Review Article

Etiopathology and Blood Biochemistry Alterations in Canine Pyometra: A Review

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Abstract

Canine pyometra is the condition characterized with accumulation of pus/purulent secretions in the uterus of bitches mostly during progesterone dominant phase. It may occur in any breed at any age but nullipara animals of age more than 4-6 years predisposes most. Disease is generally characterized with cystic endometrial hyperplasia. Increased progesterone, good endometrial secretory environment for bacterial growth, bacterial invasion (most promptly E. coli) etc. are key etiological factors. Released endotoxins and synchronized pathogenesis lead to organ damage, altered blood-biochemistry with enzymatic profile with variety of clinical symptoms as mucopurulent vaginal discharge, polyurea, polydypsia, vomition, hyperthermia followed by hypothermia and in more severe case death may occur. Altered blood biochemistry includes increased BUN, hyperproteinemia and hyperglobinemia, elevated level of urea nitrogen, creatinine, total plasma cholesterol, mild to moderate elevation in Alanine aminotransferase (ALT) and Alkalinephosphatase (ALP) and plasma progesterone concentration etc. These etiopathological factors and altered blood biochemical parameters may prove beneficial in diagnosis and prognosis of the case on the basis of which one can adopt better strategies to treat and manage the cases of canine pyometra.

Key words: Blood Biochemistry, BUN, Canine, Cholesterol, Endometritis-Pyometra Complex, Progesterone

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Introduction

Canine pyometra is the condition characterized with accumulation of pus/purulent secretions in the uterus of bitches with an open (open pyometra) or closed cervix (closed pyometra) mostly occur during diestrous i.e. during progesterone dominant phase (Amstutz *et al*., 1998; Baithalu *et al*., 2010). It tends to affect middle and old age unspayed bitches, but can also affect younger females with a mean age of approximately 2 yrs (Baithalu *et al*., 2010) especially who receives hormonal treatments (Whitehead, 2008). A Swedish

study showed that 23.24% to 25% of all bitches developed pyometra before the age of 10 years (Hagman, 2004). One can say that the disease may be developed in bitches at any age. However, there is a normal relationship between nulliparity and pyometra, nulliparous bitches and bitches of more than 4-6 years of age (Jennifer *et al*., 2003) seem to be predisposed (Chastain *et al*., 1999). Some breeds are more prone to uterine infection like; golden retriever, miniature schnauzer, saint bernard, rottweiler and bernese mountain dog etc but some are less prone to develop the disease as german shepherds, dachshunds and Swedish hounds etc (Egenvall *et al*., 2001) while according to Jennifer *et al*. (2003) there is no breed disposition.

The cystic endometrial hyperplasia-pyometra complex (CEH/P), also known as the endometritis-pyometra complex (EPC), is one of the most serious and most common uterine diseases in bitches (Kida *et al*., 2006). Pyometra is a hormonally mediated, generally a post oestrous/diestrous reproductive disorder associated with a variety of life-threatening clinical and pathological manifestations as abnormal uterine endometrium (Amstutz *et al*., 1998), multi-systemic diseases (Bartoskova *et al*., 2007; Bosschere *et al*., 2002) and/or pseudopregnancy (Pretzer, 2008). Normally Serum progesterone increases from 1–3 ng/ml during the LH (Leutinizing hormone) surge to 15–80 ng/ml in cycle days 20 to 35, then slowly declines (Concannon, 2011). Not only estrogen keeps the cervix relaxed for longer period in the luteal phase but also the elevated progesterone level after ovulation provokes endometrial growth (endometrial hyperplasia), glandular secretion (estrogen induces proliferation and progesterone does branching and coiling of the glands responsible for onset of increased secretion) which provides good media for bacterial growth (Baithalu *et al*., 2010), suppresses uterine contractions (Cox, 1970) and suppress the cellular immunity (Sugiura *et al*., 2004) leads to pyometra. Thus, it has been well established that hyperplasia of endometrial glands and reduced myometrial contractions during diestrous due to progesterone increases the risk of pyometra (Orozco *et al*., 2005; Pretzer, 2008). These effects are reported to be cumulative in each subsequent estrous cycle results in more glandular activity and inflammation in the uterus (Ukwueze and Orajaka, 2014).

Some authors consider the difference between cystic endometrial hyperplasia-pyometra (CEH-Pyometra) and metritis based on stage of reproductive cycle. Metritis is developed postpartum, when serum progesterone levels are low while pyometra is usually occurred during diestrous phase, when progesterone level is high (Roberts *et al*., 2003). However, uterine infection may occur at any stage of estrous cycle or during pregnancy (Baithalu *et al*., 2010). Both, the pregnancy in one horn and pyometra in other horn could have developed because of effect of progesterone (Orozco *et al*., 2005; Risso *et al*., 2014) but such cases are uncommon.

Etiopathology

Canine pyometra is characterized by hyperplasia of the endometrium with infiltration of inflammatory cells and it may cover all layers of the uterus (Kempisty *et al*., 2013) along with uterine bacterial infection. It

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consists of three clinical forms as pyometra, chronic endometritis and cystic glandular endometrial hyperplasia (Zdunczyk *et al*., 2006). The pathophysiology may be centred on hormonal imbalance, bacterial invasion, involvement of some growth factors and reduced immunity.

Endometrial hyperplasia is the result of cystic deformation of endometrial glands and stromal proliferation of fibroblasts with inflammatory reaction (De Bosschere *et al*., 2001) which shows increased secretory activity. Cystic Endometrial Hyperplasia (CEH) often precedes the disease, but can also be found in many older bitches without any sign of pyometra (Ukwueze and Orajaka, 2014). Regulation of estrogen and progesterone receptor expression in endometrial glands may play an important role in pathogenesis of pyometra complex in the bitch. Hormonal disorders resulting from the long proliferation phase, persistence of ovarian follicles or ovarian cysts, active ovarian neoplasia and bacterial infections have a remarkable influence on the uterine degeneration and proven to be the main cause of endometritis-pyometra complex (Kida *et al*., 2006; Kempisty *et al*., 2013). Further in normal estrous cycle progesterone induces downregulation of estrogen receptors in endometrium so process of endometrial gland proliferation stopped but this mechanism failed in CEH. Due to increase expression of estrogen, the endometrium remains receptive to even low levels of circulating estrogen. Therefore, the hormone as estrogens which are used in misalliance, promote over proliferation of endometrium and lengthen the period in which the uterine cervix remains open and thus influence the development of the pyometra. Mentioned proliferative and secretive changes are the effect of endometrial overreaction to oestrogens and progesterone (hyperoestrogenisation) (Kempisty *et al*., 2013). More interestingly, the effects of the progesterone are enhanced and stimulatory with estrogen primed uterus (Baithalu *et al*., 2010). Thus, estrogen therapy at the time of high progesterone level may predispose bitches to pyometra. Thus, the hormonal therapies which include either progesterone for estrous suppression or estrogen for estrous induction, pregnancy termination/mismating (Robert *et al*., 2003) or pseudopregnancy may explain the development of pyometra in young bitches. The use of progesterone for oestrus/ovulation synchronization in bitches can also be a cause of pyometra. It is suggested to administer the progesterone only after 50 days gestation as earlier administration can induce pyometra and masculinisation of female foetuses. Bitches which manifest persistent oestrus have poor chances for fertility and great chances to develop pyometra (Kempisty *et al*., 2013).

Escherischia coli, *Staphylococcus* spp., beta-haemolytic streptococci, Pasteurella multocida and Klebsiella spp. are the organisms mostly isolated from pyometra cases, either in pure culture or as mixed infections (Dhaliwal *et al*., 1998). In approximately 90% of cases, *Escherichia* are the main pathological agent (Susi *et al*., 2006; Kumar *et al*., 2016) in dogs and cats (Coggan *et al*., 2008). It has been postulated that *E. coli* along with *Streptococcus* sp are normal flora of vagina which ascend into the uterus through dilated cervix during estrous (Biswas *et al*., 2012) and induce the disease during metoestrus by acting on the progesterone primed endometrium directly via toxins or indirectly by the release of inflammatory mediators (Johnston *et*

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al. 1985; Noakes *et al*., 2001). The uterine infection may also be occurred at the time of coitus as a result of a pre-existing vaginal infection or endometrial hyperplasia, or both (Risso *et al*., 2014). Smith (2006) demonstrated that *E. coli* causes pyometra in canines on day 11 to 20 and 20 to 30 after luteinizing hormone peak, as at that time uterus is most susceptible to infection. It has also been proven that during early metestrous progesterone induces the development of endometrium receptors for *E. coli* and infection at this time provokes the adhesion and colonization of *E. coli* leading to pyometra (Kempisty *et al*., 2013). This bacterium produces endotoxins which has capacity to initiate the cytokine cascade along with release of inflammatory mediators. CNF (cytotoxic necrotizing factor) may be a virulence factor, as its production is associated with severe damage of endothelium and enhanced inflammatory reactions. Also *E. coli* is found normally present in the urine and feces of affected bitches (Tsumagari *et al*., 2005).

Growth factors and their receptors (growth factors receptors, GFs) has been reported to play a significant role in various genital pathologies in bitches (Gama *et al*., 2009). Insulin-like growth factors (IGFs) and the insulin-like growth factor-binding proteins (IGFBP) play role in proliferation of endometrium (Dolka *et al*., 2010) along with growth and development of ovarian follicle and corpus luteum and in turns synthesis of oestrogens and progesterone (Chamberlain and Spicer, 2001) thus possibly involve in endometrium hyperplasia (De Cock *et al*., 2002; Fabian *et al*., 2005). A significant effect of epidermal growth factor (EGF) has also been demonstrated on the proliferation of endometrial cells in the mouse uterus (Komatsu *et al*., 2003; Kida *et al*., 2010). Caron *et al*. (2009) demonstrated that transforming growth factors (TGF-β) undergo expression during regression of the basic decidua layer of uterine mucosa. The levels of EGF and TGFα were found to depend on the phase of the sexual cycle i.e. on sex hormones such as oestrogen and progesterone (Komatsu *et al*., 2003; Tamada *et al*., 2005; Kida *et al*., 2010). It ensures their possible involvement in pathogenesis of canine pyometra.

As prevention against pathogen invasion, the female genital system developed appropriate immune mechanisms. Innate immunity represents commonest and fastest protective mechanism upon pathogen invasion (Silva *et al*., 2010). Innate immunity is based on the activity of Toll-like receptors (TLR), which recognize pathogen associated molecular patterns (PAMP) synthesized by microbes and initiate a inflammatory reaction cascade (Horne *et al*., 2008) responsible for the development of a immune response. They were detected on the endometrial cells in humans as well in cattle (Silva *et al*., 2010). Thus, Toll-like receptors can be taken as marker for identifying the endometrial pathogenic lesions in bitches suspected of endometritis-pyometra complex. CEH/P complex may also be induced by improper uterine biopsies, scarification and uterine irritants such as suture material (Noakes *et al*., 2001). Regarding cytological changes, degenerated endometrial cells with large cytoplasmic vacuoles (Kempisty *et al*., 2013) and decrease in nuclear size are more marked during pyometra in comparison with the normal physiological condition (Groppetti *et al*., 2010).

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Blood Biochemistry

In diseased bitches, elevated WBC-count (peripheral leukocytosis with neutrophilia) often exceeding 30,000 cells/mm^3, degenerative left shift is common clinical findings (Mahesh *et al*., 2014; Ukwueze and Orajaka, 2014). Haematology reveals a mild normocytic, normochromic, nondegenerative anemic condition (Robert *et al*., 2003) which reflects the chronicity of the disease and toxic suppression of bone marrow. The packed cell volume (PCV) may be 30-35% (Baithalu *et al*., 2010) to 63% (Ukwueze and Orajaka, 2014) in cases of pyometra.

Liberated endotoxins lead to organ damage, alter blood-biochemistry with enzymatic profile and display variety of clinical symptoms (Hagman, 2004, Biswas *et al*., 2012). In pyometric bitch's abnormal serum biochemistry as increased BUN, hyperproteinemia and hyperglobinemia with hypoalbuminaemia found to be evident (Dabhi and Dhami, 2006; Hagman *et al*., 2009; Biswas *et al*., 2012 and Gupta *et al*., 2013) which may result due to dehydration and chronic antigenic stimulation of the immune system (Robert *et al*., 2003). Immune complex deposits along the glomerular basement membrane causes a mixed membrano glomerulo-nephropathy (Stone *et al*., 1988) which allows leakage of plasma protein into the glomerular filtrate i.e. urine analysis may show isosthenuria and proteinuria without pyuria or hematuria (Robert *et al*., 2003).

In many cases of canine pyometra they also have a prerenal azotemia. Urea nitrogen and creatinine both are the catabolic waste products normally excreted by the kidneys and therefore both are indicators of glomerular filtration rate (Neel and Grindem, 2000). These could be useful to judge the prognosis of pyometra, since degree of damage to kidneys depends upon the level of toxaemia and dehydration caused due to pyometra (Gupta *et al*., 2013; Kumar *et al*., 2016; Biswas *et al*., 2012). The plasma urea nitrogen level may be >35 mg/dl and creatinine may be > 1.5 mg/dl in pyometric bitches (Sharma 2004; Gupta *et al*., 2013). The plasma concentrations of both urea nitrogen and creatinine decreases within 8-15 days following ovario-hysterectomy surgery as compared to pre-operative values in pyometric bitches and this decline may be because of reversal of toxaemia (Gayakawad *et al*., 1999).

According to Hagman (2004) the total plasma cholesterol concentration remains greater in pyometric bitches than in healthy ones and it is higher in close pyometra than open pyometra which reflect the intrahepatic cholestasis in bitches with pyometra. These changes may be due to hepatocellular damage and cellular hypoxia because of toxaemia and dehydration. Moreover, the cholesterol levels have also been demonstrated as decline significantly following ovario-hysterectomy (Gupta *et al*., 2013; Hagman, 2004; Hagman *et al*., 2009). There may be also mild to moderate elevation in Alanine aminotransferase (ALT) and Alkalinephosphatase (ALP) concentration (Baithalu *et al*., 2010; Gupta *et al*., 2013). Dabhi *et al*. (2007) and Hagman *et al*. (2009) reported increased plasma ALP upto 3-4 times in pyometric bitches as compared to healthy ones. These findings reflect the damage to the vital organs as well bone marrow due to toxaemia in bitches with pyometra. Some authors reported the decreased levels of the enzyme alanine

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aminotransferase (ALT) (may be due to hepatic membrane damage and reduced liver enzyme synthesis) and increased levels of aspartate aminotransferase (AST) associated with pyometra (De schepper *et al*., 1987; Biswas *et al*., 2012). The plasma progesterone concentration is found significantly higher but plasma oestradiol-17β concentration is found lower in pyometric bitches than in normal ones and both the hormone levels are found slightly higher in close pyometra than the open pyometra cases (Karmakar *et al.*, 2002; Dabhi *et al*., 2007; Gao *et al.*, 2011; Gupta *et al*., 2013). However, Nomura *et al.* (1984) and Wakankar (1993) found that the progesterone levels, in general, were not always higher in pyometric bitches than in normal ones in luteal phase.

Thus, elevated levels of plasma BUN, creatinine, proteins, ALT, AP, cholesterol and progesterone etc in pyometric bitches and returning to their normal values after removal of ovaries with uterus (reversal of toxaemia and other pathologies) indicates that these blood biochemical parameters may prove well of diagnostic and prognostic value (Gupta *et al*., 2013). Similarly, well understanding of the cause and development of the disease along with the pathological changes due to it may prove helpful to adopt the better strategies to treat and manage the diseased animal.

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