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Selected Oxidative Stress Markers in Stress-related Skin Diseases of Dogs

Thesis presented by

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Abstract

The present clinical studies were carried out to investigate selected oxidative stress markers in stress-related skin diseases of dogs. The studies were applied on a total number of 83 dogs of different breeds, their ages ranged from 2 months to 13 years.

Dogs were classified into: apparently healthy group, flea allergy dermatitis group (FAD), demodicosis group, dermatophytosis group, pyoderma affected group, tick infested group and allergy contact dermatitis (ACD) affected group. Clinical, hematological, biochemical constituents and oxidative stress biomarkers were analyzed.

Clinical presentations of examined groups revealed significant heamato-biochemical alterations include increase in red blood cells count(RBCs) in allergy contact dermatitis affected group, decrease in hemogram activity in demodecosis infected dogs, leucocytosis in FAD, demodecosis, pyoderma and ACD, neutrophilia in demodecosis, pyoderma and ACD infected group, neutropenia in tick infestation, eosinopenia in demodicosis and pyoderma groups but eosinophilia in FAD and tick infestation group, and monocytopenia in ACD also, lymphocytopenia in demodecosis and ACD dogs. Serum biochemical analysis revealed an increase in alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitogen (BUN). Oxidative stress markers analysis revealed significant increase in superoxide dismutase (SOD) in demodecosis and ACD groups along with significant decrease in glutathione peroxidase (GPx) in demodecosis, dermatophytosis and pyoderma groups, decrease in Catalase (CAT) level in pyoderma group and plasma zinc levels in FAD, dermatophytosis and ACD groups.

Key words: Oxidative stress, Heamato-biochemical, Canine, Skin affections.

To my family

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Contents

List of Tables

List of Figures

List of Photos

Introduction

Skin, the integumentry system is the largest organ of the body. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, as well as prevention of excess water loss from the body and a role in thermoregulation. The skin is continuous, with the mucous membranes lining the body's surface **(Kanitakis, 2002).**

In canine practice, skin disorders are among the most common health problems affecting dogs. The condition of dog's skin can be an important indicator of general health status **(Merck and Merial 2007).**

Canine skin affections originated mainly from infectious and non-infectious etiologies, External parasites are very frequently causes of skin diseases in young dogs: canine demodicosis, sarcoptic mange, flea infestation and ticks infestation, followed by bacterial skin infection (pyoderma), allergic diseases (allergic contact dermatitis, atopic dermatitis and cutaneous food allergic reaction), and fungal skin diseases (Dermatophytosis) **(Verde, 2005).**

The haematobiochemical evaluation and mineral status of dogs suffering from skin affections may be valuable attributes to identify underlying problems which might be contributing factors in the development of the disease **(Chandan and Surojit 2014)**.

Free radicals insult, induce or contribute to adverse effects on the skin, including erythema, edema, wrinkling, photoaging, inflammation, autoimmune reactions, hypersensitivity, keratinization abnormalities, preneoplastic lesions, and skin cancer **(Nachbar and Korting 1995).**

Reactive oxygen species (ROS) are a family of oxygen-based free radicals that contain or are capable of producing an unpaired electron. Overproduction or inadequate removal of ROS can result in oxidative stress, leading to altered metabolism, dys-regulated signal transduction events, and biomolecular damage, all of which contribute to pathological changes in cell and tissue function. Biomolecular damage that occurs because of elevated ROS levels is

manifested as lipid peroxidation, DNA mutations/breakage, enzyme inactivation/activation, and protein oxidation/degradation **(Özben, 1998)**.

Antioxidants function to delay or prevent ROS-induced cellular damage, and work by reducing local oxygen concentrations, impairing chain initiation reactions, binding catalysts such as metal ions that generate ROS, and attenuating hydrogen abstraction by active radicals **(Paulsen and Carroll 2013).**

Antioxidants include low molecular-weight compounds such as β-carotene, tocopherols and the thiol-containing compound glutathione (GSH), as well as high-molecular-weight antioxidant enzymes and protein that modulate the action of ROS or their byproduct include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), the thioredoxin/thioredoxin reductase system and metallothioneins **(Suntres, 2011).**

Antioxidant enzymes, SOD and GPX, constitute the primary antioxidant defense system against ROS. Both increased and decreased levels have been reported in different diseases because of enhanced ROS production, either by up-regulation of enzyme activity or utilization of the antioxidant enzymes to counter the ROS **(Dotan *et al.*, 2004).**

Therefore, this work was carried out to:

1) Investigate general clinical examination for detection of specific selected skin disease process involved in oxidative stress in dogs.

2) Evaluate laboratory changes including hematologic, serum biochemical, skin scraping and bacteriological examination.

3) Determinate specific oxidative stress markers related to skin affections.

Review of Literature

Kanitakis (2002) mentioned that the skin is the largest organ of the body. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, as well as prevention of excess water loss from the body. The skin is continuous, with the mucous membranes lining the body's surface.

1. Skin Anatomy and Histology:

Scott, *et al.* (2000) mentioned that the skin should be considered as a real vital organ, insuring the whole body protection (other organs and tissues), it has connections with other organs and with the interior of the body (usually by mucosae), and connections at the level of the eyes, tympanic membrane, nose, mouth, ano-genital area.

Favarato and Conceição (2008) reported that hair cycle activity, in some dog breeds, is strongly related to temperature variation and photoperiod, leading to decreased hair density in the warmer months, which helps the heat loss in these animals.

Bourguignon, *et al.* (2013) mentioned that the skin is divided into three layers: epidermis, dermis and hypodermis. Epidermis, the outermost layer of the skin, is composed by keratinocytes, melanocytes and Langerhans cells. Dermis, the layer under the epidermis, is composed by a conjunctive matrix where reticular, elastic and collagen fibers are found. Dermis cellular structure is composed by fibroblasts, mast cells and histiocytes. It also contains epidermal appendages (hair, nails and sebaceous gland), arrector pili muscles and blood and lymph vessels. Hypodermis or subcutaneous tissue provides support and cushioning against physical trauma. It is composed of a loose connective tissue and elastic fibers interspersed by adipocytes**.**

2. Skin Function:

Scott, *et al.* (2000) mentioned that the most important function of the skin is to make an internal environment for all other organs by maintaining an effective barrier to the loss of water, electrolyte, and macromolecules. A corollary function is the exclusion of external injurious agent, chemical, physical, and microbiological from entrance into internal environment. The flexibility, elasticity, and toughness of the skin aloe motion and provides shape and form. The skin surface also has antibacterial and antifungal functions. Skin is a primary sense organ for touch, pressure, pain, itch, heat, and cold.

Bourguignon, *et al.* (2013) mentioned that the skin is the largest organ of the body with many different functions as immune protection, sensory perception, vitamin D production and it acts as a barrier between the animal and the environment.

3. Skin Affections in Dogs:

3.1. Non-Infectious Skin Affections in Dogs:

3.1.1. Allergic Skin Diseases:

3.1.1.1. Adverse Food Reaction:

Hillier and Griffin (2001) mentioned that adverse food reaction refers to any abnormal clinical response assigned to consumption of food or its additives.

Jackson (2001) reported that adverse food reactions are described in veterinary medicine since 1920, reporting the occurrence of gastrointestinal signs and skin reactions in response to food allergens. No breed, sex or age predisposition to the occurrence of clinical signs was reported in such type.

Hensel (2010) reported that the reaction is classified as food allergy (immune-mediated) or food intolerance (non-immune-mediated). The majority of reactions in animals are food intolerances and they can be of pharmacological or metabolic origin, poisoning, idiosyncrasy, toxicity or anaphylactic reaction to the food.

Bloom (2011) found that there are some dogs with pruritic skin diseases or otitis which resolves with restrictive diet, but it remains unproved the

immunologic cause or hypersensitivity (allergy) associated with cutaneous adverse food reactions (CAFR). Since the etiopathogenesis was not elucidated yet, the term food allergy should be avoided, and CAFR is more appropriate.

3.1.1.1.2. Clinical signs of Adverse Food Reaction:

Jackson (2001) stated that pruritus is the most important sign reported and it affects mainly the face, perineum and ears (otitis externa).Gastrointestinal signs as vomiting and diarrhea can also be observed.

3.1.1.1.3. Diagnosis of Adverse Food Reaction:

Jackson (2001) mentioned that the best diagnostic approach for CAFR in dogs is feeding them with a diet, with only one source of protein that the animal has never been in contact before (novel protein). The diagnosis is obtained with the resolution of clinical signs after the diet trial and with the return of these signs when the previous diet is offered again. The diet trial should be implemented for at least six weeks. Homemade diets are more appropriate for the CAFR trial described above, but there are also commercially available prescription diets.

Hensel (2010) stated that prescription diets, commercially available in some countries, are made of an unusual protein source and a non-allergenic carbohydrate source such as potato or oat meal. Lamb, duck, rabbit and Kangaroo meat are protein sources usually found in these diets.

3.1.1.2. Canine Atopic Dermatitis in Dogs:

Olivry and Hill (2001) reported that the refractory period for allergic contact dermatitis is reported to be rarely less than two years, so one would not expect it to appear in very young animals.

Halliwell (2006) mentioned that Canine atopic dermatitis (CAD) is an allergic, hereditary, and inflammatory and pruritic skin disease, with characteristic clinical signs associated with immunoglobulin E (IgE) production against environmental allergens.

Snyder (2007) stated that allergic condition was represented by 7.7%, Flea allergic dermatitis was represented by 0.6%. Contact allergic dermatitis was represented by 1.1%. Food allergy was represented by 4.1%. Atopy was

represented by 1.7%. Urticaria wheals after penicillin injection were represented by 0.24%.

Olivry, *et al.* (2010) reported that most dogs with atopic dermatitis begin to manifest signs between six months and three years of age.

3.1.1.2.1. Clinical signs of Canine Atopic Dermatitis:

Olivry and Sousa (2001) stated that there is no sex predisposition and clinical signs may or not be seasonal, depending on the allergen involved. Usually, patients have a history of pruritus with or without secondary skin or ear infections. Primary lesions include macules and papules, but frequently, patients are presented with secondary lesions from self-inflicted trauma as excoriations, alopecia, lichenification and hyperpigmentation. Lesions affect the face, concave part of the pinna, ventral aspect of the neck, axilla, groin, abdomen, perineum, ventral aspect of the tail, limbs joints, medial aspects of limb extremities, feet and ears.

3.1.1.2.2. Diagnosis of Canine Atopic Dermatitis:

DeBoer and Hillier (2001) mentioned that in dog as in human beings there is no pathognomonic sign of atopic dermatitis that could provide a diagnosis based only in history and physical examination. Diagnosis depends on patient fitting in several criteria associated with the condition and on elimination of differential diagnoses. Following clinical diagnosis, laboratory or clinical tests as allergy tests and histopathology, reinforces the diagnosis.

3.1.1.3. Allergic Contact Dermatitis:

White (1991) stated that allergic (ACD) and irritant (ICD) contact dermatitis are two very similar conditions mediated by direct contact with environmental substances and, therefore, they affect sparsely haired, predominantly ventral skin.

Tim, *et al.* (2009) mentioned that ACD is a type 4 (cell-mediated) hypersensitivity reactions to small, low molecular weight chemicals (haptens) that bind to host proteins. Haptenated proteins are phagocytosed, processed, and presented by antigen presenting cells, especially epidermal Langerhan's cells, to T cells bearing the appropriate T cell receptors. These recirculate to the