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Leptin as a biomarker in patients Infected with *Hymenolpis nana* in Al-Najaf province, Iraq.

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ABSTRACT

The study was conducted on 60 out patients and 30 healthy males to determine the influences of infected with *Hymenolpis nana* on levels of leptin, iron, ferritin and Hb , RBCs and PCV in males infected with *H. nana* in compared with healthy group. Who have visited Al-Sadder medical city and Al-Hakeem Hospital in Al- Najaf Province during the period from March till August 2015. Diagnosis infection with this parasite by using the wet amount microscope for stool from patients. The results showed significant decrease ($P<0.05$) in leptin, iron and ferritin in *H. nana* infection patients in compared to control group. Furthermore the results showed serum Hb, RBCs and PCV were significant decreased ($P< 0.05$) in *H. nana* infection patients in compared to control group.

Keywords: Hb, ferritin, Leptin, Iron

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INTRODUCTION

Anemia may be caused by celiac disease and particularly common in patients with untreated celiac disease in the past. [1,2,3,4]

Leptin, the 16-kDa product of the obese (*ob*) gene was originally described as a regulator of food intake and energy expenditure. Leptin is constitutively produced by adipocytes but may also be expressed in stomach, muscles, placenta and mammary epithelial cells [5, 6].

Intestinal parasitic infestation represents a considerable medical and public health problem in the developing countries and up to 10% of the population of the developing world is infected with intestinal worms [7,8].

Intestinal parasites are responsible for morbidity and mortality worldwide, especially in low income countries and in people with other diseases [9], and are more prevalent in hot and humid environments, with poor sanitation, contaminated water, poor housing and overcrowded [10]. Such environments are common in the suburbs of many African cities.

Hymenolepis nana, a dwarf tapeworm, is the most common human tapeworm infection, with an estimated 50 to 75 million carriers worldwide. This tapeworm is endemic in Asia, Africa, and southern and eastern Europe, and its life cycle involves humans or rodents as the definitive host and arthropods as the intermediate host, Humans and rodents are infected when they ingest cysticercoid-infected arthropods or embryonated eggs from contaminated food, water, or hands. Upon ingestion, eggs hatch and release a 6-hooked larva called the qazoncosphere (hexacanth), which penetrates the intestinal villi and develops into a cysticercoid larva [11].

Unlike that of all other species of tapeworm, this worm's entire life cycle can be completed in the bowel, that is, autoinfection, so infection can persist for years if left untreated. *H nana* infection is most often asymptomatic; however, symptoms can attend heavy worm burdens. Medical professionals should always be aware that parasite infections can be present in asymptomatic disease [8, 11].

The prevalence of intestinal hymenolepiasis in a community may be a useful indicator of the degree of fecal contamination of an environment and/or the level of hygiene practice [11]. Iron-deficiency anemia may be due to either decrease absorption of iron or due to increased loss iron. [12]

Anemia is one of the most commonly recognized disorders. It is estimated to affect half the school-age children and adolescents in developing countries. Iron deficiency anemia affects about 1.3 billion people with highest prevalence and morbidity in young children and pregnant women [13].

Anaemia is often associated with parasitic disease such as malaria and hookworm infections, Hookworms contribute to anaemia because it induces iron deficiency by chronic intestinal blood loss. The two species of hookworms *Ancylostoma duodenale* and *Necator americanus* cause about 0.2 mL and 0.15 mL blood loss per day respectively. Hookworms also release anticlotting factors (i.e., coagulase, a blood thinner) which ensures continuous blood flow, high intensity Trichuris and Ascaris infections have been known to influence nutritional status [14]

Most diagnoses are made by identifying the appearance of the worm or eggs in feces. Due to the large quantity of eggs laid, physicians can diagnose using only one or two fecal smears [15]. While Fecal contamination is one of the most serious environmental health problems in poor countries, where 3 million children die of enteric diseases each year and even more suffer from debilitating diseases due to intestinal parasites, although the infection is often asymptomatic, its effects may contribute substantially to child morbidity when associated with malnutrition, pneumonia, enteric diseases and vitamin A deficiency [16]. The most significant cause of iron-deficiency anemia in third world children is parasitic worm's hookworms, whipworms, and round. Worms cause intestinal bleeding, which is not always noticeable in faeces, and is especially damaging to growing children [17]. Malaria, hookworms and vitamin A deficiency contribute to anemia during pregnancy in most underdeveloped countries. In women over 50 years old, the most common

cause of iron-deficiency anemia is chronic gastrointestinal bleeding from nonparasitic causes, such as gastric ulcers, duodenal ulcers or gastrointestinal cancer [18, 19, 20].

SUBJECTS AND METHODS

Specimens

From March till August 2015, 60 samples were collected from patients and 30 healthy male who attended the clinics in AL-Sadder teaching Hospital and AL-Hakeem Hospital in AL-Najaf governorate, Stool samples were collected into clean, wide-mouth specimen bottles, from male patients and five ml of blood samples were also collected from each patients by vein-puncture, four ml put into specimen tubes and remains for 30 minutes at room temperature. After that the samples were centrifugation at 3000 rpm for 5 minutes (Backman/counter, Germany) to separate the serum and collected in other sterile tubes, each sample of serum was divided into three parts; each of them was kept in deep freeze at -20°C till used for the determination of leptin, iron and ferritin. The remainder one ml of blood was drawn in tube with anti-coagulated EDTA (Abott /Jordan) which was used for determination the hematological parameters Hb, PCV and RBCS.

Specimen Processing

Freshly voided stool specimens were processed and examined microscopically using X40 objective lens for intestinal parasites as described by [21]. Ten X40 objective fields of the stool smears were examined before a slide was considered negative.

Determination of serum leptin level

Estimated serum leptin of human by using ELISA Kit supplied by Ray Biotech, Inc. China with number code Cat#: ELH-Leptin-001 .

Serum Iron (Colorimetric Test)

The colorimetric test method was used to estimate the serum of iron via RANDOX reagents, code HB012. (Randox Kit, U.K) by cypress diagnostics biochemistry analyzer.

Ferritin ELISA

This test was intended to quantify the serum levels of ferritin through the immunoenzymatic technique Enzyme-Linked Immunosorbent Assay (ELISA) using bio Elisa reader ELx 800 (bio kit, U.S.A.) .The human Accu Bind ferritin ELISA kit was achieved according to the manufacturing company (Monobind Inc , U.S.A.) .

Statistical Analysis

Data were analyzed using the software packages Graph pad prism for Windows (5.04, Graph pad software Inc. USA), Data are presented as the mean \pm standard error (SE). The comparison between the patients and healthy groups were analyzed by T-test. A p-value < 0.05 was considered significant.

RESULTS

Relation between leptin of Hymenolipiasis Patients and Healthy Group

The result of fig.1 shows comparison between Hymenolipiasis patients and healthy group where a significant decrease ($P < 0.05$) of serum iron concentration in Hymenolipiasis patients 5.234 ± 0.425 ng/ml as compared to healthy group 9.412 ± 0.914 ng/ml.

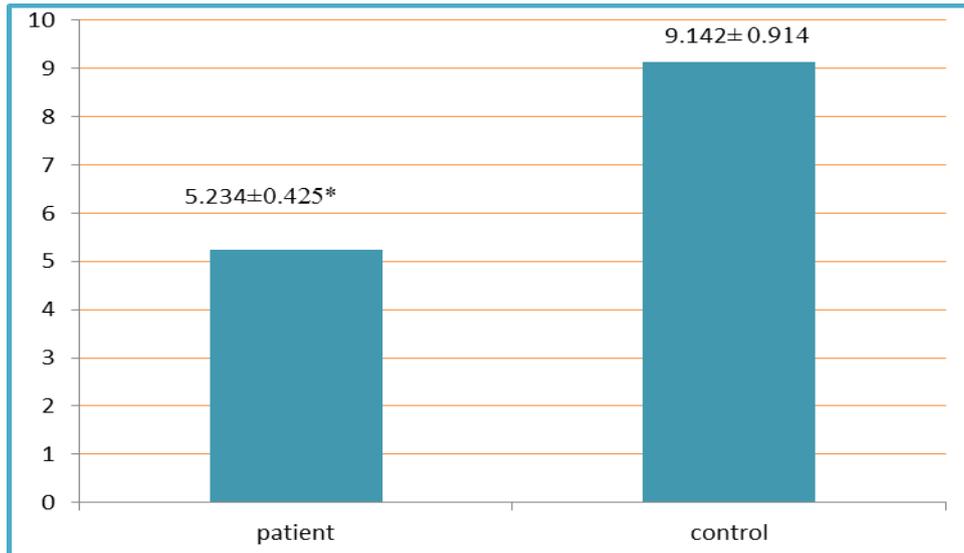


Fig.1. comparison between Leptin level of Hyminalipiasis patients and Healthy group.

*Significant difference (P<0.05) between control group and patients.

Relation between Ferritin of Hyminalipiasis Patients and Healthy Group

The Result of fig.2 showed comparison between Hyminalipiasis patients and healthy group where as significant decrease (P<0.05) of serum ferritin concentration in Hyminalipiasis patients 12.312 ± 0.481 ng/ml in compared to healthy group 25.613 ± 1.106 ng/ml.

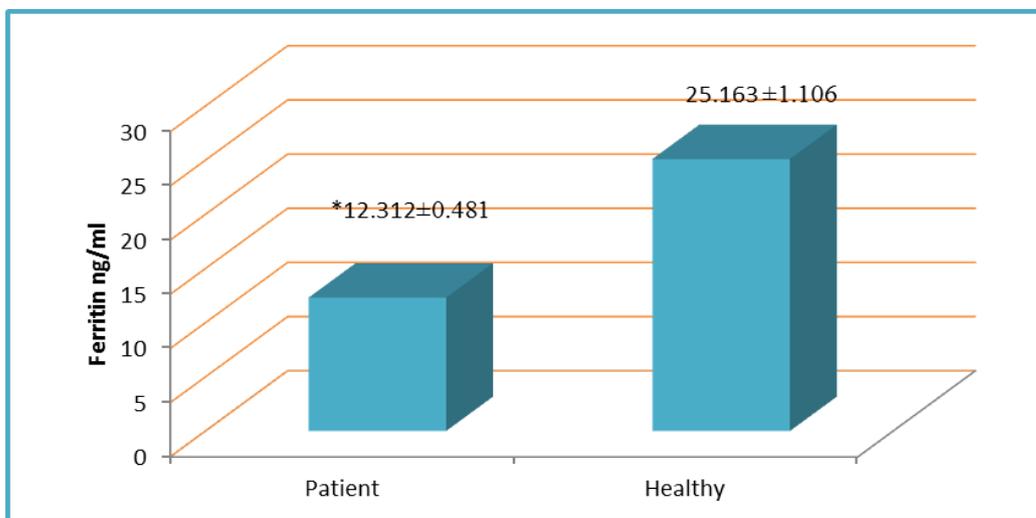


Fig.2. comparison between ferritin level of Hyminalipiasis patients and Healthy group

*Significant difference (P<0.05) between control group and patients.

Hematological Criteria

The result of fig.3 showed comparison between Hyminalipiasis patients and healthy group where as significant decrease (P<0.05) of Hb, PCV and RBCs count in Hyminalipiasis patients 8.948 ± 0.581 gm/dL in compared to healthy group 13.012 ± 0.151 gm /dL, 28.781±0.751 (%) in compared to healthy group 39.424± 0.610 (%), 3.985±0.631 × 10⁶/ mm³ in compared to healthy group 5.132±0.190 × 10⁶/ mm³, as seen in figure(3).

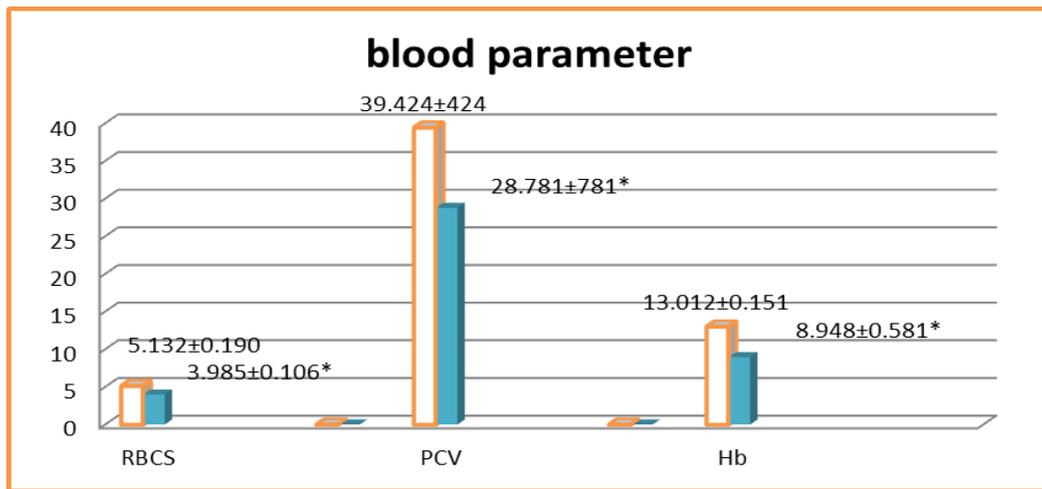


Fig.3. comparison between blood parameter (Hb, PCV and RBCS) of Hyminolipiasis patients and Healthy group.

*Significant difference (P<0.05) between control group and patients.

Relation between Iron of Hyminolipiasis Patients and Healthy Group

The result of fig.4 shows comparison between Hyminolipiasis patients and healthy group where as significant decrease (P<0.05) of serum iron concentration in Hyminolipiasis patients 30.423 ± 0.301 Ug/dL as compared to healthy group 62.136 ± 2.216 Ug/dl.

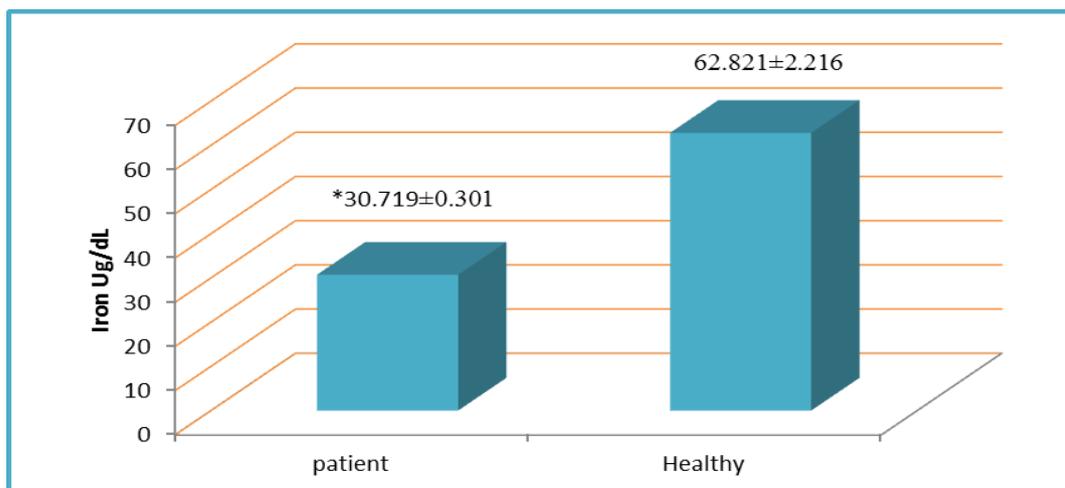


Fig.4. comparison between Iron level of Hyminolipiasis patients and Healthy group.

*Significant difference (P<0.05) between control group and patients.

CONCLUSION AND DISCUSSION

The results revealed the serum leptin, iron, ferritin, count of red blood corpuscular; hemoglobin concentration and packed cell volume significantly decrease in Hyminolipiasis infection patients compared to healthy group.

The decrease in leptin level in patients with parasitic infection maybe due to a role of leptin in host resistance to infection had been suggested in children and adult with congenital deficiency of the leptin receptor [22], or maybe due to deletion of the leptin receptor in the intestinal epithelium and increased susceptibility to intestinal parasitic infections, Intestinal parasitic infections may cause damage in intestinal mucosa such as inflamation, ulceration, and pathological changes in the villi of epithelial cells in the period of

infection. During the chronic period of the pathology, epithelial cell damage and intestinal abscesses have also been reported [23, 24].

The consuming of iron by *H. nana* cause a decrease in the iron levels, the source of iron maybe from the hemolysis of red blood cells from lesion occur by the worm. The possibilities are that the pathway of iron metabolism in the presence or absence of other micronutrients is different or that the presence of unabsorbed iron in the intestinal tract increases the production of free radicals and render the gut unsuitable for the establishment of infection [25]. This is supported by the fact that the effect of iron was most pronounced in those receiving iron without other micronutrients, indicating that micronutrients with antioxidant properties, e.g. vitamins A, C and E, are able to neutralize the free radicals generated by the iron, the decrease in ferritin levels maybe due to an increase in consuming iron by this parasite and this leads to decrease in the storage of iron as ferritin or increased utilized by worm. The present results indicated significant decrease in RBCs count, significant decrease in the concentration of Hb and PCV in patients with *H. nana* infection compared to control group, this result maybe due to hemolysis of RBCs by *H. nana* worm and this maybe caused decrease in the number of RBCs ,the hemolysis of RBCs maybe lead to decrease in the Hb concentration ,this finding supports the hypothesis that anemia in most frequent extra-articular manifestation of the disease [25 ,14]. Or the decreased of Hb, PCV and RBCs count caused by this parasite maybe due to a deficiency of iron, folic acid, and protein [26]. The relationship between parasitic infestation and anemia is a pathogeno-physiologic type [27].

From this study conclusion that serum levels of leptin decrease in patients infected with *H. nana* due to effects of early iron deficiency on the dopamine system and due to effects occur in receptor of leptin by this parasite.

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