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Haptoglobin baseline value in jennies and the effect of ovariectomy on its serum concentration

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ABSTRACT

The study was conducted to determine the baseline concentration of serum haptoglobin (Hp) in jennies during the breeding and nonbreeding season and to evaluate the effects of ovariectomy on serum Hp concentrations in jennies. Eighteen adult jennies were divided in three groups: nine jennies (OVA) were ovariectomized using laparoscopic surgery, six jennies (LAP) were exploratory examined by laparoscopic surgery, and three jennies were used as a control group. Blood samples were collected from the animals at Day −6, −2, −1, 0, 1, 2, 5, 8, 15, 22, 29 and 36 of surgery. Serum samples were analyzed by an ELISA specifically developed for determining equine Hp. The mean weekly Hp concentration ranged between 149.76 ± 7.55 and 178.94 ± 6.67 mg/L. The Hp concentrations of clinically healthy jennies revealed no significant variation among time, and there was no effect of reproductive season on Hp concentrations in jennies. Serum Hp concentration was elevated at the first day after operations in the OVA and LAP group. Five days after the operation, the Hp concentration reached the maximum in the LAP and OVA group (278.84 ± 34.22 and 359.88 ± 35.45 mg/L, respectively) and decreased at Day 8 after the operations. On Day 22, 29 and 32 after the operations, the concentration of Hp in LAP and OVA animals was close to its concentration in the control group. In conclusion, Hp is not related to reproductive status of jennies and it can be used as an indicator for cell and tissue damage after surgical operations.

1. Introduction

Acute phase proteins are liver-derived serum proteins, the concentration of which alters in response to infection or inflammation (Eckersall, 1995). Serum amyloid A, fibrinogen and haptoglobin (Hp) are reported to be acute phase proteins in horses (Patterson et al., 1988; Pepys et al., 1989). Hp, an α2-globulin, is useful as a clinical parameter for the evaluation of the occurrence and severity of inflammatory diseases in horses (Kent and Goodall, 1991; Taira et al., 1992) and other species such as sheep, cattle and pigs (Skinner and Roberts, 1994; Eckersall et al., 1996; Chan et al., 2004).

The baseline concentration of serum Hp has been recorded for cattle (Skinner et al., 1991; Uchida et al., 1993; Salonen et al., 1996), horses (Kent and Goodall, 1991; Taira et al., 1992), ponies (Eurell et al., 1993), pigs (Lipperheide et al., 1997), camels (Nazifi et al., 2006) and sheep (Skinner and Roberts, 1994; Aziz and Taha, 1997), but there is no information about the basal concentration of serum Hp in donkeys.

Hp was found in female reproductive system of women and some laboratory and farm animals, it was detected in women uterus (Berkova et al., 2001) and follicular fluid (Porta et al., 1999), rat ovaries (O'Bryan et al., 1997), mice uterus and ovaries (Friedrichs et al., 1995), rabbit uterus (Olson et al., 1997), and buffalo follicular fluid (Bergamo et al., 1995).

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The most important biological function of haptoglobin consists in the host defense responses to infection and inflammation (Dobryszycka, 1997), it acting as anti-inflammatory (Jue et al., 1983), antibacterial (Eaton et al., 1982), antioxidant (Gutteridge, 1987), inhibitor of prostaglandin synthesis (Sadrzadeh and Bozorgmehr, 2004), estrogen-binding properties (Mucchielli, 1982) and it has an effect on vitamin C metabolism (Langlois et al., 1997).

Alterations in serum Hp concentrations have been observed following surgery such as castration (Taira et al., 1992), exploratory laparotomy (Eurell et al., 1993) and splenectomy (Hanzawa et al., 2002). Serum Hp has been reported to increase two to three fold through the first week after surgery (Kent and Goodall, 1991; Hanzawa et al., 2002).

In equine species, bilateral ovariectomy is performed as an elective surgery used to prevent estrus associated behaviors and pregnancy and to manage colic associated with estrus (Trotter and Embertson, 1992; Hooper et al., 1993). It is also a therapeutic procedure in pathological conditions such as ovarian hematoma, teratoma and granulosa cell tumor (Carson-Dunkerley and Hanson, 1997; Palmer, 2002; Catone et al., 2004).

The purpose of this study was to determine the baseline concentration of serum Hp in jennies during the breeding and nonbreeding season. In addition, the effect of laparascopic ovariectomy on serum Hp in jennies was investigated.

2. Materials and methods

2.1. Animals

Eighteen jennies (*Equus asinus*) 3–5 years old (120–150 kg) were used in this study. The animals were housed together in a large paddock in the animal house of the College of Veterinary Medicine, University of Mosul, and had free access to feed and water. The jennies were generally and clinically healthy within normal body temperature, heart and respiratory rate, normal blood picture and free from internal, external and blood parasites. The jennies were divided randomly into three groups; the first group has nine jennies, which were ovariectomized using laparoscopic surgery, the jennies in the second group ($n = 6$) were exploratory examined by laparoscopic surgery, while the animals in the third group ($n = 3$) were used as a control group (no surgical application).

2.2. Pre-surgical preparation

The surgical operation was done during the first week of June. The operations were carried out on three different days, on each day all procedures were applied to three jennies from the first group, two jennies from the second group and one jenny from the third group. The pre-surgical preparations were applied to all animals (including control group). The animals were fasted for 12 h to reduce the volume of intestinal contents and to improve the working area within the abdomen. Both paralumbar fossae were clipped and prepared using routine aseptic techniques. The jennies were sedated with xylazine hydrochloride (Pantex, Holland) (1 mg/kg b.w., i.v.) prior to being restrained in stocks. Local anesthesia was achieved by direct infiltration of the laparoscopic portal sites with approximately 20 mL of 2% lidocaine (Laborate Pharmaceutical, India).

2.3. Surgical procedure

Ovariectomy and laparoscopic exploratory examination were carried out through three instrument portals in the paralumber fossa as described by Aziz et al. (2008).

2.4. Postoperative care

After the operation, the animals (including control group) were given Penicillin–Streptomycin (10,000 IU/kg Penicillin and 10 mg/kg Streptomycin, intramuscularly) (Combi-Kel 20 + 20, Kela Laboratoria, Belgium), daily for 4 days. The skin sutures were removed one week after surgery.

2.5. Blood samples

To estimate the baseline concentration of Hp, blood samples were collected from nine healthy jennies biweekly during the period from November to May. In this period the jennies were passing through an anoestrus season (November–January), transitional period (February–March) and oestrus season (April–May).

To estimate the effect of ovariectomy on serum Hp, blood samples were collected from 18 animals subjected to the surgery experiment at Day −6, −2, −1, 0, 1, 2, 5, 8, 15, 22, 29 and 36 of surgery.

Blood samples were collected into sterile serum separation tubes by jugular venipuncture using an 18 gauge needle. The samples were allowed to clot at room temperature for 30 min. After 24 h of keeping at 5 °C, the blood tubes were centrifuged at 3000 rpm for 15 min and the serum was collected. Serum samples were stored at −20 °C until assayed.

2.6. Hp assay

The ELISA for the determination of equine Hp was developed according to a previously described competitive ELISA for pig Hp (Hiss et al., 2003). The assay reagents were adapted accordingly. In brief, equine serum was purified to obtain Hp. The purified protein was used to create a standard curve and for biotinylation. The Hp antiserum used in the equine ELISA was generated in rabbits immunized against pigs, horses, humans and dogs Hp (Hiss et al., 2003). The assay procedure was as follows: Microtiter plates (EIA Plate 9018, Corning Costar, Cambridge, MA, USA) were coated with sheep anti-rabbit-Fc antibodies (1.5 μg/mL 50 mM $NaHCO_3$ pH 9.6). For the assay, 50 μL of biotinylated equine Hp (1/600 in assay buffer: 0.12 M NaCl, 0.02 M Na_2HPO_4, 0.01 M EDTA, 0.1% hydrolyzed gelatine, 0.005% chlorhexidine digluconate 20%, 0.05% Tween® 20, 0.002% phenol red, 0.1% bovine hemoglobin, pH 7.4) were added per well. In a second step, serum samples (1/10,000 in assay buffer) were added in duplicate (50 μL/well). After