration was found in the TGF- β and cortisol level at any point of time.

Methods

Subject

Thirty active, healthy adults (27 men and 3 women) who were participating in 28th Indian scientific expedition to Antarctica volunteered to take part in the study. Their physical characteristics are outlined in Table 1. Participants were informed of the aims and procedures of the study before providing written informed consent. Institute's ethical committee clearance was obtained for the study (Table 1).

Diet

The food with 28th Indian Antarctic expeditioners was varied and plentiful. The on-board (Ship) and base ration was adequate. It was mainly canned; a meal of frozen meat was available daily throughout the expedition. Potatoes were supplied as dehydrated strips. Vitamin tablets were freely available. The expeditioners have good food quality

in terms of the balanced diet; though the food intake during sea voyage drastically reduced. It was further improved when the expeditioners reached in Maitri base but due to non availability of fresh food the dietary stress was also experienced by the expeditioners.

Environment

The environment in the Antarctic is influenced by its solar altitude and latitude. In the winter, from the beginning of May until the first week in August, the darkness is absolute, precludes traveling and confines the men to the base. In the summer, from mid-October to the middle of February, the sun does not set. During the remaining 5 months there are variable combinations of light and darkness during the 24 h.

The duration of this study spanned only summer Season: (1) January to Mid February on-board in southern ocean and (2) Mid February to Mid March at the Indian research base Maitri with daylight and outside temperatures ranging from -1 to -10 °C. The solar radiation was often so intense that the men, dressed in a moderate thickness of clothing, could work with bare hands in air temperature as low as -10 °C.

Saliva Collection

The Preliminary saliva samples were collected in India in late October of the year preceding the study for the 2009. The second time saliva samples were collected in southern ocean after being 1 month onboard on 31st January 2009 and the third sampling was done at the Maitri station after staying for 1 month on 3rd March 2009. The subjects were asked to not eat immediately prior to providing their saliva sample and to maintain their normal dietary habits over the course of the study period. The samples were collected at the same time of the day to control for diurnal variations. They were measured for volume, immediately frozen and stored at -70 °C until analysis. Routine monthly saliva samples were collected and immediately stored at -70 °C.

Enzyme Linked Immunosorbent Assay (ELISA) for Salivary IgA, and IgM

IgA and IgM were measured by double antibody sandwich ELISA method as per manufacturer's instruction (ICL, Inc, OR, USA). Briefly IgA and IgM were measured in saliva by capture ELISA using human immunoglobulin reference saliva (ICL, Inc, OR, USA), goat anti-human IgA, and IgM conjugated to Horseradish peroxidase were added. Following washing steps, the enzyme bound to the immunosorbent was assayed by the addition of a chromogenic substrate, 3',3',5,5'-tetramethylbenzidine (TMB). After 20 min of incubation at room temperature, stop solution

Table 1 Participant Characteristics

Variable	Subject
No.	30 (27 male, 3 female)
Median age (year) (range)	36 (22–61)
Smokers	4
Alcohol intake (occasionally)	15

(2 N H₂SO₄) was added. The Absorbance was measured at 450 nm in ELISA Reader (Biotek, USA). The quantity of the Immunoglobulins in test samples were interpolated from the standard curve constructed from the standards and correlated for sample dilution.

Enzyme Linked Immunosorbent Assay (ELISA) for TGF- β 1 Estimation

Estimation of TGF- β 1 in saliva samples were carried out as solid phase enzyme immunoassay with ELISA kit (Pierce, USA) based on multiple antibody sandwich principle. The ELISA plates were coated with mouse monoclonal antibody specific for human TGF- β 1 to detect the specific cytokine present in the standard and saliva samples. The anti- TGF- β 1 rabbit polyclonal antibody conjugated to biotin was added to each well respectively followed by streptavidin/HRP incubation for 20 min. At the end color was developed using peroxide/TMB substrate solution. The substrate initiated a peroxidase catalyzed color change, which was stopped within 15 min by acidification with stop solution. Absorbance was measured by ELISA reader (Biotek, USA) at 450 nm.

Estimation of Cortisol Level

Salivary cortisol was estimated as per manufacturer's instruction (Diagnostic Biochem Canada Inc). Briefly, anti-cortisol antibody-coated micro well plates were incubated with 20 μ l of either standard or saliva samples followed by 100 μ l of the Cortisol-Horseradish Peroxidase Conjugate and incubated on a plate shaker at 200 rpm for 45 min at room temperature. Plates were washed thrice with wash buffer. At the end color was developed using peroxide/TMB substrate solution that was stopped within 15 min by acidification with stop solution. Absorbance was measured by ELISA reader (Biotek, USA) at 450 nm.

Statistical Analysis

Values are represented as mean $\pm$ standard error (SE). Comparison between results from on-board personnel, Maitrians and controls were performed using the repeated measures ANOVA. Entire analysis was conducted using SPSS 15 software. The p value of ≤ 0.05 was considered significant.

Results

Secretory Immunoglobulin Levels in Saliva

The effect of ship borne journey and extreme environmental conditions of Antarctica to the mucosal immune

system was investigated on the basis of immunoglobulin profile in the saliva samples of Indian expeditioners and compared with their respective base line values as controls. Samples were analyzed for SIgA, SIgM and TGF- β by sandwich ELISA. The levels of SIgA and SIgM were as follows.

SIgA Levels

Before proceeding to Antarctica the levels of SIgA were 13.52 ± 1.43 mg/dL. These levels increased to 19.71 ± 1.50 mg/dL after being 1 month on-board in the Southern Ocean. The levels were significantly higher in comparison to the control level ($p < 0.01$). The IgA levels further increased to 30.88 ± 2.02 mg/dL after staying for 1 month off-board at Maitri station. The off-board levels (Maitrians) of SIgA were significantly higher in comparison to the control and on-board levels ($p < 0.001$). On the whole the levels of SIgA increased with the duration of ship borne journey and Antarctic stay (Fig. 1).

SIgM Levels

The basal SIgM levels were 3.20 ± 0.17 mg/dL before proceeding to Antarctica. The levels moderately increased to 3.25 ± 0.71 mg/dL after being 1 month on-board which was not significant. The SIgM levels were decreased to 2.89 ± 0.15 mg/dL after staying for 1 month off-board at Maitri station which was significantly lowered in comparison to the on-board SIgM levels ($p < 0.05$). These data indicate that the SIgM levels were decreased during Maitri stay (Fig. 2).

TGF- β 1 Levels

Salivary TGF- β 1 levels were assessed in the Indian expeditioners as a signature cytokine of mucosal immunity. The

Fig. 1 Changes in salivary IgA level in Indian Antarctic expeditioners before leaving India (Control), after 1 month ship journey (on board personnel) and after 1 month stay at Maitri Antarctica (Maitrians). Data presented as Mean $\pm$ SE. * $p < 0.01$ versus control; # $p < 0.001$ versus control and on-board personnel

base line salivary TGF- β 1 level was 72 ± 19 pg/ml in the expeditioners before proceeding to Antarctica. The level moderately increased to 85 ± 28 pg/ml during On-board Ship journey in Southern Ocean. However after staying for 1 month at Maitri the level decreased to 67 ± 17 pg/ml. After applying the repeated measures Anova, significant alteration was not observed at any point of salivary TGF- β 1 levels (Fig. 3).

Salivary Cortisol Level

Salivary cortisol levels were assessed in the Indian expeditioners as physiological stress indicator. The base line salivary cortisol level was 4.803 ± 0.07 ng/ml in the expeditioners before proceeding to Antarctica. The level became 4.793 ± 0.04 ng/ml during On-board Ship journey in Southern Ocean. However after staying for 1 month at Maitri the level decreased to 4.625 ± 0.07 ng/ml. There was no significant alteration observed at any point of salivary cortisol levels (Fig. 4).

Fig. 2 Changes in salivary IgM level in Indian Antarctic expeditioners before leaving India (Control), after 1 month ship journey (on board personnel) and after 1 month stay at Maitri Antarctica (Maitrians). Data presented as Mean $\pm$ SE. * $p < 0.05$ versus on-board personnel

Fig. 3 Changes in salivary TGF- β level in Indian Antarctic expeditioners before leaving India (Control), after 1 month ship journey (on board personnel) and after 1 month stay at Maitri Antarctica (Maitrians). Data presented as Mean $\pm$ SE

Discussion

In the present study, we provided evidence that Antarctic environment increased SIgA level while decreased SIgM level. This result suggests that acute exposure to Antarctica may improve mucosal immune protection. SIgA has the important function of blocking entrance of pathogenic organisms and preventing infections. Reduction in SIgA levels might increase the risk of upper respiratory tract infection [1, 14, 15]. Here, we showed that ship borne journey increased SIgA levels, while SIgM levels were not altered as compared to base line level. The SIgA level was further increased when expeditioners spent 1 month at Maitri, Antarctica.

Antarctic exposure in Australian wintering personnel causes decreased IgA level in the month of March and April as compared to the other months [11]. However in our study SIgA level was increased on board as well as at Maitri research base. Similar observation was reported by Gleeson et al. [10] in Australian wintering personnel. Secretion from the salivary glands is controlled by both the sympathetic and parasympathetic nervous systems [16, 17]. When the sympathetic nerve activity is dominant, small amounts of viscous saliva is secreted; when parasympathetic nerve activity is dominant, watery serous saliva is secreted [16, 17]. There are reports which suggest that Extreme environmental conditions like isolation, cold and fear may affect autonomic nervous system [18]. This could be the reason for differing values of SIgA or SIgM level in Antarctic sojourners. Our data is further supported by the study of Weber-Mzell et al. [30] where they reported the prevalence of increased SIgA levels in Caucasians males due to influence of gender, age and seasons.

Literature search revealed scarcity of papers on effect of harsh environmental conditions on salivary cytokines. Most papers on cytokines and immunoglobulins in saliva

Fig. 4 Changes in salivary cortisol level in Indian Antarctic expeditioners before leaving India (Control), after 1 month ship journey (on board personnel) and after 1 month stay at Maitri Antarctica (Maitrians). Data presented as Mean $\pm$ SE

are related to oral infections, periodontal diseases and autoimmune conditions such as Sjögren's syndrome [19–24]. Previous measurements of cytokines and immunoglobulins showed that stress do alter the immunity [25–27]. TGF- β 1 profoundly affects mucosal and systemic immunity and inflammation, whether by inducing B cell switching to IgA or by regulating homing of T lymphocytes into the mucosal compartment [14]. However, most conclusions have been based upon in vitro studies and do not necessarily reflect the effects of TGF- β 1 in-vivo. Van Ginkel et al. [13] in a study reported the in vivo role of TGF- β 1 in IgA production in mucosal and systemic immune compartments employing TGF- β 1-/- mice. This study showed that IgA levels in both serum and mucosal secretions were significantly reduced in TGF- β 1-/- mice. In other in vitro study, effects of TGF- β on IgA production by LPS stimulated B cells have been studied. TGF- β itself could augment polyclonal IgA production. These results suggest that TGF- β has a differential role in the induction of IgA production [28]. Salivary cortisol was assessed as a measure of physiological stress. The previous studies suggest that there was decreased level of serum cortisol in the personnel who stayed in Antarctica for summer [29]. However we did not found any significant difference in salivary cortisol level at any point of time during the summer expedition. From the above data it can be inferred that SIgA levels could be a possible biomarker for stress like Antarctic harsh environment. However, further studies with larger subjects are required before making firm conclusions on these markers.

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Conflict of Interest Authors declare no conflict of interest.

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