

RESEARCH ARTICLE

Evaluation of Circulatory Concentration of Cytokines Platelet-Derived Growth Factor-BB, Transforming Growth Factor Beta-1, and IL-10 in Canine Generalized Demodicosis

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ABSTRACT

The purpose of this study was to investigate the changes in circulating concentrations of PDGF-BB, TGF- β 1 and interleukin-10 (IL-10) in dogs with generalized demodicosis. Twelve dogs with generalized demodicosis were diagnosed based on clinical findings and microscopic examination of the cutaneous scrapings. Six healthy dogs were included in the study as a control. Commercial specific ELISA assays were used to measure circulating concentrations of plasma PDGF-BB, and serum TGF- β 1 and IL-10. Marked and significant increase in plasma PDGF-BB and serum TGF- β 1 concentrations was evidenced in diseased dogs, while increase in serum IL-10 concentration was non-significant. The levels of all three markers dropped down to near normal/healthy status after the treatment of diseased dogs. These results indicate that the increased concentrations of circulating PDGF-BB, TGF- β 1, and IL-10 play a pivotal role in the pathogenesis of the canine demodicosis.

Keywords: Dog, ELISA assay, Generalized demodicosis, IL-10, PDGF-BB, TGF- β 1, Immunosuppression.

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INTRODUCTION

Demodicosis, also named as demodectic mange, red mange, or follicular mange, can be defined as a common but exigent, inflammatory, noncontagious parasitic dermatosis caused by over the population of the host-specific follicular mites of various *Demodex* species (Ravera *et al.*, 2015; Shrestha *et al.*, 2015). Canine generalized demodicosis (CGD) may be a severe and potentially life-threatening disease. It usually starts in dogs of 18 months of age to 4 years of age, and the lesions may be similar to localized demodicosis. Cutaneous changes in young and older dogs include clogged hair follicles, papules, pustules, follicular casts, plaques, crusts, edema, deep folliculitis, and furunculosis (Mueller, 2004). Generalized demodicosis may commonly be complicated with secondary bacterial folliculitis and/or furunculosis (Kuznetsova *et al.*, 2012). It is a complex disease whose exact pathogenesis remains unclear. Immunosuppression is directly associated with the development of the disease, whether caused by other infirmities or not (Gortel, 2006). Cytokines have an important role in the regulation of the immune response, and their expression pattern seems to contribute to the clinical presentation of the disease (Tani *et al.*, 2002). Therefore, the present study was aimed to investigate the changes in circulating concentrations of PDGF-BB, TGF- β 1 and interleukin-10 (IL-10) in dogs with generalized demodicosis before and after treatment.

MATERIALS AND METHODS

The dogs with clinical manifestations of different dermatological afflictions brought to the Veterinary Clinical

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Complex of the College in Anand and VCARE, Vadodara, were included in the study. A total of 12 positive cases of generalized demodicosis were selected for the routine therapeutic trial. Six healthy dogs negative for demodectic mites were also used as a control for comparison. Blood samples were collected from all the diseased and healthy dogs initially and again after treatment from diseased dogs by venipuncture from the cephalic vein into plain glass tubes for serum, and into tubes containing K3EDTA as an anticoagulant for plasma samples. The blood samples were immediately transferred to the laboratory. Plasma was separated from blood by centrifugation at 2000 x g for 15 minutes at 4°C. The blood samples for serum separation were allowed to clot

at room temperature for 2 hours and then were centrifuged for 20 minutes at 1000 x g at 4°C and serum was aliquoted. The plasma and sera samples were stored at -20°C in a deep freeze until analyses.

Concentrations of serum TGF- β 1, IL-10, and plasma PDGF-BB were measured by enzyme-linked immunosorbent assay (ELISA) using the canine TGF- β 1, IL-10, and PDGF-BB assay kits. Each assay was performed according to the supplier's instructions. The data was compiled into Excel spreadsheets (Office 2010, Microsoft, India) and analyzed using SPSS for Windows (Version 24.0, IBM India). The values of diseased and healthy dogs, as well as before and after the treatment of diseased dogs, were compared by the paired t test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The circulating concentrations as the mean \pm standard error of serum TGF- β 1, IL-10, and plasma PDGF-BB observed in healthy, and CGD dogs, and in CGD dogs before and after treatment are presented in Table 1 and 2, respectively. In the present study, the mean concentration of PDGF-BB was found to be significantly increased in the demodectic dogs as compared to healthy dogs (78.83 \pm 23.64 vs. 19.70 \pm 5.86 pg/mL, $p < 0.05$) (Table 1). The post-treatment mean concentration of PDGF-BB in diseased dogs was found to be decreased significantly (15.82 \pm 4.23 pg/mL, $p < 0.05$) as compared to pre-treatment value (78.83 \pm 23.64 pg/mL) (Table 2). PDGF-BB is known to stimulate growth, division, migration, and proliferation of the cells (Rosenkranz and Kazlauskas, 1999; Yarim *et al.*, 2013). PDGF exerts a stimulating effect on collagen synthesis (Rothe and Falanga, 1989), and the pivotal role of PDGF-BB in the wound healing process in post-irradiation surgical incisions has been well documented. A significant result in the present study was that generalized demodicosis cause an increase in the PDGF-BB concentration in dog plasma, most probably because of triggering of keratinocytes, fibroblasts, vascular endothelial cells, and macrophages. Therefore, increased PDGF-BB in dogs with generalized demodicosis suggest that this growth factor may play a role in the immune response to *Demodex canis* and skin repair mechanism in this disease.

Table 1: Circulating concentrations (Mean \pm SE) of PDGF-BB, TGF- β 1, and IL-10 in demodectic dogs and control dogs

Cytokines	Diseased dogs	Healthy dogs	p value
PDGF-BB	78.83 \pm 23.64	19.70 \pm 5.86	0.03*
TGF- β 1	106.34 \pm 17.87	62.36 \pm 7.86	0.04*
IL-10	69.03 \pm 28.78	19.89 \pm 5.95	0.12NS

NS = Non-significant, *Significant ($p < 0.05$).

Table 2: Circulating concentrations (Mean \pm SE) of PDGF-BB, TGF- β 1, and IL-10 in demodectic dogs (pre- and post-treatment)

Cytokines	Pre-treatment	Post-treatment	p value
PDGF-BB	78.83 \pm 23.64	15.82 \pm 4.23	0.02*
TGF- β 1	106.34 \pm 17.87	59.39 \pm 12.40	0.04*
IL-10	69.03 \pm 28.78	40.10 \pm 23.60	0.45NS

NS = Non-significant, *Significant ($p < 0.05$).

The mean serum concentration of TGF- β 1 was found to be significantly increased in the demodectic dogs as compared to healthy dogs (106.34 \pm 17.87 vs 62.36 \pm 7.86 pg/mL, $p < 0.05$). The mean concentration post-treatment in diseased dogs was found to be decreased significantly (59.39 \pm 12.40 pg/mL, $p < 0.05$) as compared to pre-treatment value (106.34 \pm 17.87 pg/mL) (Table 1, 2). It has been reported that TGF- β 1 has a critical role in the proliferation and differentiation of mesenchymal cells, extracellular matrix production, wound healing, and immunosuppression (Yarim *et al.*, 2013). The importance of TGF- β in immune regulation and tolerance has been increasingly recognized (Tani *et al.*, 2002; Patel *et al.*, 2018). It has been demonstrated that dogs with generalized demodicosis have a lower CD4+ to CD8+ T cell ratio than dogs having localized demodicosis (Singh *et al.*, 2010). In parallel, Walton *et al.* (2008) reported the infiltration of CD8+ T lymphocytes coupled to over-expression of TGF- β 1 in the dermis of severe crusted scabietic men. Patel *et al.* (2018) however, did not find mRNA expression of TGF- β as appropriate for monitoring the progress of the demodicosis.

Several studies have shown alterations in the circulatory concentration of PDGF-BB and TGF- β 1 in skin disorders. Yarim *et al.* (2013) reported a strong positive correlation between PDGF-BB and TGF- β 1 concentrations in dogs with generalized demodicosis. Ludwicka *et al.* (1995) reported an increased concentration of PDGF-BB and TGF- β 1 in patients with scleroderma. The increased TGF- β 1 concentration in dogs with generalized demodicosis in our study may be associated with an increase in CD8+ T cell population due to canine generalized demodicosis and may be related to skin repair mechanism in this disease.

In present study, the mean serum concentration of IL-10 was found to be non-significantly increased (69.03 \pm 28.78 pg/mL, $p > 0.05$) in the demodectic dogs as compared to healthy dogs (19.89 \pm 5.95 pg/mL, Table 1), and the post-treatment mean concentration of IL-10 decreased non-significantly (40.10 \pm 23.60 pg/mL, $p > 0.05$), as compared to pre-treatment value (69.03 \pm 28.78 pg/mL, Table 2). IL-10 is multifunctional cytokine produced by T cells and regulates the activity of cells in the immune system and may have a crucial role in the regulation of different types of T cells (Mosmann and Sad,

1996). The association between immunological disorders and the development of demodicosis has a poorly known mechanism (Tani *et al.*, 2002; Singh *et al.*, 2010). Our findings were in agreement with the previous scientific studies wherein the potential association of immunosuppressive cytokines with canine demodicosis has been demonstrated (Tani *et al.*, 2002; Felix *et al.*, 2013; Yarim *et al.*, 2013; Kumari *et al.*, 2017).

CONCLUSION

The results of the present study showed increased circulating PDGF-BB and TGF- β 1 concentrations in dogs with generalized demodicosis compared to healthy controls. Immunosuppression is an imperative feature of canine demodicosis. In this light, elevated IL-10 levels may be considered a relevant factor to explain why some animals develop demodicosis; hence, further studies are needed for a better understanding of immunosuppression in CGD.

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REFERENCES

- Felix, A.O.C., Guiot, E.G., Stein, M., Felix, S.R., Silva, E.F., and Nobre, M.O. (2013). Comparison of systemic interleukin 10 concentrations in healthy dogs and those suffering from recurring and first time *Demodex canis* infestations. *Vet. Parasitol.*, 193 (1): 312-315.
- Gortel, K. (2006). Update on canine demodicosis. *Vet. Clinics: Small Anim. Practice*, 36(1): 229-241.
- Kumari, P., Nigam, R., Singh, A., Nakade, U.P., Sharma, A., Garg, S.K., and Singh, S.K. (2017). *Demodex canis* regulates cholinergic system mediated immunosuppressive pathways in canine demodicosis. *Parasitology*, 144(10): 1412-1416.
- Kuznetsova, E., Bettenay, S., Nikolaeva, L., Majzoub, M., and Mueller, R. (2012). Influence of systemic antibiotics on the treatment of dogs with generalized demodicosis. *Vet. Parasitol.*, 188(1-2): 148-155.
- Ludwicka, A., Ohba, T., Trojanowska, M., Yamakage, A., Strange, C., Smith, E.A., and Silver, R. M. (1995). Elevated levels of platelet derived growth factor and transforming growth factor-beta 1 in bronchoalveolar lavage fluid from patients with scleroderma. *The J. Rheumatol.*, 22(10): 1876-1883.
- Mosmann, T.R., and Sad, S. (1996). The expanding universe of T-cell subset: Th1, Th2 and more. *Immunol. Today*, 17(3): 138-146.
- Mueller, R.S. (2004). Treatment protocols for demodicosis: an evidence-based review. *Vet. Dermatol.*, 15(2): 75-89.
- Patel, J.K., Parwari, M., Nauriyal, D.S., and Joshi, C.G. (2018). Assessment of cytokine profile in the peripheral blood mononuclear cells of dogs with generalized demodicosis. *Indian J. Vet. Med.*, 38(1&2), 67-71.
- Ravera, I., Ferreira, D., Gallego, L.S., Bardagi, M., and Ferrer, L. (2015). Serum detection of IgG antibodies against *Demodex canis* by western blot in healthy dogs and dogs with juvenile generalized demodicosis. *Res. in Vet. Sci.*, 101: 161-164.
- Rosenkranz, S., and Kazlauskas, A. (1999). Evidence for distinct signaling properties and biological responses induced by the PDGF receptor α and β subtypes. *Growth Factors*, 16(3): 201-216.
- Rothe, M., and Falanga, V. (1989). Growth factors: their biology and promise in dermatological diseases and tissue repair. *Archives of Dermatol.*, 125(10): 1390-1398.
- Shrestha, D., Thapa, B., Rawal, G., Dhakal, S. and Sharma, B. (2015). Prevalence of demodectic mange in canines of Kathmandu Valley having skin disorder and its associated risk factors. *Int J. Appl. Sci. & Biotech.*, 3(3): 459-463.
- Singh, S.K., Dimri, U., Sharma, M.C., Sharma, B., and Saxena, M. (2010). Determination of CD4+ and CD8+ T cells in the peripheral blood of dogs with demodicosis. *Parasitology*, 137(13): 1921-1924.
- Snedecor, G.W., and Cochran, W.G. (1994). *Statistical Methods*. 14th edn. Oxford and IBH Publishing House, New Delhi, India.
- Tani, K., Morimoto, M., Hayashi, T., and Taura, Y. (2002). Evaluation of cytokine messenger RNA expression in peripheral blood mononuclear cells from dogs with canine demodicosis. *J. Vet. Med. Sci.*, 64(6): 513-518.
- Walton, S.F., Beroukas, D., Roberts-Thomson, P., and Currie, B.J. (2008). New insights into disease pathogenesis in crusted (Norwegian) scabies: the skin immune response in crusted scabies. *British J. Dermatol.*, 158: 1247-1255.
- Yarim, G.F., Yagci, B.B., and Ciftci, G. (2013). Increased circulating concentrations of PDGF-BB and TGF- β 1 in canine generalised demodicosis. *Revue de Medecine Veterinaire*, 164: 13-17.

