Histopathology of skin lesions of canine atopic dermatitis in pugs

BK Bhagya, C Ansar Kamran, Suguna Rao and BM Veeregowda

Abstract
Present study was undertaken to know the histopathological features in canine atopic dermatitis by taking skin biopsy. Skin sample was collected from 30 atopic pugs in 10 percent neutral buffer formalin and tissues were processed by routine paraffin embedding technique and three to five micrometre sections were cut and stained with hematoxylin and eosin. Histopathological findings in CAD were characterised by presence of haemorrhages in the supra epidermal, epidermal and sub epidermal region, congestion of blood vessels, accumulation of serosanguinous fluid in the supra epidermal region and edema of sub epidermal region, spongiosis, mild to severe infiltration of inflammatory cells, perivascular dermatitis, epidermal hyperplasia, hyperkeratosis, cystic dilatation of hair follicles and hyperpigmentation. Suppurative folliculitis and suppurative perifolliculitis was suggestive of secondary bacterial pyoderma.

Keywords: Histopathology, CAD, pug

Introduction
Atopic dermatitis is a common, genetically predisposed allergic skin disease [11]. It occurs in animals with, a hereditary tendency to develop IgE-mediated allergic reactions to various environmental allergens and these include, house dust mites, plant pollen, fungal spores and various other allergens. The definition of CAD suggests strong breed and familial predisposition. Pug is a breed of Chinese origin and genetically predisposed for atopic dermatitis [5].

Scott [10] established the first detailed description of histological features of CAD and CAD skin biopsies most exhibited epidermal hyperplasia, orthokeratotic or parakeratotic hyperkeratosis, hypergranulosis, spongiosis, melanosis and leucocyte exocytosis. Dermal changes were, described as congestion, vasodilatation and angiocentric inflammatory infiltrate consisting predominantly of mononuclear cells and neutrophils and bacterial infection was manifested as focal suppurative folliculitis. Present study, was undertaken to know the histopathological features of skin lesions of CAD in pugs.

Materials and Methods
In the present study, 5mm thickness skin sample was collected from 30 pugs using 5mm punch biopsy needles in 10 per cent neutral buffer formalin for histopathological examination [1] and tissues were processed by routine paraffin embedding technique and three to five micrometre sections were cut and stained with hematoxylin and eosin [3].

Results and Discussion
The study was undertaken, to know the histopathology of skin lesions in canine atopic dermatitis in pugs as, pugs are genetically predisposed for atopic dermatitis. The histopathological findings in canine atopic dermatitis were characterised by presence of haemorrhages in the supra epidermal, epidermal and sub epidermal region (33.2%) (Plate 1), congestion of blood vessels in the sub epidermal region (Plate 2) (16.6%), accumulation of serosanguinous fluid in the supra epidermal region (Plate 2) (16.6%), accumulation of serosanguinous fluid in the supra epidermal region (Plate 3) and edema of sub epidermal region (Plate 4), mild to severe infiltration of inflammatory cells in the epidermal, sub epidermal, dermal region as well as around the sebaceous glands (Plate 5&6) and cellular infiltration mainly consisted of plasma cells, lymphocytes, mast cells (33.3%) (Plate 7), eosinophils (27.7%) and polymorphs (38.8%) followed by spongiosis (56.6%) (Plate 8). Perivascular dermatitis was observed in 10% of the cases. Epidermal hyperplasia was evident in 40 per cent of cases (Plate 9) followed by hyperkeratosis (43.33%) (Plate 10) and cystic
dilatation of hair follicles (30%) (Plate 11), wherein distended hair follicles were lined by multi, layered epithelial cells enclosing concentrically arranged keratin. Hyperpigmentation (Plate 12) was observed in 6.6 per cent of the cases, where basal layer of epidermis showing more number of melanocytes with brownish melanin pigmentation. Suppurative folliculitis (Plate 13) and suppurative perifolliculitis was recorded in 3.3 percent and 16.6 percent of the cases respectively.

Results of the present study is in accordance with the findings of Rojko [9], Scott [10], Nimmo Wilkie [6], Olivry [7, 8], Gross [2] and Vaseem [13]. Numerous inflammatory cells have been reported to play role in the pathogenesis of canine atopic dermatitis and these include mast cells, eosinophils, neutrophils, lymphocytes, dendritic antigen presenting cells and cells of the monocyte or macrophage lineage [3]. Perivascular dermatitis is characterized by inflammatory cells around vessels of the dermis. In atopic dermatitis, inflammation appears in perifollicular or periadnexal areas as blood vessels are numerous in that location [2]. Allergic skin diseases produce multifocal spongiosis and it is characterized by edema of the intercellular spaces of the epidermis and superficial follicular wall. Inflammatory cell influx accompanies spongiotic change. Epidermal and follicular infundibular hyperplasia is a common and pervasive characteristic of chronic skin diseases of all causes. Hyperplastic skin diseases are characterized by the proliferation of epidermal and follicular infundibular keratinocytes. Epithelial hyperplasia, or acanthosis, may be due to internal factors (metabolic, hereditary) or external injury (self-trauma). Hyperkeratosis is defined as an increase in the cornified layer, and technically may be orthokeratotic or parakeratotic. Diffuse parakeratosis may be a feature of allergic conditions [9].

Hyperpigmentation may occur in any area where there has been inflammation or irritation to the skin and post inflammatory occurs as a sequela to an underlying skin disease such as pyoderma or hypersensitivity. Post inflammatory hyperpigmentation reflects increases in cytokine amounts and activity following infection or inflammation. Several cytokines such as arachidonic acid, prostaglandin (PG) E₂, and PGD₂, increase melanocyte proliferation. Melanocyte dendricity is enhanced by PGE₂, leukotriene (LT) C₄, LTD₄, and thromboxane 3 (TX₃). Arachidonic acid, TX₃, LTD₄, and LTC₄ enhance the activity of tyrosinase. Melanocyte production is stimulated by LTD₄ [12]. Suppurative folliculitis in the present study is suggestive of secondary pyoderma [10]. In the present study, infiltration of neutrophils was observed in 38.8 percent of cases. The main function of neutrophils is the capture and destruction of foreign material, especially bacteria, and are not consider to be major players in the pathogenesis of AD. Neutrophils were the major component of the intense mixed leukocytic dermal infiltrate in dogs with pyoderma [9]. Additional lesion observed in the present study was perifollicular infiltration of inflammatory cells, which is in accordance with findings of Rojko [9] and purulent or purulogranulomatous perifolliculitis is also feature of pyoderma.

Plate 1: Section of skin showing sub epidermal haemorrhage (H&E 100X)

Plate 2: Section of skin showing sub epidermal congestion (H&E 100X)

Plate 3: Section of skin showing accumulation of serosanguinous fluid in the supraepidermal region and infiltration of inflammatory cells (H&E 100X)
Plate 4: Section of skin showing sub epidermal edema (H&E 100)

Plate 5: Section of skin showing infiltration of mononuclear cells in the sub epidermal region (H&E 100X)

Plate 6: Section of skin showing infiltration of inflammatory cells in the dermal sebaceous glands (H &E 400X)

Plate 7: Section of skin showing infiltration of mast cells with occasional eosinophils (H&E400X)

Plate 8: Section of skin showing spongiosis, epidermal hyperplasia, subepidermal haemorrhage and infiltration of inflammatory cells (H&E 100X)

Plate 9: Section of skin showing epidermal hyperplasia (H&E 100X)

Plate 10: Section of skin showing segmental hyperkeratosis (H&E 200X)

Plate 11: Section of skin showing cystic dilatation of hair follicles (H&E 100X)
Conclusion
In conclusion, commonly observed histopathological findings in the lesional skin of canine atopic dermatitis were congestion, haemorrhage, infiltration, spongiosis, hyperkeratosis, and hyperpigmentation. Hyperpigmentation is suggestive of inflammation whereas folliculitis is because of secondary bacterial infection.

References
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