

Liver Abscesses in Dromedary Camels (*Camelus dromedaries*): Oxidative Stress Biomarkers and Proinflammatory Cytokines

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Abstract

Background: The characteristic clinical manifestations, oxidative stress markers and proinflammatory Cytokines in liver abscess in camel are poorly defined.

Objective: Our objectives were to investigate liver abscess in camel at the slaughter house and to address its effect on blood cellular and biochemical values particularly oxidative stress markers and proinflammatory cytokines.

Methods: Thirty-five camels with liver abscesses and 15 healthy camels were included in this study. Complete blood picture, and selected biochemical parameters were carried out. Bacteriological examination was also carried out.

Results: Clinical signs were recorded. The hematological picture of diseased camels revealed reduction in the total erythrocytic count and hemoglobin level associated with elevation in leucocytic count and neutrophils percentage. The biochemical analysis of serum samples revealed increase in the levels of liver enzymes associated with reduction in the levels of total proteins, albumen and glucose levels in diseased camels when compared to their levels in healthy ones. Increased levels of lipid peroxidation and glucose 6-phosphate dehydrogenase with significant reduction in the levels of superoxide dismutase, catalase and glutathione were evident when compared to their levels in healthy camels. There was elevation in the levels of TNF- α , IFN- γ , IL-1 α , IL-1 β , IL-6 and IL-10 in diseased camels when compared to their levels in normal ones. The isolated bacteria from liver abscess were *Fusobacterium necrophorum*, *Corynebacterium psuedotuberculosis*, *Escherichia coli*, and *Staphylococcus* spp.

Conclusions: Oxidative stress and proinflammatory Cytokines could be used as biomarkers of liver abscess in camel.

Keywords: Liver; Camel; Oxidative stress; TNF- α ; IFN- γ ; IL-1 α ; IL-1 β ; IL-6; IL-10

Introduction

Liver disease is relatively common but often occurs in the absence of specific clinical signs. The liver has great powers of regeneration and more overt clinical signs associated with its failure do not appear until some 70-80% of the functional capacity is lost. Obscure signs of liver disease are therefore much more common than overt signs of liver failure [1,2]. Liver abscesses have capsules that vary in thickness, and range in size from a minute pinpoint to over 15 cm in diameter. The distribution of abscesses in the liver lobes shows no consistent pattern [3-5].

A growing body of evidence suggests that the formation of reactive oxygen species is a common occurrence associated with most if not all disease processes. The overall importance of reactive oxygen species to the progression and severity of various disease states varies greatly depending on the conditions and whether the disease is acute or chronic. Free radical researches in animals are in progress and further investigations are needed to establish the involvement of reactive oxygen species in diseases affecting different animal species and the pathology they produce [6-8]. Oxidative stress is a process based upon the action of free radicals formed by oxygen or other molecules and fragments [9]. Free radicals are highly reactive substances produced continuously during metabolic processes and participate to a great extent in physiological events such as immune response, metabolism of unsaturated fatty acids and inflammatory reaction. However, their excess results in impairment of DNA, enzymes and membranes [10-12] and may induce changes in the activity of the immune system and in the structure of basic biopolymers, which may be related to mutagenesis and aging processes [13]. The liver is well protected against free radicals.

It is one of the best antioxidant supplied organs. An important function of the liver is the detoxification of drugs, chemicals and toxic materials, with the subsequent release of free radicals [14,15]. To the best of our knowledge little is known about liver abscess as a disease condition in camels, therefore the main objective of this study was to throw the light on liver abscess in camel at the slaughter house and to address the effects on blood cellular and biochemical values particularly oxidative stress markers and proinflammatory cytokines.

Materials and Methods

Animals

A total of 50 one-humped camels (*Camelus dromedarius*) were involved in this study. Their age ranged between 3.5 and 7 years old. All camels were examined carefully before slaughtering. The camels divided into two groups. The 1st was healthy camels (n=15) and the second was camels with liver abscess (n=35).

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Samples and sampling protocol

Blood samples were collected from all camels just before slaughtering during the inspection at the yard belonging to the slaughter house. Two blood samples were obtained from each animal. The first was 10 ml of blood obtained via the jugular vein using a sterile syringe into heparinized blood collecting vacutainer tubes and used for hemogram evaluation using the electronic cell counter. The second blood samples were obtained in plain vacutainer tubes in order to obtain serum for biochemical analysis of the selected parameters mainly total plasma proteins, albumin, globulin, glucose, blood urea nitrogen and liver enzymes including Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Gamma Gultamic Transferase (GGT), Glutamic Dehydrogenase (GLDH), Alkaline phosphatase (ALP) and bilirubin. Blood biochemical parameters were measured spectrophotometrically using the automated Udhim system and commercially available test kits (Roche Diagnostics GmbH, Mannheim and Randox Laboratories GmbH, Krefeld).

Bacteriologic culture of the pus collected from the abscesses was done in all camels according to standard techniques. The samples were cultured under aerobic and anaerobic conditions. The isolated microorganisms were identified using VITEK2 Compact, Biomeriux, France.

Preparation of erythrocyte hemolysate

Immediately after collection, blood samples were centrifuged at 1500 rpm for 15 min at 4°C. The plasma and Buffy coats were removed by aspiration. The sediment containing blood cells was washed three times by resuspending in isotonic phosphate-buffered saline, followed by re-centrifugation and removal of the supernatant fluid and the Buffy coats. The crude red cells were lysed in nine volumes of ice-cold distilled water to prepare a 10% erythrocyte hemolysate.

Lipid peroxidation (MDA)

Lipid peroxidation in RBC hemolysate was determined as thiobarbituric acid reactive substances (TBARS) according to Placer et al. [16]. The method is depended on forming a color complex between the products of lipid peroxidation and thiobarbituric acid (TBA). Briefly, 0.2 ml of RBC hemolysate was added to 1.3 ml of 0.2 mol/l Tris, 0.16 mol/l KCl buffer (pH 7.4). TBA (1.5 ml) was added and the mixture was heated in a boiling water bath for 10 min. After cooling, 3 ml of pyridine-butanol (3:1, v/v) and 1 ml of 1 mol/l NaOH were added. The absorbance was read at 548 nm against bi-distilled water as a blank. In this assay, 1,1,3,3-tetramethoxypropane was used as a standard. Lipid peroxidation in the RBC hemolysate was expressed as nmol of erythrocytic malondialdehyde (eMDA)/g Hb.

Glutathione (GSH)

GSH concentration in the RBC hemolysate was measured using the

method of Beutler et al. [17]; this method is based on the development of a stable yellow color when 2-nitrobenzoic acid is added to sulfhydryl compounds. The amount of reduced product, thionitrobenzene, was measured at 412 nm and expressed as mmol/g Hb.

Superoxide dismutase (SOD)

SOD activity was estimated in the RBC hemolysate according to the method described by Misra and Fridovich [18]. This method is based on the ability of SOD to inhibit the autoxidation of epinephrine to adrenochrome in an alkaline medium (pH 10.2). OD was measured at 480 nm and expressed as U/mg Hb.

Catalase (CAT)

CAT activity was measured in the RBC hemolysate by the method of Beers and Sizer [19]. Decomposition of H₂O₂ was followed directly by the decrease in absorbance at 240 nm, and the difference in absorbance per min/mg Hb was taken as a measure of the CAT activity.

Glucose-6-phosphate dehydrogenase (G6PD)

G6PD level was measured using commercially available test kits supplied by Biodiagnostic-Egypt, according to the methods described by Beutler [20].

Determination of Cytokine response

IFN- γ , TNF- α , IL-1 α , IL-1 β , IL-6 and IL-10 were determined from undiluted serum samples using commercially available ELISA Kits (Biosource, Diagnostic Corporation, USA). The plates were read at 450 nm and a correction wavelength of 550 nm was measured on a computerized automated microplate ELISA reader (Bio TEC, ELX800G, USA). Values expressed in picograms per milliliter were extrapolated using linear regression from a standard curve of known amounts of human cytokines.

Statistical analysis

The statistical significance between means was compared using Student's t-test; and (P \leq 0.05) was considered significant. All data are presented as means \pm standard error (SE) of the means. All tests were performed using computer package of the statistical analysis system [21].

Results

The diseased camels with liver abscess showed significant polypnea, icteric mucous membranes, variable physical activities, variation of body temperatures, diarrhea & dehydration in some cases (16/35) and anorexia (Table 1). The hematological picture of diseased camels revealed significant (P \leq 0.05) reduction in the total erythrocytic

Variables	Healthy camels (Control)	Camels with Liver abscess
Respiratory rates/min	12.36 \pm 2.3	20.4 \pm 3.2*
Heart rates/min	36.3 \pm 3.2	46.5 \pm 3.3*
Temperature	37.1 \pm 0.32	38.3 \pm 0.52 (3 camels had fever, 32 camels had normal temperature)
Mucous membranes	Rosy red color	Icteric color
Physical activities	excellent	Varied from fair to bad
General appearance	Bright	Varied from bright (16/35) to dull (19/35)
Appetite	Normal	Anorexia
Dehydration	Normal hydration	Observed in (16/35)
Diarrhea	Absent	Observed in (16/35)

*Means are significantly different at the level (P \leq 0.05)

Table 1: Physical parameters in examined Dromedary Camels.

count and hemoglobin level. In addition there was significant ($P \leq 0.05$) increase in leucocytic count and neutrophils percentage (Table 2). Concerning the biochemical analysis, there were significant ($P \leq 0.05$) increase in the levels of AST, ALT, BUN, bilirubin, GGT and GLDH values in diseased camels when compared to their values in healthy camels. Moreover, there was significant ($P \leq 0.05$) reduction in the levels of total proteins, albumen and glucose levels in diseased camels when compared to their values in healthy ones (Table 3). In the present study there was significant ($P \leq 0.05$) increase in the levels of lipid peroxidation (MDA) and G6PD with significant ($P \leq 0.05$) reduction in the levels of SOD, CAT and GSH when compared to their values in healthy camels. Furthermore, there was significant ($P \leq 0.05$)

Parameters	Healthy camels (Control)	Camels with Liver abscess
RBC ($\times 10^9/\mu\text{l}^3$)	11.15 \pm 3.22	8.45 \pm 2.22*
Hb (gm/dl)	8.98 \pm 0.88	7.78 \pm 1.12*
PCV (%)	46 \pm 0.08	48 \pm 0.06*
WBC ($\times 10^3/\mu\text{l}^3$)	14.20 \pm 5.31	24.20 \pm 7.11*
Neutrophils(%)	57.0 \pm 3.44	82.0 \pm 3.56*
Lymphocytes (%)	25.3 \pm 3.56	24.3 \pm 1.26
Monocytes (%)	2.4 \pm 0.32	2.3 \pm 0.22
Basophils (%)	1.1 \pm 0.12	1.0 \pm 0.13
Eosinophils (%)	2.4 \pm 0.21	2.6 \pm 0.32

*Means are significantly different at the level ($P \leq 0.05$).

Table 2: The Mean hematological values in examined Dromedary Camels.

Variables	Healthy camels (Control)	Camels with Liver Abscess
Total Plasma Proteins (gm%)	6.34 \pm 0.44	5.10 \pm 0.5*
Albumen (gm%)	3.74 \pm 1.2	3.00 \pm 0.4*
BUN (gm%)	12.50 \pm 0.34	21.20 \pm 3.4*
Glucose (mmol/l)	37.67 \pm 3.10	30.50 \pm 2.5*
Bilirubin (mmol/l)	1.10 \pm 0.45	1.90 \pm 0.5*
AST (mmol/l)	139.50 \pm 23.4	197.21 \pm 21.4*
ALT (mmol/l)	17.30 \pm 4.9	55.11 \pm 5.5*
ALP (IU/l)	45.60 \pm 2.6	88.20 \pm 4.5*
GGT (IU/l)	15.63 \pm 2.2	66.30 \pm 6.35*
GLDH (IU/l)	13.56 \pm 5.21	55.45 \pm 6.8*

*Means are significantly different at the level ($P \leq 0.05$).

AST: Aspartate Amino Transferase, ALT Alanine Amino Transferase, ALP: Alkaline phosphatase, GGT: Gamma GultamicTransferase, GLDH: Glutamic Dehydrogenase

Table 3: The mean values of blood biochemical parameters in control and diseased Dromedary Camels.

Variable	Healthy camels (Control)	Camels with Liver Abscess
MDA (nmol/g Hb)	12.5 \pm 1.21	26.6 \pm 1.23*
SOD (U/mg Hb)	5.14 \pm 0.45	3.52 \pm 0.22*
Catalase (U/mg Hb)	16.77 \pm 1.23	6.32 \pm 0.85*
R.GSH (mmol/gmHb)	7.5 \pm 0.56	3.8 \pm 0.48*
G6PD (IU/g Hb)	26.43 \pm 0.21	39.23 \pm 0.31*
TNF- α (pg/ml)	14.45 \pm 0.54	22.25 \pm 0.43*
IFN- γ (pg/ml)	18.65 \pm 1.23	33.23 \pm 1.25*
IL-1 α (pg/ml)	9.23 \pm 0.23	14.21 \pm 0.23*
IL-1 β (pg/ml)	19.32 \pm 1.84	26.23 \pm 0.52*
IL-6 (pg/ml)	13.54 \pm 0.89	16.23 \pm 0.32*
IL-10 (pg/ml)	9.92 \pm 0.32	13.54 \pm 0.23*

*Means are significantly different at the level ($P \leq 0.05$).

Table 4: The mean values of oxidative stress markers and proinflammatory cytokines in control camels and those with Liver abscess.

elevation in the levels of TNF- α , IFN- γ , IL-1 α , IL-1 β , IL-6 and IL-10 when compared to their values in normal camels (Table 4). The isolated bacteria from liver abscess in different camels was *Fusobacterium necrophorum* (n=20), *Corynebacterium psuedotuberculosis* (n=8), *Escherichia coli* (n=3), and *Staphylococcus spp.* (n=4).

Discussion

Liver disease is relatively common but often occurs in the absence of specific clinical signs. The liver has great powers of regeneration and more overt clinical signs associated with its failure do not appear until some 70-80% of the functional capacity is lost [1].

In the present study the diseased camels showed significant polypnea, icteric mucous membranes, variable physical activities, dehydration in some cases and anorexia. These results are partially similar to those stated by Andrews et al. [22] in cattle and Braun et al. [23] who stated that clinical signs which may develop in animals with hepatic abscesses are non-specific and include weight loss, reduced weight gain, reduced milk production, bouts of fever and anorexia, and signs of pain when they move or lie down. The present findings disagree with Harman et al. [24], Nagaraja and Chengappa [5] in cattle as they mentioned that liver abscesses are detected only at the time of slaughter, because cattle, even those that carry hundreds of small abscesses or several large abscesses, seldom exhibit any clinical signs.

Generally, hematology and liver function tests are not reliable indicators of liver abscesses [25,26]. The hematological picture of diseased camels revealed significant ($P \leq 0.05$) reduction in the total erythrocytic count and hemoglobin level. In addition there was significant ($P \leq 0.05$) increase in leucocytic count and neutrophils percentage when compared to their values in healthy camels. These results are in consistent with those obtained by Doré E, et al. [27] in Holstein dairy cattle as they reported that when there are several abscesses, or a large abscess, leukocytosis with neutrophilia and increased fibrinogen levels develop.

Concerning the biochemical analysis, there was significant ($P \leq 0.05$) increase in the levels of AST, ALT, BUN, bilirubin, GGT and GLDH values in diseased camels when compared to their values in healthy camels. Moreover, there was significant ($P \leq 0.05$) reduction in the levels of total proteins, albumen and glucose levels in diseased camels when compared to their values in healthy camels. These results are in concurrence with those obtained by Craig et al. [28] in cattle and West [29] in horses.

Reactive oxygen intermediates are formed in many parts of liver cells. A balance between free radical reactions and antioxidant activities is very important for normal liver functioning. This balance is altered in pathological processes [30]. The antioxidant system consists of antioxidant enzymes, including superoxide dismutase, catalase and glutathione peroxidase, glutathione, ancillary enzymes such as glutathione reductase, glutathione S-transferase and glucose 6-phosphate dehydrogenase, metal-binding proteins such as transferrin, ceruloplasmin and albumin, vitamins such as alpha-tocopherol, ascorbate and beta-carotene, flavonoids and urate [6,31].

In the present study there was significant ($P \leq 0.05$) increase in the levels of lipid peroxidation (MDA) and G6PD (26.6 \pm 1.23 and 39.23 \pm 0.31, respectively) associated with significant ($P \leq 0.05$) reduction in the levels of SOD, CAT and GSH (3.52 \pm 0.22, 6.32 \pm 0.85 and 3.8 \pm 0.48, respectively) when compared to their values in healthy camels (12.5 \pm 1.21, 26.43 \pm 0.21, 5.14 \pm 0.45, 16.77 \pm 1.23 and 7.5 \pm 0.56, respectively). These results are in agreement with Britton et al. [32] who

stated that production of free radicals has been implicated in a variety of liver diseases where they can damage cellular macromolecules and therefore, may participate in hepatocellular injury. In addition, free radical-initiated lipid peroxidation may play a role in hepatic fibrogenesis, perhaps through an effect of aldehydic peroxidation products on Kupffer cells and lipocytes. In the same concern Poli [33] and Bianchi et al. [34] mentioned that oxygen free radicals might play a role in the pathogenesis of tissue damage in many pathological conditions including liver diseases where antioxidant tissue systems are reduced. The leading mechanism of free radical toxicity is the peroxidation of membrane phospholipids. Lipid peroxidation is initiated by the formation of lipid peroxide or hydroperoxides. Peroxy radicals are formed in the presence of oxygen to start a chain reaction (propagation). Various pathogenic effects occur as a result of the degradation of membrane lipids. The interaction of degradation products with various cellular macromolecules and the production of new reactive oxygen species during the course of the chain reaction process [35] may lead to membrane damage, protein damage, enzyme dysfunction and DNA or RNA damage [8]. Moreover the present results are also in concurrence with those obtained by De Jong et al. [36] in liver of rat and Czuczajko et al. [37] in patients with chronic liver diseases. The current results disagree with Abd Ellah et al. [38] in cattle. The authors stated that erythrocytic oxidative status (GSH-Px and G6PD) was not affected by hepatic dysfunction, and the effects only observed in hepatic tissues as there was significant increase in hepatic G6PD activity and a significant decrease in hepatic GSH-Px activity in cows suffering liver abscesses. The significant decrease in GSH, SOD and CAT levels in the cases of liver abscess may be attributed to increased free radical stress in the liver tissue, which inhibits the enzymes activity [38]. It has been reported that increased oxidative stress results in impairment of enzymes containing thiol groups and cell membranes [33,39]. Free radicals can oxidize proteins, the amino acids being oxidized to their hydroxy derivatives; for example, phenylalanine can be oxidized to hydroxyphenylalanine, such oxidation can cause enzymatic inactivation [40]. It has been reported that G6PD activity in blood and in liver tissues may serve as a useful biochemical test specific for fatty liver in cows [41]. Elevated expression of G6PD is also important in the support of major antioxidant pathways, as the generated NADPH is the reducing coenzyme for peroxidases in the case of fatty liver [42].

Hepatic cellular dysfunction and death also have the ability to initiate immunological reactions, including both innate and adaptive immune responses [43]. In the present study there was significant ($P \leq 0.05$) elevation in the levels of TNF- α , IFN- γ , IL-1 α , IL-1 β , IL-6 and IL-10 (22.25 ± 0.43 , 33.23 ± 1.25 , 14.21 ± 0.23 , 26.23 ± 0.52 , 16.23 ± 0.32 and 13.54 ± 0.23 , respectively) when compared to their values in healthy camels (14.45 ± 0.54 , 18.65 ± 1.23 , 9.23 ± 0.23 , 19.32 ± 1.8 , 13.54 ± 0.89 and 9.9 ± 0.32 , respectively). The present results are in consistent with Ishida et al. [43] in mice. The authors stated that hepatocyte stress and/or damage could result in the release of signals that stimulate activation of other cells, particularly those of the innate immune system, including Kupffer Cells (KC), Natural Killer (NK) cells, and NKT cells. These cells contribute to the progression of liver injury by producing proinflammatory mediators and secreting chemokines to further recruit inflammatory cells to the liver. It has been demonstrated that various inflammatory cytokines, such as tumor necrosis factor TNF- α , interferon (IFN- γ), and interleukin IL-1 β produced during liver injury are involved in promoting tissue damage. However, innate immune cells are also the main source of IL-10, IL-6, and certain prostaglandins, all of which have been shown to play a hepatoprotective role [44,45]. Moreover it was stated by García-Ruiz et

al. [46] that liver injury and altered metabolism in liver disease also may be generated by inflammation-promoting substances called cytokines. For example, a cytokine called tumor necrosis factor-alpha (TNF- α) causes cell damage by promoting oxidative stress in mitochondria, the key energy-producing structures in the cell.

From this study it could be concluded that liver abscess syndrome creates oxidative stress and immunological responses in dromedary camels. Treatment therapy should be modified by adding antioxidant mixture. Oxidative stress and cytokines could be used as a biomarkers of liver abscess syndrome in Dromedary camel (*Camelus dromedaries*). Further investigations and extra efforts should be made to confirm this in other species. If this could be achieved, it would yield a valuable tool to diagnose liver abscess syndrome and would open up new perspectives in research fields dealing with liver abscess.

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