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ORIGINAL ARTICLE

Correlation of serum IgE with stress in Indian dromedaries affected with skin wounds

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The present investigation was planned to explore stress induced changes in the levels of IgE by correlating them with the important parameters of stress like cortisol and free radical scavengers in the serum of Indian dromedaries. The mean values of serum IgE and cortisol were significantly ($p \leq 0.05$) higher in wound affected dromedaries than the respective healthy values. A positive significant ($p \leq 0.05$) correlation was observed between the values of serum IgE and cortisol. The mean values of serum vitamin C, vitamin E and glutathione decreased whereas of serum catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase increased significantly ($p \leq 0.05$) from their respective healthy mean values. A negative significant ($p \leq 0.05$) correlation was observed between the values of serum IgE and vitamin C; serum IgE and vitamin E and serum IgE and glutathione activities. A positive significant ($p \leq 0.05$) correlation was observed between the values of serum IgE and catalase; serum IgE and superoxide dismutase; serum IgE and monoamine oxidase; serum IgE and glutathione reductase; serum IgE and xanthine oxidase; serum IgE and oxidase; and serum IgE and peroxidase activities. It was concluded that stress was able to induce marked changes in the levels of IgE in the Indian dromedaries. Wounds altered the immune status as well as levels of cortisol and free radical scavengers in the serum.

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The majority of the health issues of the animals can be related to an immune system disorder. Immunity and inflammatory processes of the body can be lowered by prolonged stress resulting in slowed wound healing and other health consequences. Stress can be assessed in the form of

cortisol and free radical scavengers levels whereas immune status can be assessed by IgE levels in the blood. It is important to investigate the impact that variation in serum cortisol or any free radical scavenger has on immunoglobulin E (IgE), which is a class of antibody playing an important role in allergy, and is especially associated with type 1 hypersensitivity. Although IgE is typically the least abundant isotype, it is capable of triggering the most powerful immune reactions. Much attention has been paid on the determination of serum IgE levels in allergic disease and helminthic infection (Kataria and Kataria, 2004). On the basis that hyper-IgE syndrome is characterized by recurrent staphylococcal abscesses of the skin, lungs, joints, and viscera, it is the need of hour to record possible changes in the serum IgE levels along with cortisol and other markers of stress like free radical scavengers.

Free radicals damage immune system and are contributing factors for many diseases. They wipe out cytokine (communication) pathways. Oxygen-derived free radicals are important in both natural and acquired immunity. Oxidative stress may be detrimental in acquired immunity by activation of nuclear factor kappa B, which governs gene expression involving various cytokines, chemokines, and cell adhesion molecules, among others (Knight, 2000**).** Free radical scavengers include antioxidants and enzymes. Animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells along with alteration in the immune status of animals by increasing susceptibility to infectious agents and by reducing the rate of wound healing (Cacioppo *et al*.,2002). The developing oxidative stress can only be detected by laboratory methods. Nature of work put the dromedaries in troubles of having common affections like nasal peg wounds, saddle gall etc. Chronic affections may predispose them to stress inviting a cascade of pathologies. For their stress free management in arid tracts, it has become important to carry out studies on various inflammatory skin affections in light to immune system and oxidative status. Owing to the paucity of literature on this aspect the present investigation was planned to explore stress induced changes in the levels of IgE by correlating them with the important parameters of stress like cortisol and free radical scavengers in the serum of Indian dromedaries. .

MATERIALS AND METHODS

The blood samples (60) were collected during a survey study on camel diseases to harvest the serum during morning hours from adult male Indian dromedaries (*Camelus dromedarius*) of arid region (Rajasthan, India) managed in similar conditions of feeding and watering by the private farmers kept for the purpose of farming and load carrying. The camels were divided into two groups i.e. healthy and wound affected. Blood was drawn from 15 healthy and 45 skin wound affected adult male dromedaries during moderate (Mean maximum temperature ranged between 27and 29^{o}C) ambience. The skin wounds were present in different parts of the body in different animals due to various causes.

The total serum IgE levels were determined using coat-A-count total IgE IRMA kit (DPC, USA) as per the instructions supplied with kit. It was a solid phase double antibody immunoradiometric assay in which ^{125}I labelled anti IgE monoclonal antibodies in liquid phase acted as tracer and a polyclonal anti IgE antibody was immobilized to the wall of polystyrene tube. The activity was counted in ^{125}I Gamma counter for one minute. Total IgE concentrations were determined by the graph plotted from calibrators

provided with kit. The serum cortisol was determined by using the Gamma coat (^{125}I) cortisol radioimmunoassay kit procedure based on the competitive binding principles of radioimmunoassay (DiaSorin, USA). Serum samples and standards were incubated with cortisol tracer in antibody-coated tubes (Rabbit anti-cortisol serum coated) where the antibody was immobilised onto the lower inner wall of the Gamma Coat Tube. After incubation the contents of the tubes were decanted and the tube was counted in a ^{125}I Gamma counter (ECIL, India).

Free radicle scavengers in the serum included vitamin C, vitamin E, catalase, monoamine oxidase, glutathione reductase, superoxide dismutase, glutathione, and peroxidase. They were determined by the methods of Varley (1988); Nair and Magar (1955); Goldblith and Proctor (1950); Green and Haughton (1961); King (1965); Winterbourn *et al*. (1975); Owens and Belcher (1965) and Snell and Snell (1954) with modifications (Kataria and Kataria, 2009). Xanthine oxidase and oxidase were determined by the methods of Litwack *et al.* (1953) and Snell and Snell (1954) with modifications (Kataria and Kataria,2009), respectively.

The mean values obtained from apparently healthy animals were considered as control and rest other mean values were compared from the respective control mean values. The statistical significance was determined as per Snedecor and Cochran (1967). Correlations were made using MSTAT computer programme.

RESULTS AND DISCUSSION

The mean values of serum IgE, cortisol and free radical scavengers in the serum of healthy and wound affected Indian dromedaries are presented in table 1.

The mean values of serum IgE and cortisol were significantly ($p \leq 0.05$) higher in skin wound affected dromedaries than the respective control values. A positive significant ($p \leq 0.05$) correlation was observed between the values of serum IgE and cortisol. The mean values of serum vitamin C, vitamin E and glutathione decreased whereas of serum catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase increased significantly ($p \leq 0.05$) from their respective control mean values. A negative significant ($p \leq 0.05$) correlation was observed between the values of serum IgE and vitamin C; serum IgE and vitamin E and serum IgE and glutathione activities. A positive significant ($p \leq 0.05$) correlation was observed between the values of serum IgE and catalase; serum IgE and superoxide dismutase; serum IgE and monoamine oxidase; serum IgE and glutathione reductase; serum IgE and xanthine oxidase; serum IgE and oxidase; and serum IgE and peroxidase activities.

There is paucity of the work to correlate the parameter of immune system with stress in dromedaries. In present study the significant increase in serum cortisol indicated that affected dromedaries were stressed. Simultaneous increase in serum IgE was correlated well with increased cortisol concentrations. The study demonstrated that the changes in the levels of serum cortisol affected the IgE levels. Toda *et al.* (2007) observed a significant correlation between serum total IgE and cortisol in allergic human subjects. These results exhibited a bidirectional feedback between the immune system and hypothalamus-pituitary-adrenal axis. The levels of cortisol can regulate IgE levels (Herrschan *et al*.,1992). The mean value of serum IgE in healthy dromedaries was similar to those given by Kataria and Kataria (2004).

In present study serum vitamin C, vitamin E and glutathione levels were lower in wound affected dromedaries which indicated their depletion in the process to prevent or to reduce oxidative stress.

Oxidative stress resulted in affected camels probably because neutrophil and macrophage phagocytosis stimulates various cellular processes including the "respiratory burst" whereby increased cellular oxygen uptake results in the production of the potent oxidants. Although some of them are beneficial as they are bactericidal agents, hypochlorous acid and hydroxyl radical (Knight, 2000), but this also causes depletion of antioxidants. Antioxidants essentially reverse several immune deficiencies, resulting in increased levels of interleukin-2, elevated numbers of total lymphocytes and T-cell subsets, enhanced mitogen responsiveness, increased killer cell activity, augmented antibody response to antigen stimulation, decreased lipid peroxidation, and decreased prostaglandin synthesis (Knight, 2000).

Vitamin C is a potent anti-oxidant which protects the body against oxidative stress (Padayatty *et al*., 2003). Stress, trauma or injury can use up large quantities of vitamin C (Long, 2003). Vitamin E or α-tocopherol is the most important lipid-soluble antioxidant which protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. Glutathione (GSH) is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds (Ramboer *et al*., 1972), as well as maintaining other antioxidants such as vitamins C and E in their reduced or active forms. Kataria *et al.* (2010) also reported depletion of antioxidants in brucella affected cows and attributed this to oxidative stress.

Superoxide dismutase, monoamine oxidase, catalase, glutathione reductase, xanthine oxidase, oxidase and peroxidase activities in the serum increased in wound affected dromedaries which indicated towards stress to the animals. The cytoplasm of all cells contain superoxide dismutase, key antioxidant enzyme, which is known to reverse fibrosis, perhaps through reversion of myofibroblasts back to fibroblasts (Vozenin-Brotons *et al*.,2001).Its higher concentration in the serum is an indicator of oxidative stress (Bauer and Bauer, 1999). Monoamine oxidases catalyse the oxidative deamination of monoamines and play an important role in the inactivation of neurotransmitters, and their dysfunction could result in stress (Meyer *et al*., 2006). Stress can be noticed in the form of its increased activity in serum. Higher serum concentration of this enzyme in wound affected animals indicated towards oxidative stress. Catalase functions to catalyse the decomposition of hydrogen peroxide to less reactive gaseous water and oxygen (Gaetani *et al.,* 1996 and Chelikani *et al.,* 2004). Glutathione reductase is an enzyme that reduces glutathione disulfide to the sulfhydryl form GSH, which is an important cellular antioxidant (Meister, 1988). The activity of glutathione reductase is used as an indicator for oxidative stress. Xanthine oxidase is a form of xanthine oxidoreductase that generates reactive oxygen species (Ardan *et al*., 2004). It is not a scavenger of free radical but along with free radical scavengers it can be used as a marker of oxidative stress. An oxidase is any enzyme that catalyzes an oxidation/reduction reaction involving molecular oxygen as the electron acceptor. In these reactions, oxygen is reduced to water or hydrogen peroxide. Therefore it is considered as good marker of oxidative stress. Peroxidase is a hemoprotein catalysing the oxidation by hydrogen peroxide of a number of substrates such as ascorbate, ferrocyanide, cytochrome C etc. Serum peroxidase activity is considered as the main indicator of the antioxidant activity (Podil'chalk *et al*., 1996).

Table 1. Mean ± SEM values of serum IgE, cortisol and free radical scavengers in wound affected Indian dromedaries

Parameters	Healthy (n=15)	Wound affected (n=45)
IgE, U ml^{-1}	2.39± 0.02	35.09± 0.08
Cortisol, $nmolL^{-1}$	26.90± 1.0	170.0 ± 8.0 [b]
Vitamin C, µmol L^{-1}	25.5 ± 1.0	10.01[b]± 0.9
Vitamin E, µmol L^{-1}	6.3±0.2	2.0[b] ±0.09
Glutathione, µmol L^{-1}	4.6±0.2	1.4 [b] ±0.05
Superoxide dismutase, kU L^{-1}	135.10 ±6.5	300.4[b] ±10.6
Monoamine oxidase, U L^{-1}	314.0± 8.0	480[b] ± 15.2
Catalase, kU L^{-1}	74.8 ±3.9	130.3[b] ±10.1
Glutathione reductase, k U L^{-1}	2.6± 0.1	8.0 [b] ± 0.5
Peroxidase, mU L^{-1}	77.0±5.0	166[b] ±7.7
Xanthine oxidase, mU L^{-1}	55.0±2.0	123[b] ±9.2
Oxidase, U L^{-1}	61.0±3.0	140[b] ±5.9

Superscript 'b' on the means showed the significant ($p \leq 0.05$) difference from the respective mean value of healthy animals.

Figures in the parentheses indicate number of animals.

Correlation of IgE with free radical scavengers, positive or negative, was due to the fact that in inflammatory processes, free radicals or reactive oxygen species are known to induce mediator release from mast cells and basophils (Strenzke *et al*., 2001). This could be the probable reason of increased IgE levels in wound affected camels.

It was concluded that stress was able to induce marked changes in the levels of IgE in the Indian dromedaries. Skin wound affections altered the immune status as well as levels of cortisol and free radical scavengers in the serum. Serum IgE level was correlated with serum cortisol which potentiated the fact that stress and immune systems had bidirectional feedback. Decrease in the levels of antioxidants along with raised cortisol and IgE strongly pointed towards the existence of stress in dromedaries. The present investigation also attempted to provide baseline values of serum IgE, cortisol and free radical scavengers at one platform in healthy dromedary camel to help in the clinical diagnosis. This study also recommends the use of antioxidants in wound affections in dromedaries.

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