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STUDY OF HEMATO-BIOCHEMICAL CHANGES IN POST-PARTURIENT HEMOGLOBINUREA IN IRAQI LOCAL COWS

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Abstract

The present study was conducted in the Salah-Aldin Governate in Iraq, during the period of one year from 1/1/2019 to 30/12/2019, the study was carried out on eight clinical cases of Post Parturient hemoglobinuria (PPH) in local cows was diagnosed in area affected by PPH with clinical signs of disease after parturition, sufficient from increased respiration and pulse rate, pale mucous membrane, anorexia, color of the urine was red, dark red to coffee-colored. And 10 cows were selected clinically healthy and parturition considered as healthy or control animals. all study cows in average age (4 – 8) years and in early lactation after parturition within 4 weeks, and about in their (3^{rd} - 8^{th}) lactation. times, after the clinical, hematological and biochemical examination the study revealed an increase in highly significant ($P \leq 0.01$) in respiratory and pulse rates in post-parturient Hburia cows when comparative with control cows, while ruminal movement is significant decreased ($P \leq 0.05$) in post-parturient Hburia when compared with control cows. Hematological studies revealed RBCS count, PCV % and Hb values were decreased highly significant ($P \leq 0.01$) and a significant increased ($P \leq 0.05$) in WBCs in post-parturient Hburia while MCV, MCH, and MCHC, was a significant decrease ($P \leq 0.05$) in Post parturient Hburea when compared with control cow.
Biochemical studies revealed increase highly significant ($P \leq 0.01$) in parameters of serum biochemical concentration such as Iron, Glucose, Total protein, AST, Erythrocyte malondialdehyde and significant increase in urea, creatinine, globulin, ALT, and ALP in post-parturient Hburia, while appeared decreased highly significant ($P \leq 0.01$) in concentration parameters of Phosphor, Copper, Erythrocyte reduced glutathione and decreased significant ($P \leq 0.05$) in Albumin concentration in post-parturient Hburia, but didn't revealed the present studies any significant differences in concentrations of Calcium and total proteins between the two groups. In conclusion, a deficiency of phosphorus in the body, after parturition could be responsible for the development of post-parturient haemoglobinuria in cows.
***Keywords*:** Postparturient Haemoglobinurea, Biochemical changes, Hematological changes, Iraqi local Cows.

Introduction

Post-Parturient hemoglobinuria (PPH) also known as hypophosphatemia, post-parturient hemoglobinuria, red water, is an acute life threatening disease of high yielding Cows occurs immediately after parturition with in 4 weeks after parturition, characterized by hypophosphatemia, intra vascular haemolysis, anemia, haemoglobinuria, passing coffee-colored urine, pale mucus membrane, tachypenea, tachycardia, reduced ruminal motility, weakness and marked decrease in milk production (Akhtar *et al.*, 2006; Mahmut *et al.*, 2009; Constable *et al.*, 2017; Purohit *et al.*, 2018). The disease occurring in older early lactating dairy cows, The 3rd – 6th lactation are considered to have the highest incidence of the disease (Bhikane and Syed, 2014; Soren *et al.*, 2014). Phosphorus deficiency in early lactation can be result of high phosphorus losses through the mammary gland with inadequate dietary phosphorus supply hypophosphataemia results in decreased red blood cell (RBC) glycolysis and ATP synthesis which predisposes red blood cells to altered structure and function and an increase in fragility and haemolysis, with resultant haemoglobinaemia and hemoglobinuria (Singari *et al.*, 1991; Constable *et al.*, 2017). The important risk factors to occurrence the PPH are age of animal, stage of pregnancy, number of lactation, postpartum period, ingestion of cruciferous plants were considered putative risk factors for development of PPH in cows (Resum *et al.*, 2017). Also, copper deficiency is an etiological factor of post-parturient hemoglobinuria as its deficiency reduces activity the copper containing enzyme superoxide-dismutase, which is a part of the erythrocyte protective mechanism against oxidative stress (Purohit *et al.*, 2013). Hematologicaly the PPH show decrease RBCs count, low Hb concentration and PCV value (Soren *et al.*, 2014; Sharma *et al.*, 2014; Kumar *et al.*, 2014), and higher erythrocytic sedimentation rate resulted from intra-venous hemolysis (Mahmood *et al.*, 2013). Biochemically, Karapınar *et al.* (2006) detected low level of phosphorus (0.5 and 1.5 mg/ dL) in cows serum with hemoglobinuria. Durrani *et al.* (2010) reported higher urea and creatinine concentrations in the post-parturient hemoglobinuria affected animals. also , These higher level of urea and creatinine is reported by (Tewari *et al.*, 2014) in PPH affected animals which might be due to the damage to kidneys resulting from anemic hypoxia due to extensive intra-vascular hemolysis.

Materials and Methods

The area of the present study conducted in the Salah Aldin Governate in Iraq, during one year from 1/1/2019 to 30/12/2019.

Animals of the present investigation were carried out on eight clinical cases of Post Parturient hemoglobinuria (PPH) in local cows was diagnosed in area affected by PPH with clinical signs of disease after parturition, sufficient from increased respiration and pulse rate, pale mucous membrane, and anorexia. The color of the urine was red, dark red to coffee-colored. For confirmation, the animals were subjected to laboratory examination. Differential diagnosis was ruled out through laboratory tests. Blood samples from ten clinically healthy cows parturition were also collected once to keep them as healthy or control animals. all study cows in average age (4–8) years and in early lactation after parturition, within 4 weeks, and about in their (3^{rd}-8^{th}) lactation times. Clinical examination of all cows put under investigation was performed according to the method

described by (Kelly, 1984; Constable, 2017), Including temperature, respiration, pulse rate, and ruminal movement.

The blood samples collected from all study cows according to (Pugh, 2000; Benjamin, 1978), from the jugular vein in an amount (15 ml). three sets blood samples were obtained from each animal, the first set of samples were collected (2.5 ml) put on labeled test tube containing (EDTA) for examination, Red blood cell count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV) and Total Leukocyte Counts (WBCs). The second one (5 ml) put in heparinized blood sample tubes also coagulated blood samples were analyzed for Erythrocyte malondialdehyde (Ohkawa *et al*., 1979) and Erythrocyte reduced glutathione (Beutler, 1971) levels in red blood cells. The second one (7.5 ml) in a test tube without anticoagulant to serum isolation for a purpose the of biochemical values examination, After keeping the serum-containing test tubes in standing conditions half an hour, they were centrifuged at 3000 rpm for 15 minutes for proper separation of serum from coagulated blood. Serum was separated and stored in aliquots at -20°C until it examined.

Hematological examination includes Red blood cell count (RBCs X106/100 ml), hemoglobin concentration (Hb g/l), packed cell volume (PCV%), WBCs count (X103/100ml), mean corpuscular volume (MCV fl), mean corpuscular hemoglobin (MCH pg), mean corpuscular hemoglobin concentration (MCHC g/dl), were determined according to (Schalm, 1986; Coles, 1986).

Biochemical analysis determination of serum concentrations of phosphorus, calcium, iron, copper, glucose, urea, Creatinine, Bilirubin, Total proteins, Albumin, Globulin, Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), were carried out by using commercial test kits supplied by (Assel S.R.L., Italy) by using the atomic absorption spectrophotometer according to the method described by (Daly, 1972; Khayam-Bashi, 1977; Ogawa,1984). the heparinized blood samples (10 ml) were centrifuged at 500×g for 15 minutes at 4°C. The plasma and buffy coats were removed by aspiration. The sediment containing blood cells was washed three times by re-suspending in isotonic phosphate-buffered saline, followed by re-centrifugation and removal of the supernatant fluid. The crude red cells were lysed in nine volumes of ice-cold distilled water to prepare a 10% erythrocyte hemolysate estimation of malondialdehyde (Ohkawa *et al.*, 1979) and reduced glutathione (Beutler, 1971) levels in red blood cells.

Data were analyzed using the packaged SPSS program for windows version 10.01. presented as mean ± Standard Error (SE). Differences between groups were determined by the T-test. The significance level was set at P≤ 0.05. (SPSS, 2000).

Results

Table 1 : Clinical examinatiom parameters (Mean ±SE) in Healthy and Post-partureint Hemoglubinurea Cows.

Groups / parameters	Healthy Cows (n:10) Mean ± SE	Cows with PPH (n:8) Mean ± SE	P Value
Temperature °C	38.39 ± 0.09	38.68 ± 0.08	0.092
Pulse rate/min	65.50 ± 1.18	91.25 ± 1.76 **	0.000
Respiratory rate/min	23.60 ± 0.52	40.62 ± 0.92 **	0.000
Ruminal movement/2 min.	3.00 ± 0.18	1.80 ± 0.12 *	0.034

** Highly significant (P ≤ 0.05) * Significant (P ≤ 0.05) NS Non significant (P ≥ 0.05)

Table 2 : Hematological Examination parameters (Mean ±SE) in Healthy and Post-partureint Hemoglubinurea Cows.

Groups / parameters	Healthy animals (n:10) Mean ± SE	Cows with PPH (n:8) Mean ± SE	P Value
RBC ($x10^6$/µl)	6.04 ± 0.22	3.08 ± 0.16 **	0.000
Hb (g/ dl)	9.73 ± 0.30	4.76 ± 0.17 **	0.000
PCV (g/ dl)	30.40 ± 1.55	16.25 ± 1.09 **	0.000
WBCs ($x10^3$/ µl)	10.48 ± 0.31	12.80 ± 0.78 *	0.022
MCV (Fl)	51.98 ± 5.06	50.84 ± 4.89 *	0.024
MCH (Pg)	16.24 ± 0.57	15.69 ± 0.94 *	0.047
MCHC (g/dl)	32.95 ± 2.25	29.88 ± 1.52 *	0.014

**Highly significant (P ≤ 0.01) *Significant (P ≤ 0.05)

Table 2 : Biochemical examination parameters (Mean ±SE) in Healthy and Post-partureint Hemoglubinurea Cows.

Groups / parameters	Healthy animals (n:10) Mean ± SE	Cows with PPH (n:8) Mean ± SE	P Value
P (mg/dl)	5.74 ± 0.21	2.11 ± 0.07 **	0.000
Ca (mg/dl)	7.55 ± 0.91	7.90 ± 0.13 NS	0.290
Fe (µg/dl)	90.30 ± 3.95	136.4 ± 2.48 **	0.000
Cu (µg/dl)	94.10 ± 1.74	72.62 ± 4.07 **	0.001
Glucose (mg/dl)	64.90 ± 1.66	83.50 ± 2.00 **	0.000
Urea (mg/dl)	33.30 ± 0.97	51.62 ± 3.14 *	0.003
Creatinine (mg/dl)	1.73 ± 0.13	2.10 ± 0.10 *	0.048
Total bilirubin (mg/dl)	0.41 ± 0.03	4.12 ± 0.45 **	0.000
Total protein (g/dl)	6.59 ± 0.24	6.67 ± 0.17 NS	0.813
Albumin (g/dl)	3.46 ± 0.04	3.08 ± 0.07*	0.002

Glubulin (g/dl)	3.13 ± 0.23	3.56 ± 0.10 *	0.04
ALT (IU/L)	25.40 ± 0.33	40.75 ± 3.71*	0.003
AST (IU/L)	109.9 ± 3.80	164.8 ± 5.29 **	0.000
ALP (IU/L)	87.50 ± 3.86	126.8 ± 6.48 *	0.004
Eryth. Malond. (n molles/ml)	197.9 ± 5.09	340.6 ± 6.65 **	0.000
Eryth. Reduc. Glutath. (%)	68.60 ± 2.10	47.25 ± 1.63 **	0.000

**Highly significant ($P \leq 0.05$) *Significant ($P \leq 0.05$) NSNon significant ($P \geq 0.05$)

Clinical findings

Clinical findings of the examined cows in Table (1), showed slightly increase but a non- significant in temperature value in post-parturient Hburia with control cows, but revealed increase highly significant ($P \leq 0.01$) in respiratory and pulse rates in post-parturient Hburia cows when comparative with control cows, while ruminal movement is significant decreased ($P \leq 0.05$) in post-parturient Hburia when compared with control cows.

Hematological examination

Hematological studies revealed Table (2), RBCs count, PCV % and Hb values was decreased highly significant ($P \leq 0.01$) in post-parturient Hburia while MCV, MCH and MCHC, was a significant increase ($P \leq 0.05$) in Post parturient Hburea when compared with ControlCows. But showed a significant decrease ($P \leq 0.05$) in WBCs count in Post parturient Hburea with control cows.

Biochemical examination

Biochemical studies revealed Table (3) increase highly significant ($P \leq 0.01$) in means of serum biochemical concentration such as Iron, Glucose, Total protein, AST, Erythrocyte Monaldehyde and significant increase in urea, creatinine, globulin, ALT and ALP in post-parturient Hburia, while appeared decreased highly significant in concentration means of Phosphor, Copper, Erythrocyte reduced glutathione and decreased significant ($P \leq 0.05$) in Albumin concentration in post-parturient Hburia, but didn't reveal the present studies any significant differences in means concentration of Calcium and Total proteins between the two groups.

Discussion

The result of current studies were shown Table (2) their changes in clinical findings between post-parturient Hburea and healthy animals that observed significant increase in respiratory and pulse rates and slightly in temperature with decreased significantly in rumen movement when compared with healthy animals, these clinical findings are typical of those reported for post-parturient hemoglobinuria in cows (Bhat, 2010; Constable *et al*., 2017). In this study, revealed RBC, PCV, and Hb concentrations in the cows with post-parturient hemoglobinuria were significantly decreased when compared with healthy cows, Thompson and Badger (1999) reported that RBC, Hb, and PCV in cows with PPH decreased significantly. Muhammad et al, (2001) reported a significant decrease in erythrocyte count, hemoglobin, and hematocrit in affected animals, which indicates a positive correlation between the total erythrocyte count and both hemoglobin and hematocrit leading to severe anemia, the intravascular hemolysis due to impaired glycolytic pathway and depletion of (ATP) in erythrocytes results from phosphorus deficiency. The subnormal concentration of (ATP) predisposes red blood cells alters the function and structure, causing loss of normal formability, and an increase in fragility, leading to hemolysis (Malik and Samad, 1996). also, the current studies shown a significant increased in WBCs Count, The increasing of WBCs occurs due to increased Neutrophils due to increased stress in cases of PPH (a metabolic disorder) conseders the source of the release of corticosteroids that results in increased neutrophils and depressed lymphocytes (Singari *et al.*, 1991).

In biochemical parameters examination in current studies showed significant differences in mineral indices, it shown decreased in phosphor and copper concentration in cows that sufficient from post-parturient Hburea, this result degree with (Karapınar *et al.*, 2006), he found that the serum phosphorus level in the cows with post-parturient Hburea was markedly decreased, the causes of postparturient hemoglobinuria may be related to dietary phosphorus deficiency or heavy lossing of phosphorus through milk (Constable *et al.*, 2017). However, calcium levels were within the normal level; a similar finding was also reported by (Mahmut *et al.*, 2009). Increased serum enzymes ALT, AST and ALP concentration in current studies could be attributed to the drastic fall in hemoglobin levels as a result of intra-vascular hemolysis which resultant generalized hypoxia, cause damage to cell membranes, resulting in leakage of these enzymes in the serum (Cornelius, 1980). Also, the Liver enzymes and bilirubin levels were significantly elevated, These changes are consistent findings associated with intravascular hemolysis (Meyer and Harvey, 2004). The values of Urea and Creatinine in present studies appeared of increased in means of concentrations in diseased cows by post-parturient Hburea when compared with health animals this results degree with (Benjamin, 1978) considered that creatinine concentrations over 2 mg/dL lead to a reduced kidney filtration rate, which affects in a way similar to that of blood urea. Both creatinine and urea levels were elevated and positively correlated to each other in post-parturient hemoglobinuria affected cows. According to (Digraskar *et al.,* 1991), creatinine and urea are waste products that the glomerular normally filter from the blood and these are interrelated. Latimer *et al.* (2003) also reported that if the kidneys are not working correctly, these substances accumulation in the body and elevated blood levels of creatinine and urea are indications of pathological function of kidneys.

The presented studies showed increased significantly of glucose concentration and erythrocyte malondialdehidehide and decreased in erythrocyte reduced glutathione concentration in post-parturient Hburea cows this result degree with (Gahlawa *et al.*, 2007; Mata and Bhardwaj, 1985), Phosphorus deficiency decreases the utilization rate of glucose and (ATP) production by erythrocytes leading to decrease in synthesis, as well as reduction of glutathione which predisposes the erythrocytes to oxidants adverse effects, the resultant oxidative stress probably leads to lipid peroxidation of the erythrocyte cell membrane with eventual intravascular hemolysis. increase of malondialdehyde levels

in erythrocytes of hemoglobinuria animals indicated that there was enhanced lipid peroxidation in red cell membranes of PPH affected animals. In erythrocytes, the reduced glutathione protects hemoglobin from oxidative denaturation and its membrane against lipid peroxidation (Trotta *et al.,* 1982), the extremely lowered levels of reduced glutathione in erythrocytes of post-partureint haemoglobinuric cows indicated a very low antioxidant status in diseased cows, there was an appreciable increase in reduced glutathione contents of erythrocytes. In conclusion, a deficiency of phosphorus in the body, after parturition could be responsible for the development of post-parturient haemoglobinuria in cows.

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