

# Increased circulating concentrations of PDGF-BB and TGF- $\beta$ 1 in canine generalised demodicosis

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## SUMMARY

The purpose of this study was to investigate the changes of circulating concentrations of platelet derived growth factor BB (PDGF-BB) and transforming growth factor beta 1 (TGF- $\beta$ 1) in dogs with generalised demodicosis. Fifteen dogs with generalised demodicosis diagnosed on clinical findings and microscopic examination of the cutaneous scrapings and 10 healthy dogs were included in the study. Circulating concentrations of PDGF-BB and TGF- $\beta$ 1 were measured by commercial specific ELISA assays. Marked and significant increases in plasma PDGF-BB and in serum TGF- $\beta$ 1 concentrations were evidenced in diseased dogs. Circulating PDGF-BB concentrations were closely and positively associated with TGF- $\beta$ 1 concentrations in affected dogs ( $r = 0.92$ ,  $p < 0.001$ ). These results indicate that the increased concentrations of circulating PDGF-BB and TGF- $\beta$ 1 play a pivotal role in the pathogenesis of the canine demodicosis.

**Keywords :** dog, generalised demodicosis, PDGF-BB, TGF- $\beta$ 1, immune response, skin repair.

## RESUME

**Augmentation des concentrations circulantes de PDGF-BB et TGF- $\beta$ 1 au cours de la démodicose généralisée chez le chien**

Le but de cette étude était d'étudier les variations des concentrations circulantes du PDGF (Platelet derived growth factor)-BB et du TGF (Transforming growth factor)- $\beta$ 1 chez les chiens atteints d'une démodicose généralisée. Pour cela, 15 chiens présentant une démodicose généralisée diagnostiquée sur la base des signes cliniques et de l'examen microscopique des frottis cutanés et 10 chiens sains ont été inclus dans l'étude. Les concentrations circulantes de PDGF-BB et TGF- $\beta$ 1 ont été mesurées au moyen de trousse de dosage ELISA commercialisées spécifiques du chien. Des augmentations marquées et significatives des concentrations plasmatiques du PDGF-BB et des concentrations sériques du TGF- $\beta$ 1 ont été mises en évidence chez les chiens malades. De plus, les concentrations circulantes de PDGF-BB et de TGF- $\beta$ 1 ont été fortement et positivement corrélées chez les chiens affectés ( $r = 0.92$ ,  $p < 0.01$ ). Ces résultats indiquent que l'augmentation des concentrations circulantes de PDGF-BB et TGF- $\beta$ 1 jouent un rôle central dans la pathogénie de la démodicose canine.

**Mots-clés :** chien, démodicose généralisée, PDGF-BB, TGF- $\beta$ 1, réponse immune, réparation de la peau.

## Introduction

Canine generalised demodicosis is severe and inflammatory skin disease of dogs mostly caused by the mite *Demodex canis* and is clinically characterised by the severe itching, alopecia, crusting, follicular papules and pustules [8, 37]. The disease has been divided into two forms based on the extent of the affected body area; localised and generalised. In localised form, skin lesions are in one body area, in face or feet and prognosis is good. Generalized form is characterized by skin lesions spread over the whole body and have a poor prognosis [37]. Although the clinical findings are well defined, the pathogenesis of the generalised demodicosis is not fully known. Increasing evidence suggests that cell-mediated immune responses play a key role in the disease development [4, 7, 11, 37]. As a result of the inflammatory events, the release of growth factors from cells such as T cells, platelets, macrophages, lymphocytes, fibroblasts and endothelial cells is stimulated [16, 21, 24, 30].

Platelet derived growth factor (PDGF) is stored in the  $\alpha$  granules of platelets from which is released after activation

of this cells and variety of other cell types including macrophages, fibroblasts, keratinocytes, endothelial cells and connective tissue cells [1, 34] and plays a pivotal role in cell growth, division, migration and proliferation [10, 33]. Moreover, PDGF not only acts as chemoattractant for monocytes, fibroblasts, neutrophils and myocytes [10, 34], but also contributes to wound healing process [12, 22, 25]. Upregulation of PDGF type  $\beta$  receptor expression has been implicated in skin of patients with systemic sclerosis which is an autoimmune disease of the connective tissue [17].

Transforming growth factor beta 1 (TGF- $\beta$ 1) is produced by T cells, platelets, macrophages, keratinocytes and lymphocytes and regulates their proliferation, differentiation and survival [21, 24, 40] and also stimulates monocyte chemotaxis and growth factor production [42]. TGF- $\beta$  plays an important role in the synthesis of matrix proteins such as fibronectin and collagen [12, 14, 31].

Many pathological conditions in humans and animals may cause an increase of the circulating concentrations of growth factors such as PDGF-BB and TGF- $\beta$  [6, 20, 27]. It has

	Breed	Sex	Age (months)
<b>Affected group (generalised demodicosis)</b>	Kangal	Female	18
	Kangal	Female	24
	Kangal	Female	36
	Border collie	Male	18
	Border collie	Male	24
	German shepherd	Female	12
	German shepherd	Female	18
	Yorkshire terrier	Male	24
	Yorkshire terrier	Female	17
	Golden retriever	Female	36
	Golden retriever	Female	24
	English setter	Female	20
	Catal burun	Male	18
	Labrador	Female	30
	<b>Control group</b>	Yorkshire terrier	Male
Yorkshire terrier		Female	26
German shepherd		Male	20
German shepherd		Male	22
German shorthaired pointer		Male	17
Labrador		Male	26
Labrador		Female	30
Rottweiler		Male	24
Mixed breed		Female	20
Mixed breed		Female	36

TABLE I: Signalment of dogs with generalised demodicosis (n = 15) and healthy dogs (n = 10).

been documented that overexpression of TGF- $\beta$  in peripheral blood mononuclear cells from dogs with canine demodicosis infection [41]. So far, there are no reports evaluating the circulating PDGF-BB and TGF- $\beta$ 1 concentrations in canine generalised demodicosis. The aim of the present study is to evaluate the PDGF-BB and TGF- $\beta$ 1 concentrations in canine generalised demodicosis.

## Materials and methods

### ANIMALS AND SAMPLING

Twenty-five stray dogs, males and females, belonging to several breeds, 1 to 3 years old (Table I) obtained from the various local dog shelters in Ankara city, Turkey, during April to July 2010 were used in this study. Fifteen dogs had typically clinical signs of generalised demodicosis, including alopecia, scales, crusts, erythema, widespread folliculitis and hyperpigmentation and constituted the affected group. The control group consisted of 10 clinically healthy dogs. The clinical diagnosis of demodicosis was confirmed by microscopic examination of deep skin samples collected from affected areas of dogs. *Demodex canis* mites were found in skin scrapings from affected areas.

Blood samples were collected by venipuncture from the cephalic vein into plain glass tubes for preparation of serum samples, and into tubes containing sodium citrate as an anticoagulant for preparation of plasma samples. The

samples were transferred immediately to the laboratory. Plasma was promptly separated from blood by centrifugation at 1000 x g for 15 minutes at 4°C. The blood samples for serum collection 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. Prepared plasma and serum were immediately stored at -80°C until analyses.

### MEASUREMENTS OF PDGF-BB AND TGF- $\beta$ 1 CONCENTRATIONS

Concentrations of plasma PDGF-BB and serum TGF- $\beta$ 1 were measured by enzyme-linked immunosorbent assay (ELISA) using the canine PDGF-BB and TGF- $\beta$ 1 assay kits (Uscn Life Science Inc., Wuhan). Each assay was performed in triplicate, according to the supplier's instructions. The ELISA plates were analyzed using Microplate Reader (Digital and Analog Systems, RS 232, Roma, ITALY) at an optical density of 450 nm. The detection limits for the assays were 3.9 ng/L for plasma PDGF-BB and 7.8 ng/L for serum TGF- $\beta$ 1. Intra-assay and interassay coefficients of variation were 4.9% and 7.8%, respectively, for the PDGF-BB dosage; 3.9% and 6.4%, respectively, for the TGF- $\beta$ 1 dosage. Standard solutions ranged from 15.6 to 4000 ng/L for the PDGF-BB test and from 31.2 to 2000 ng/L for the TGF- $\beta$ 1 test.

### STATISTICAL ANALYSIS

The statistical analysis was conducted using SPSS statistical software for Windows (SPSS-PC, SPSS Inc., Chicago, Illinois,

USA). The values of PDGF-BB and TGF- $\beta$ 1 are presented as the mean  $\pm$  standard deviation (SD). Measurements were compared by the non-parametric Mann-Whitney U-test. Pearson's correlation was performed to investigate the possible associations between PDGF-BB and TGF- $\beta$ 1. A p value of  $< 0.05$  was considered statistically significant.

## Results

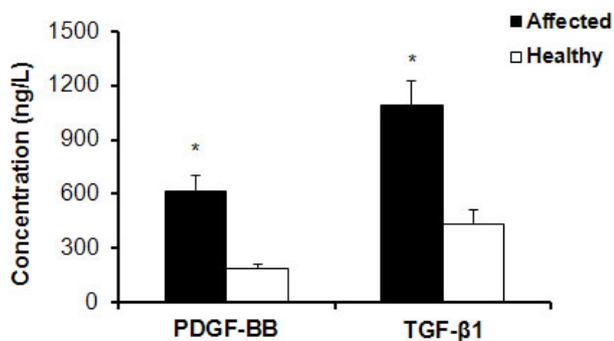


FIGURE 1 : Concentrations of plasma PDGF-BB and serum TGF- $\beta$ 1 in dogs with generalized demodicosis (n = 15) dogs and healthy ones (n = 10). Results are expressed as means  $\pm$  standard deviations. \*p < 0.001

As shown in figure 1, the circulating PDGF-BB and TGF- $\beta$ 1 concentrations were dramatically increased in dogs with generalised demodicosis compared to the healthy controls (p < 0.001). Moreover, there was a close and positive correlation between the circulating concentration of PDGF-BB and TGF- $\beta$ 1 in dogs with generalised demodicosis (r = 0.92, p < 0.01) (figure 2). Particularly, all dogs exhibiting a PDGF-BB concentration above 750 ng/L also had a high TGF- $\beta$ 1 concentration (above 1000 ng/L).

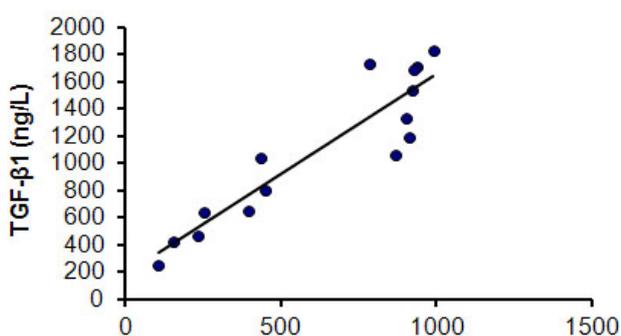


FIGURE 2 : Correlation between circulating PDGF-BB and TGF- $\beta$ 1 concentrations in dogs with generalised demodicosis (n = 15).

## Discussion

Numerous studies have documented that immunosuppressive factors are responsible for the canine demodicosis [2, 3, 19, 43] and T cells play an important role in the disease [7]. Various studies have shown that T lymphocytes synthesize TGF- $\beta$  [21, 24, 40]. It has been reported that TGF- $\beta$  have critical role in proliferation and

differentiation of mesenchymal cells, extracellular matrix production, wound healing and immunosuppression [24, 30, 31]. TGF- $\beta$  has also been reported to play significant roles in activation and proliferation of T lymphocytes [5, 16, 36]. It has been established that the TGF- $\beta$  is elevated in many pathological conditions in humans and animals [6, 20, 27].

In the present study, circulating concentrations of TGF- $\beta$ 1 were found to be markedly increased in dogs with generalised demodicosis compared to healthy ones. TANI et al. [41] reported that TGF- $\beta$  mRNA expression in peripheral blood mononuclear cells is higher in dogs with localised demodicosis than healthy ones and they also indicated that this may play a key role in the differentiation of 2 forms of the disease. Recently, it has been demonstrated that dogs with generalised demodicosis have a lower CD4+/CD8+ T cell ratio than dogs having localised demodicosis [38]. In parallel, WALTON et al. [44] have reported that the infiltration of CD8+ T lymphocytes coupled to over-expression of TGF- $\beta$ 1 in dermis of severe crusted scabietic men. TGF- $\beta$  also stimulates collagen production by fibroblasts and induces the maturation of keratinocytes [13, 15, 18, 23, 29]. SPORN et al. [39] reported that subcutaneous implantation of transforming growth factors obtained from bovine in the backs of rats resulted in elevated total protein, accumulated collagen and repaired DNA. When these studies are taken into consideration, the increased TGF- $\beta$ 1 concentration in dogs with generalised demodicosis in our study may be associated with an increase in CD8+ T cell population due to canine generalised demodicosis and may be related to skin repair process in this disease.

PDGF-BB is known to stimulate growth, division, migration and proliferation of the cells [10, 33]. PDGF exerts a stimulating effects on collagen synthesis [35] and the pivotal role of PDGF-BB in the wound healing process in postirradiation surgical incisions has been well documented [25]. Additionally, administration of PDGF into wound site has been reported to be effective in the modulation of the wound healing process in experimental wound model in pigs [22]. PDGF-BB has been reported to accelerate new tissue formation in experimentally diabetic rats [9]. NARAYANAN and PAGE [26] showed that synthesis of collagen in cultured human gingival fibroblasts was stimulated by exogenous PDGF. A similar effect have also been found on collagen synthesis by TGF- $\beta$  [13-15, 32]. A significant result in present study was that generalised 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 generalised demodicosis suggest that this growth factor may play a role in the immune response to *Demodex canis* and skin repair mechanism in this disease.

A strong positive correlation was found between PDGF-BB and TGF- $\beta$ 1 concentrations in dogs with generalised demodicosis. PDGF-BB has been reported to promote

TGF- $\beta$ 1 overexpression in vascular smooth muscle cell [28]. Our results on dogs with canine demodicosis are in accordance with those of PAN et al. [28]. Studies have shown alterations of PDGF-BB and TGF- $\beta$ 1 in the skin disorders. Overexpression of PDGF type  $\beta$  receptor has been demonstrated in skin of patients with systemic sclerosis which is an autoimmune disease of the connective tissue [17]. Additionally, increased PDGF and TGF- $\beta$ 1 concentrations have been observed in patients with scleroderma [20].

Although canine generalised demodicosis is recognised as severe skin disease of dogs, studies on growth factors in this disease have been limited. To our knowledge, no published research has reported the circulating concentrations of PDGF-BB and TGF- $\beta$ 1 in canine demodicosis. In the present study, increased circulating PDGF-BB and TGF- $\beta$ 1 concentrations were found in dogs with generalised demodicosis compared to healthy controls. Probably, the increase in these growth factors is related to the immunological response of the dogs to the mite *Demodex canis* and skin repair process in this disease. In conclusion, the results of the present study suggest that cellular immune responses in affected dogs may be exacerbated by *Demodex canis* and increased concentrations of circulating PDGF-BB and TGF- $\beta$ 1 play a pivotal role in the pathogenesis of the canine demodicosis.

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