



Original Article

# Histological Changes and Ghrelin, P-Selectin, and Leptin Parameter in Patients with Hepatic Hydatidosis in Iraq

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## Abstract

Humans and animals are affected by hydatid cyst disease as a worldwide zoonotic disease, which is caused by the metacestode stage of *Echinococcus* spp. This study was performed to evaluate the histological change of liver and blood concentrations of biomarkers, such as ghrelin, p-selectin, and leptin, in humans infected with hydatid cyst. A total of 30 surgical specimens of liver and blood of infected humans and 30 healthy individuals as a control group were evaluated. Liver tissue sections in cases infected with hydatid cyst and control group, histological abnormalities in the liver, including fibrosis, increased inflammatory cells, dystrophic areas, and necrosis were compared in this study. In addition, serum leptin levels were significantly lower in patients with hydatid cyst disease than in the control group ( $P$ -value<0.05), whereas p-selectin and ghrelin levels significantly decreased in patients ( $P$ -value<0.05). The results of this research can be effective in improving and promoting the treatment programs of hydatidosis.

**Keywords:** Ghrelin, Histological change, Hydatid disease, Kufa, Leptin, P-selectin

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## 1. Introduction

*Echinococcus granulosus* causes hydatidosis which is a zoonotic parasitic disease that infects numerous hosts, especially herbivores, such as cows, camels, goats, sheep, and humans (1). This parasite is transmitted by the eggs of adult tapeworm *E. granulosus*, which can infect both humans and animals (2). The larvae stage of the life cycle in the intermediate host develops in the tissues of organs, particularly the liver and lungs. A significant aspect is the presence of adult worms in the intestines of dogs, wolves, and foxes as definitive hosts. Hydatid cyst affects the liver and lungs in 90% of intermediate hosts. Surgery is performed on the patient, and the therapy consists of two medications, namely Mebandazole and Albendazole (3).

Humans and other mammals can be infected by *E. granulosus* through ingesting eggs from the primary host's feces, eating contaminated plants, drinking polluted water, or touching or playing with infected dogs. The six-hooked oncosphere hatched from the egg penetrates the intestinal wall with its secretions and travels via small veins or lymphatic vessels to the liver, lungs, brain, and other organs. When the embryo penetrates these organs, transforms into a larval stage and produces the hydatid cysts. The fertile cyst forms the oncospherical stage to reach the metacestode stage. Often these fertilized cysts are ingested by definitive hosts (e.g., dogs) after slaughtering contaminated animals (4).

Today, various pieces of research have been performed on therapeutic solutions for the effects of

hydatidosis on human health. Despite the wide range of medications used to chemically treat the disease against hydatid cysts, it has been shown that they are only partially effective. Another way to treat hydatid cyst is surgery, which in some cases leads to unwanted complications (5).

As a result, scientists have evaluated new ideas for pharmacological treatment processes. Some even have attempted to improve chemotherapy by combining medicinal plant components with chemical medicines (6). The target of new medicine is to improve it, increase its absorption, attain higher concentration in the blood plasma, and create a stronger anti-parasite impact. Ma, Bao (7) observed that combining *Sophora moorcroftiana* alkaloids with benzole had a significant effect on the survivability of protoscolices isolated from single vacuole cysts outside of in vitro in preventing created secondary cysts in albino mice. P-selectin is a member of the selectin family that helps leukocytes adhere to active endothelium (8, 9). Serbina, Jia (10) showed that the immunological response to infection was triggered by the recruitment of leukocytes (10).

The *LEP* gene on chromosome 7 produces leptin, a signaling peptide associated with immunological and gastrointestinal system function (11). Adipose tissue mass produces leptin, a protein hormone whose levels are linked to changes in nutritional status and energy storage in a variety of nutritional situations. Serum leptin concentrations indicate total body fat content in both children and adults (12).

Ghrelin is a hormone produced mostly by the stomach, small intestine, and other organs in modest amounts. The lipid profile transports ghrelin through the bloodstream. Ghrelin and leptin levels may be affected by parasitic infection. P-selectin is a single-chain glycoprotein in platelets and endothelial cells that has a protective role in transferring leukocytes from the bloodstream to the infected organ (13).

In the present study, in addition to examining the pathological sections of organs infected with hydatid

cyst in surgical patients in Iraq, the serum levels of leptin, p-selectin, and ghrelin were evaluated in patients and the control group.

## 2. Materials and Methods

### 2.1. Sampling and Pathological Analysis

In this study, 30 surgical specimens were removed during surgery from patients with clinical hydatid cysts and 30 healthy individuals were included as a control group. From August 2018 to January 2019, all samples were obtained from patients from the hospitals in Najaf, Iraq. The samples were formalin-fixed at a concentration of 10%. All of the blocks were stained with hematoxylin and eosin and tested for the signs of inflammation.

### 2.2. Measurement of Serum Level of P-Selectin, Ghrelin, and Leptin

Blood samples from patients with hydatid cysts and healthy people as controls were obtained and centrifuged at 3,000 r/min for 5 min to isolate the serum. The supernatants were collected in new sterile tubes. Each serum sample was divided into three sections, each of which was stored at -20°C until bring used for determining the concentrations of p-selectin, ghrelin, and leptin. All these biomarkers were determined using an enzyme-linked immunosorbent assay kit (Elabsience®, Bulgaria).

### 2.3. Statistical Analysis

In this analysis, the t-test was used to compare samples using GraphPad Prism software (version 10). A *P*-value of < 0.05 was considered significant in this study (14-17).

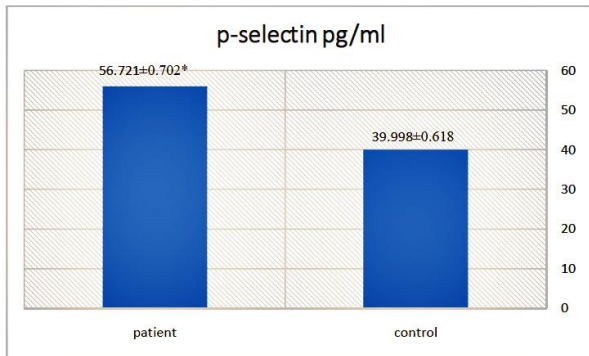
## 3. Results

In comparison to the control group, serum levels of p-selectin and ghrelin significantly increased in patients infected with hydatid cyst (56.721 0.702 pg/ml and 40.821 0.091 pg/ml), respectively (*P*-value<0.05). In contrast, the serum levels of leptin was significantly lower in patients with hydatidosis (5.632 0.192 pg/ml) than in the control group

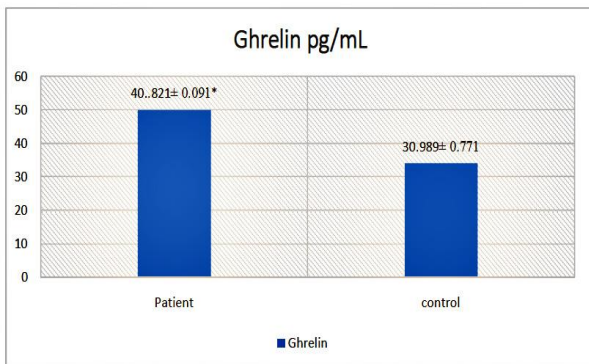
(9.281 ± 0.021 pg/ml;  $P$ -value < 0.05), as shown in figures 1-3.

The results of the present study also showed the existence of an association between hydatidosis and liver histological changes to the healthy human liver,

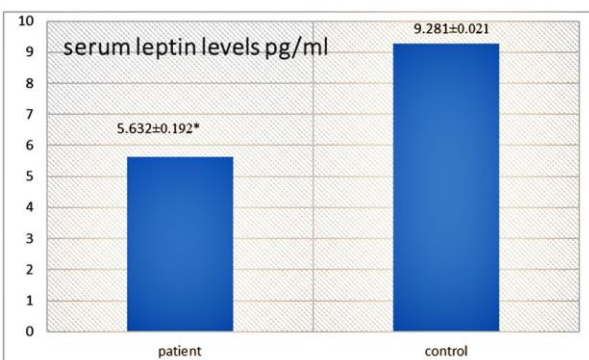
such as fibrosis, inflammation, and necrosis. It was observed that dystrophic areas in the liver tissue were infected with hydatid cysts. Moreover, increased lymphocytes and inflammatory cells were observed in the sections of infected tissues, as seen in figures 4-7.



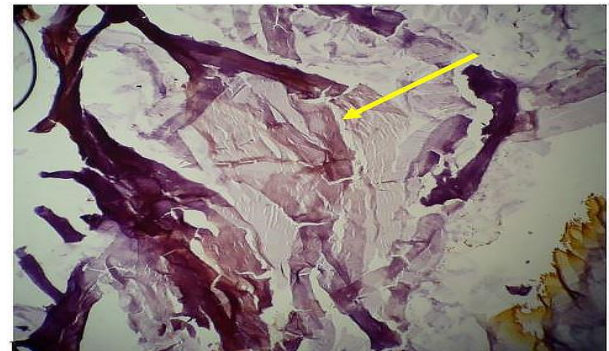
**Figure 1.** Concentration of p-selectin (pg/ml) in hydatidosis patients and control cases



**Figure 2.** Concentration of ghrelin (pg/ml) in hydatidosis patients and control cases



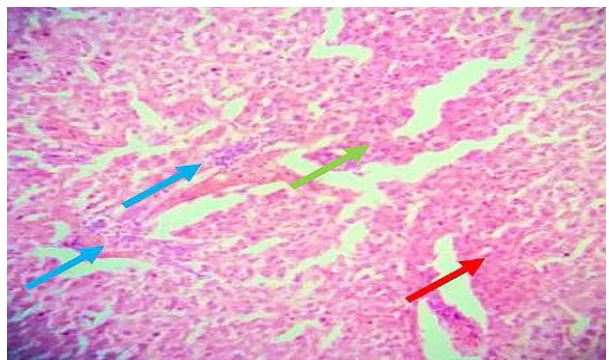
**Figure 3.** Concentration of leptin (pg/ml) in hydatidosis patients and control cases



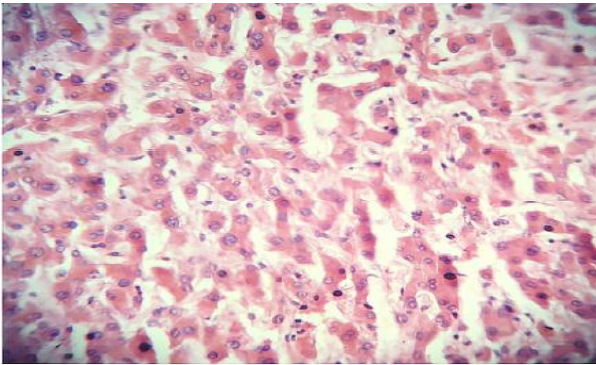
**Figure 4.** Histological spectrum of liver fibrous associated with hydatidosis infection (arrow) staining with hematoxylin and eosin, 400x



**Figure 5.** Section of the histological spectrum of liver inflammatory associated with hydatidosis infection (arrow) staining with hematoxylin and eosin, 400x



**Figure 6.** Histological section of liver necrosis associated with hydatidosis infection (arrow) staining with hematoxylin and eosin, 400x



**Figure 7.** Section of normal liver of human staining with hematoxylin and eosin, 400x

#### 4. Discussion

Hydatid cyst disease is one of the most common parasitic zoonotic diseases worldwide with a high prevalence in developing countries (1). The growth of the larval stage of *Ecchinicoccus* spp. in the intermediate host organs, such as the liver and lungs, with pathological changes, also in the examination of serum of patients with hydatid cyst, biochemical factors were changed (3). In this study, leptin, ghrelin, and p-selectin, biochemical factors, were measured in patients. It was found that ghrelin and p-selectin levels in the blood were significantly higher in patients with hydatid cyst infection than in the control group. On the other hand, the level of leptin in the blood was significantly lower. P-selectin levels in children and adults with congenital leptin deficiency may be elevated due to the effects of the disease; nevertheless, a reduction in leptin levels can be due to the function of leptin in the resistance of hosts to hydatidosis (18) or may be attributed to the removal of the receptor of leptin in the intestinal epithelium, which could explain the cause of amplified resistance to cyst stage of *Ecchinicoccus* spp. (19).

The host response to hydatid cyst infection, which needs an enhanced expression of cell adhesion molecules in order to attract effective inflammatory cells to the infection organs, can explain the much higher blood concentrations of p-selectin in hydatidosis patients (20). The findings of laboratory research

studies suggest that neutrophils may act on abnormal p-selectin lipid bilayers, however, not on intercellular adhesion molecules-1 bilayers acting as the members of the immunoglobulin superfamily, indicating that p-selectin is affected by transient neutrophil binding to the endothelium (21). Lawrence and Springer (22) has found that decreasing human p-selectin expression is critical for limiting the number of leukocytes entering tissues during chronic immune-mediated inflammation. The results of various studies have shown an increase in p-selectin among individuals infected with *Cryptosporidium parvum*, compared to the control group (23).

The insulin-ghrelin association is assumed to be formed when patients with parasitic intestinal infections have lower ghrelin concentrations to compensate for higher glucose levels, and the fact that the patients in this study had lower ghrelin levels supported the theory that this was the primary cause of appetite loss in parasite-infected patients (24). Minimizing the parasite infection-induced rise in lipid peroxidation is another possible cause-and-effect relationship that could explain the decreased endogenous ghrelin levels in infected patients (24).

The findings of the present study revealed the existence of a relationship between hydatidosis and histological alterations in the liver, such as fibrosis, inflammation, and necrosis, compared to a healthy human liver. These histopathological changes may be due to the ability of *E. granulosus* cysts in distracting liver cells, which in turn leads to a rise in fibrous tissue. In such circumstances, the liver is identified as one of the most favorable areas for the development of a hydatid cyst (25).

Torgerson (26) pointed out that the host's immunological response prevented the development of cysts and stimulated calcification cyst. Al SeÂ (27) noted changes in liver tissues involved in infection with hydatid cysts, including calcifications, blood patches, the inflammatory zone around cysts, and pale liver margins. The latest diagnosis of multilayer membrane

formation is related to inflammatory cell leakage inside the fibrous capsule (28).

According to Singh, Sharma (29), liver sections taken from near the cyst wall showed congestion, hemorrhage, hepatocyte necrosis, and atrophy, as well as fibrosis and cellular infiltration of primary macrophages, lymphocytes, and plasma cells, predominantly on the inner side of the fibrous capsule.

In other cases, the histopathological changes, including fibroplasia and cirrhosis, are formed by the immune reactions of host tissues, which are contaminated with the cyst of these helminths (30). The sinusoids and central veins are dilated and fibrosis is also detected in the portal sector Ibrahim (31). Due to the pathological changes in the liver tissue and biochemical factors in infection with hydatid cyst, it is recommended to implement a control program to reduce the disease in hosts and humans to decrease the incidence of this disease.

#### Authors' Contribution

Study concept and design: A. H. A.

Acquisition of data: S. K. A.

Analysis and interpretation of data: S. K. A.

Drafting of the manuscript: R. S. Z.

Critical revision of the manuscript for important intellectual content: N. A. A.

Statistical analysis: H. M. K.

Administrative, technical, and material support: A. H. A.

#### Ethics

The Ethics Committee of Faculty of Institutional Sciences, University of Kufa, Iraq, accepted the selection of samples, and all participants signed consent forms.

#### Conflict of Interest

The authors declare that they have no conflict of interest.

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