

Serum biochemical and hematological parameters in dogs with benign prostatic hyperplasia (BPH)

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Abstract:

BACKGROUND: Clinical prostatic diseases occur in 80% of dogs over 5 and 95% over 9 years of age. It seems that benign prostatic hyperplasia (BPH) affects Scottish terriers more severely than the other breeds. **OBJECTIVES:** This study aimed to evaluate the changes of biochemical and hematological parameters in BPH dogs. **METHODS:** Blood samples were collected from 10 male dogs (mostly terrier or mix) older than five years with weight 8.91 ± 2.5 kg. suffering from BPH which referred to Small Animal Hospital of the Veterinary Faculty of Tehran University. The diagnosis of BPH was based on clinical, laboratory surveys and ultrasonography. 10 normal male dogs with same age, breed and weight were selected as control group. Then serum acid phosphatase (TAP and PAP), CRP, urea, creatinine, total protein, albumin, globulins and hematological parameters were assayed and the results were analyzed by Independent student T-test. Also, Pearson's linear correlation test was used to determine the correlation between TAP, PAP, CRP and ESR with length and width of prostate. **RESULTS:** The length ($p=0.008$), width ($p=0.01$) of prostates was significantly higher in dogs suffering from BPH compared to the healthy dogs. TAP and PAP levels significantly increased in all dogs in BPH group (approximately 6 times) compared to the controls ($p=0.001$). Moreover, serumic CRP concentration was elevated in some BPH dogs (approximately 6 times) ($p=0.001$). While there was significant ESR elevation in some of the dogs in disease group compared to the normal dogs, no significant difference was observed in other biochemical and hematological parameters between two groups ($p>0.05$). There was a highly significant correlation between serum TAP and PAP ($p\leq 0.01$) with prostate's length and width which was more than CRP. **CONCLUSIONS:** The serum acid phosphatase, CRP and ESR were elevated in BPH dogs but the increase in serum acid phosphatase was more important than the others. It is recommended that each laboratory should use its own values of acid phosphatase in dogs.

Introduction

The prostate gland is a bi-lobed structure that lies within the pelvis just behind the bladder and directly below the rectum (Francey, 2010). Clinical prostatic diseases occur only in humans, chimpanzee, dogs (Steiner et al., 1999) and, rarely, in cats (Francey, 2010). It has been reported that it may occur in 80% of intact dogs over 5 and 95% over 9 years of age. BPH seems to affect Scottish terriers more severely than other breeds. Prostatic tumors are rather uncommon in dogs (Francey, 2010) and prostatic adenocarcinoma is the main prostatic neoplasia in humans and dogs (Swinney, 1998).

Due to the high frequency of prostatic lesions in dogs, "in vivo" diagnostic methods should be established in order to determine the specific lesion, treatment and prognosis. The human prostate gland secretes many glycoproteins. Prostatic Acid Phosphatase (PAP) and Prostatic Specific Antigen (PSA) levels are high in human patients with BPH (Corrazza et al., 1994; Wadstrom et al., 1984). PAP and PSA have been used for the identification of human prostate cancer (McEntee et al., 1987). Although PSA has been identified in normal, hyperplastic and neoplastic canine prostatic cells, it was not detected in serum or seminal from healthy dogs or those with prostatic disease (Francey, 2010). However, Amorim et al 2004 tried to evaluate PSA in serum and urine of normal dogs with human detector. Their methodology was monoclonal antibodies raised against human, thus, the sensitivity of the test for dogs was weaker (Amorim et al., 2004).

Similar to human, PAP level is hormone dependent, and its levels are variable with age (Aumüller et al., 1987; Corrazza et

al., 1994); however, Gadelha et al (2013) reported that PAP levels did not correlate with age (Gadelha et al 2013). Quantitative changes in canine PAP are less remarkable than in human, nevertheless, they are important in the evaluation of the prostatic epithelial cells secretory activity (Aumüller et al., 1987; Corrazza et al., 1994),

C-reactive protein (CRP) is an Acute Phase Protein (APP) which participates in the acute phase response. This response is a nonspecific inflammatory reaction of the host that occurs shortly after any tissue injury. Therefore, it can be considered as one of the earliest markers for any pathologic process or disease (Ceron et al., 2005; Kaneko, 2010). CRP is thought to possess high diagnostic specificity, as it is only induced by proinflammatory hypercytokinaemia. These properties should facilitate the use of canine CRP as a marker of systemic inflammatory activity for routine diagnostic, monitoring and screening purposes (Kjelgaard-Hansen et al., 2013; Kaneko, 2010). Production and response of APPs varies depending on the species. For example, a strong response occurs with CRP in dogs; however, in cats, significant increases of CRP have not been detected after an inflammatory stimulus. (Ceron et al., 2005).

The results of different experiments on whether the measurement of acid phosphatase could be used as a diagnostic test for prostatic disease (Gadelha et al., 2013) are not reliable. However, for veterinary patients (BPH dogs), the activity of acid phosphatase is not routinely used for screening purposes in Iran and there are no data about blood serum biochemical and hematological parameters in BPH dogs. Therefore, the aim of current study was to evaluate related blood serum biochemical (TAP, PAP, CRP,

urea, creatinine, total protein, albumin and globulin) and hematological parameters in dogs suffering from BPH.

Materials and Methods

The survey was conducted on dogs which referred to Small Animal Hospital of the Veterinary Faculty of Tehran University. Blood and urine samples were collected from 10 male dogs older than five years old suffering from Benign Prostatic Hypertrophy (BPH). The breeds of dogs were mostly Terrier or mix with Spitz, Shih Tzu, and Pekingese and one German shepherd. The average weight and age were 8.91 ± 2.5 kg and 113.9 ± 14.19 months, respectively. The diagnosis of benign prostatic hypertrophy was based on prostatic enlargement (judged by rectal digital palpation and ultrasonography) accompanied by one or more of the following signs: dysuria, difficulty in defecating, hematuria and sanguineous discharge from the tip of the penis unrelated to urinating. Moreover, cytology of the urine sediment was performed for diagnosis of prostatic adenocarcinoma (Corrazza et al., 1994).

Ten normal male dogs with same age, breed and weight were selected as control group. The control animals were examined according to the clinical, hematological and ultrasonography evaluation.

In all dogs, volume of each prostate was estimated by using the following formula (Francey, 2010):

$$\text{Volume [cm}^3\text{]} = (0.867 \times \text{BW [kg]}) + (1.885 \times \text{age [year]}) + 15.88.$$

Blood samples were collected into EDTA (for hematologic), citrate tubes (for ESR) and without anticoagulant for serum separation and biochemical assessment. For mea-

suring biochemical parameters, the blood tubes were inserted in water and ice flask. The whole blood was allowed to clot for 30 minutes at 25° C and then centrifuged at 2000 rpm for 15 minutes at 4 C°. The serum layer was pipetted off and then stored on ice and assayed the same day. Colorimetric methods (Commercial Iranian kit-Zeist Chimi) were used for measurement of serum Total Acid Phosphatase (TAP) and Prostatic Acid Phosphatase (PAP).

CRP concentration was measured by using a commercial canine CRP ELISA kit (Tridelta Development Ltd, Kildare, UK). The serum samples were kept at -20 C° until assayed.

In addition, other serum biochemical analysis such as urea, creatinine, total protein, and albumin were determined by using an Elitech automated analyzer (SELECTRA prom, France) and commercial kits (Pars Azmoon, Tehran, Iran). Globulin concentration was calculated by subtracting the serum albumin from the total protein concentration (Thrall et al., 2012).

ESR was measured by Westergren method. It was done by transferring 1.8 mL of blood into a vial containing a defined amount of 3.8% sodium citrate (0.2 mL) solution. The blood was mixed thoroughly and sucked into the Westergren ESR tube to the top mark (0 mark). The tube was then stood vertically for 1 hour and the level of the red cells was read as the ESR (Thrall et al., 2012).

Hematological parameters including total erythrocyte count (RBC), hematocrit value (HcT), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and total white blood cells (WBC) were determined by the NIHONKOH DEN

hematology analyzer (Italy). Differential leukocyte counts were also estimated manually as described by Meyer and Harvey (2004).

For statistical analysis, Independent t-Test (SPSS version16) was used to compare and determine statistical differences in laboratory-obtained values between two groups. All values were expressed as mean and Standard Error (SE). Also, Pearson's linear correlation test was used to determine the correlation between TAP, PAP, CRP, and ESR with length and width of prostate. A p value <0.05 was considered significant.

Results

In dogs suffering from BPH compared to healthy dogs, the prostates were higher, wider and larger (p<0.05) (Table 1).

Mean serum TAP and PAP activities were increased in BPH group significantly (71.02±2.91 and 32.19±2.83 U/L respectively) compared to control (12.14±1.63 and 5.38±1.11 U/L respectively) (p=0.001). Statistical analysis revealed that there were significant differences in serum CRP between two groups. Mean serum CRP concentration was increased in BPH group (5.5±1.4 mg/l) compared to the control (0.9±0.2 mg/l) (p=0.001) (Table2). Although serum total protein, globulin and urea were increased in BPH group, it was not significant (p>0.05). Moreover, there was no significant difference in other biochemical parameters between control and BPH dogs (p>0.05) (Table3).

There were significant rises in ESR of BPH group compared to normal dogs. Mean ±SE of ESR in BPH and control dogs was 8.8±0.57 and 6±0.47 mm/h respectively. Additionally, as it can be seen in Table 3, there were no significant differences in

Table 1. The mean±SE of length, width and volume of prostates in sonography of BPH and control groups. *-Significant.

	group BPH mean±SE (min-max)	Control group mean±SE (min-max)	p value
Length of prostate (mm)	45.78±4.78 (31.11-71.57)	28.51±2.93 (12.8-36.47)	0.008*
Width of prostate (mm)	45.78±4.78 (19.9-66.1)	26.05±2.18 (14.8-35.04)	0.01*
Volume of prostate (cm3)	41.5±3.05 (29-62)	28.5±2.93 (12.8-36.47)	0.056

Table 2. Serum acid phosphatase (TAP and PAP) activities and CRP in BPH and control groups. *-Significant.

p value	Control group mean±SE (min-max)	BPH group mean±SE (min-max)	
	12.14±1.63 (3.8-19.7)	71.02±2.91 (56.3-85)	0.001*
Serum TAP (U/L)			
	5.38±1.11 (0.9 - 13.1)	32.19±2.83 (21.2-52)	0.001*
Serum PAP (U/L)			
	0.9±0.2 (0.2 -2.3)	5.5±1.4 (1.6-10.3)	0.001*
CRP (mg/l)			

Table 3. Serum total protein, albumin, globulin, urea and creatinine in BPH and control groups.

	Control group mean±SE (min-max)	BPH group mean±SE (min-max)	p value
total protein (g/dl)	6.86±0.2 (5.96-8.4)	7.08±0.34 (5.73-9.4)	0.59
albumin (g/dl)	3.86±0.18 (2.71-4.43)	3.6±0.18 (2.56-4.18)	0.33
globulin (g/dl)	3±0.28 (1.59-4.32)	3.48±0.35 (1.84-5.4)	0.3
urea (mg/dl)	31.6±3.89 (20-52)	35.35±3.88 (19-57)	0.5
creatinine (mg/dl)	0.97±0.14 (0.5-1.7)	0.88±0.09 (0.4-1.3)	0.6

RBC indices, WBC counts, and differential leukocyte count.

There was a severe significant correlation between serum TAP and PAP (p≤ 0.01) with prostate's length and width which was more than CRP. Only correlation between CRP and width of prostate was significant (p=

Table 4. Hematological parameters in BPH and control groups. *-Significant.

	Control group mean±SE (min-max)	group BPH mean±SE (min-max)	p value
ESR mm/h	6±0.47 (4-9)	8.8±0.57 (6-12)	*0.001
HcT (%)	45.17±2.32 (33.8 -59.1)	41.29±2.34 (26.8-52.8)	0.25
RBC (106µl)	6.92±0.34 (5.5-8.9)	6.52±0.38 (4.7-8.6)	0.45
Hb (g/dl)	15.06±0.73 (-19 11.7)	15.55±0.55 (11.9-18)	0.6
WBC (103µl)	9.12±0.81 (5.82-14)	9.32±1.54 (4.4-18.9)	0.9
Neut.seg (%)	6377.3±812.8 (3621- 11900)	6276±1271.2 (2412-15050)	0.94
Neut.band (%)	70.9±33.09 (0-280)	65.8±30.27 (0-237)	0.91
Monocyte (%)	218±51.95 (0-473)	415.4±180.41 (0-1890)	0.3
Lymphocyte (%)	2175.6±269.9 (729- 3708)	2163.3±410.5 (616-5292)	0.98
Eosinophil (%)	269±127.18 (0-1235)	392.8±101.12 (0-957)	0.45
Basophil (%)	13.2±13.2 (0-132)	12.7±12.7 (0-127)	0.97

Table 5. Correlation between TAP,PAP,CRP and ESR with length and width of prostate. *-Significant.

	length	width
TAP	r=0.593 p=0.007*	r=0.562 p=0.012*
PAP	r=0.617 p=0.005*	r=0.582 p=0.000*
CRP	r=0.460 p=0.085	r=0.547 p=0.035*
ESR	r=0.322 p=0.179	r=0.415 p=0.077

0.03). There was no correlation between ESR and prostate's size (Table 5).

Discussion

The dog's prostatic glands are the best natural models for the study of human prostatic diseases (Amorim et al., 2004) because they

are the animal models that spontaneously develop prostatic hyperplasia (Gadelha et al., 2013).

Benign Prostatic Hyperplasia is best treated by the administration of estrogens or by castration (Noakes et al., 2001), because they remove the source of androgens responsible for maintaining prostatic size (Feldman and Nelson, 2004). We used castration for dogs suffering from BPH. Our experience showed that maximum atrophy of prostate was noted after 6 to 9 weeks of therapy. After castration in the dog, retained prostate exhibited a similarly high incidence of neoplasia. Thus, all of the BPH dogs in current study were treated by castration and their clinical signs improved.

Quantitative changes in canine PAP are less remarkable than in human, nevertheless, they are important in the evaluation of the prostatic epithelial cells secretory activity (Aumüller et al., 1987). In our study, serum TAP and PAP levels were particularly elevated (approximately 6 times) in all dogs with benign prostatic hypertrophy. Corrazza et al (1994) found that PAP serum concentrations were particularly elevated in dogs with benign prostatic hypertrophy, which had the same result as the current experiment. However, they showed that serum concentration of TAP and PAP increased 0.5 and 3 times respectively in some dogs suffering from BPH. In addition, they mentioned that elevated TAP and PAP is probably due to a degeneration of the prostatic secretory epithelial cells induced by an increased dihydrotestosterone concentration within the gland (Corrazza et al., 1994). Salo et al (1990) have concluded that intracapsular cancer does not elevate serum acid phosphatase levels as it was determined by radioimmunoassay or an enzymatic method.

BPH alone leads to significant rises in PAP concentration. The degree of BPH correlates with PAP level (Salo et al., 1990); however, Corrazza et al (1994) described that dogs with prostatic adenocarcinoma had significantly higher TAP, PAP serum concentrations than dogs with benign prostatic hypertrophy, normal dogs and dogs with nonprostatic disease (Corrazza et al., 1994).

In the present study, the activity of acid phosphatase was higher than the values obtained in other studies. The results of Gadelha et al. (2013) showed that the PAP values in the serum were lower than Corazza et al. (1994) and Amorim et al. (2004). As Gadelha et al (2013) described previously, this could be explained by the difference in the reagent used by the different laboratories. Also, the difference in dog's breed may be important, and it is recommended that each laboratory should establish and use its own values (Gadelha et al., 2013; Thrall et al., 2012).

C-reactive protein (CRP) is a major acute phase protein in dogs characterized by low physiological level, a marked and fast increase shortly after a systemic inflammatory stimulus (Kjelgaard-Hansen et al., 2013). Although the acute phase response, which by definition only lasts a few days, seems to play a positive role in the innate host defense mechanisms, increases in APPs have also been described in chronic inflammation (Ceron et al., 2005). Moreover, CRP can be used as a monitor and quantitative marker of the inflammatory stimulus of aseptic elective soft tissue surgery (Kjelgaard-Hansen et al., 2013).

APPs concentration in adult dogs should be interpreted with caution, as they can be influenced greatly by analytic condition. This is particularly important for APPs due

to the lack of reference materials for international harmonization of assays in small animals (Eckersall et al., 1999).

In this study, the result showed that dogs with BPH had significantly higher mean serum CRP concentration (5.5 ± 1.4 mg/l) than normal dogs (0.9 ± 0.2 mg/l) ($p < 0.001$) an increase of approximately 6-fold. Although CRP is a major APP (Francey, 2010) in BPH dogs, CRP rose moderately in current study. As our observation, relatively increased levels of CRP have been found in inflammatory bowel disease (Jergens et al., 2003) and in hematological and neoplastic diseases of the dog (Tecles et al., 2005). Increases of 95-fold were found in CRP concentration after surgical trauma compared with 40- to 50-fold increases after turpentine oil injection (Ceron et al., 2005). In our study, increase of CRP was not shown in all BPH dogs. Only 66.6% of them showed high concentration of CRP in serum which indicated that normal serum concentration of CRP does not rule out BPH in the dog.

The result showed that there was a greater correlation between serum acid phosphatase ($p \leq 0.01$) and prostate's length and width than CRP. Only correlation between CRP and width of prostate was significant ($p = 0.03$).

Although the mean of total protein and globulins in BPH groups was more than healthy dogs and albumin levels in patients was less than the others, there were no significant changes in the above parameters. If the repeated sampling was done, presumably the levels of protein parameters would increase or decrease. Albumin is a negative acute phase protein and its concentration falls gradually during infectious and inflammatory disease (Kaneko et al., 2010), also elevated levels of globulin are reported in

acute and chronic period of disease.(Kane-ko et al., 2010). Although the measurement of albumin is easier and cheaper, currently it seems to have a lower clinical value in diagnosing and monitoring inflammation (Ceron et al., 2005).

From 10 patients, in one out of two with prostatic cyst, actual elevation of protein (9.4 g/dl) was seen due to increasing globulin (5.4 g/dl).

In this study, there was no significant change in serum urea of BPH dogs. In measurement of GFR, the accuracy of serum creatinine was higher than urea because of the lack of tubular reabsorption and minimal tubular secretion (Thrall et al., 2012). In current study, dogs suffering from BPH did not show any elevation of serum creatinine concentration. It seems that kidneys were not significantly affected by BPH. As in our investigation, Rule et al (2005) mentioned that Prostatic enlargement in human was not associated with chronic kidney disease (Rule et al., 2005); however, Sataria and Staskin (2000) described that there was a relationship between BPH and chronic kidney disease due to abnormalities of the lower urinary tract and uretrovesical junction (Sataria and Staskin ,2000).

Erythrocytes sedimentation rate (ESR) is known as acute phase response as CRP. ESR is an indirect measure of the acute phase reaction. Its value lies in the fact that it is a simple and inexpensive laboratory test for assessing inflammation. In human, the researchers believe that it has even been used for the prognosis of noninflammatory condition, such as prostatic cancer, coronary artery disease and stroke(Husain and Kim, 2002).It seems that increased ESR could be important in BHP dogs because in our study, mean of ESR was elevated sig-

nificantly in them (not in all cases). There was no significant change in means of other hematological parameters. Although Corazza et al (1994) mentioned that mild leukocytosis was seen in dogs suffering from BPH (Corazza et al., 1994) our survey showed that only in 2 dogs was WBC increased. They showed neutrophilia without left shift and lymphopenia which was presumably caused by stress.

In conclusion, the serum acid phosphatase, CRP and ESR were elevated in BPH dogs; however, the increase in serum acid phosphatase was more significant than the others. It is recommended that each laboratory should establish and use its own values of acid phosphatase in dogs.

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پارامترهای بیوشیمیایی سرم و خون شناسی سگ‌های مبتلا به هایپرپلازی خوش خیم پروستاتی (BPH)

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چکیده

زمینه مطالعه: بیماری‌های پروستاتیک بالینی در ۸۰٪ سگ‌های بالای ۵ سال و ۹۵٪ بالای ۹ سال رخ می‌دهد. به نظر می‌رسد که هایپرپلازی خوش خیم پروستاتی (BPH)، سگ‌های اسکاتیش تریر را بیشتر از دیگر نژادها تحت تأثیر قرار می‌دهد. هدف: اهداف این مطالعه ارزیابی تغییرات برخی پارامترهای بیوشیمی و خون شناسی در سگ‌های مبتلا به BPH است، زیرا که در ارتباط با برخی از این پارامترها در سگ‌هایی که از BPH رنج می‌برند، اطلاعاتی وجود ندارد. روش کار: به همین منظور، نمونه‌های خون از ۱۰ سگ نر بالای ۵ سال (اکثراً تریر یا مخلوط) با وزن $91/8 \pm 5/2$ kg که از BPH رنج می‌بردند و به بیمارستان دام‌های کوچک دانشکده دامپزشکی دانشگاه تهران ارجاع شده بودند، جمع‌آوری گردید. تشخیص BPH براساس بررسی‌های بالینی، آزمایشگاهی و اولتراسونوگرافی انجام شد. ۱۰ سگ نر سالم با سن، نژاد و وزن مشابه به عنوان گروه کنترل انتخاب شدند. سپس اسید فسفاتاز سرم (PAP و TAP)، CRP، اوره، کراتینین، پروتئین تام، آلومین، گلوبولین و پارامترهای خون شناسی اندازه‌گیری گردید و نتایج بوسیله آزمون T-Student در SPSS تجزیه و تحلیل گردید. همچنین از آزمون همبستگی خطی پیرسون برای تعیین همبستگی مابین CRP، PAP، TAP و ESR با طول و عرض پروستات استفاده شد. نتایج: طول ($p=0/008$) و عرض ($p=0/001$) پروستات به طور معنی داری در سگ‌هایی که از BPH رنج می‌بردند در مقایسه با سگ‌های سالم بیشتر بود. میانگین فعالیت TAP و PAP سرم در همه سگ‌های گروه BPH به طور معنی داری (تقریباً ۶ برابر) در مقایسه با کنترل افزایش یافت ($p=0/001$). همچنین میانگین غلظت CRP سرم در برخی از سگ‌های مبتلا به BPH افزایش داشت (تقریباً ۶ برابر) ($p=0/001$). در ضمن افزایش معنی دار در ESR برخی سگ‌های بیمار در مقایسه با کنترل وجود داشت. همبستگی معنی دار زیادی مابین TAP و PAP با طول و عرض پروستات وجود داشت که بیشتر از CRP بود. نتیجه گیری نهایی: اسید فسفاتاز سرم، ESR، CRP در سگ‌های مبتلا به BPH افزایش می‌یابد اما افزایش اسید فسفاتاز سرم از سایر فاکتورها اهمیت بیشتری دارد. توصیه می‌گردد که هر آزمایشگاه بهتر است از مقادیری که خود برای اسید فسفاتاز سرم سگ‌ها به دست می‌آورد، استفاده کند.

واژه های کلیدی: اسید فسفاتاز، BPH، CRP، سگ، پروستات

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