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Hematological Study for Males Infected with Ascaris Limbercoid in Al-Najaf Province, Iraq

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Abstract: The study was conducted on 36 out patients and 20 healthy males to determine the influences of infected with Ascaris limbercoidon levels of iron, ferritin and Hb, RBCs and PCV in males infected with A. limbercoidin 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 2013. Diagnosis infection with this parasite by using the wet amount microscope for stool from patients. The results showed significant decrease ($P<0.05$) in iron and ferritin in A. limbercoidinfection patients in compared to control group**.** Furthermore the results showed serum Hb, RBCs and PCV were significant increased ($P< 0.05$) in A. limbercoid infection patients in compared to control group.

Keywords: PCV, RBCs, Diagnosis.

I. INTROCUTION

Ascaris lumbricoides is the giant roundworm of humans, belonging to the phylum Nematoda. An ascarid nematode, it is responsible for the disease ascariasis in humans, and it is the largest and most common parasiticworm in humans. One sixth of the human population is estimated to be infected by A. lumbricoides or another roundworm (Harhay, 2010) and A. lumbricoides is one of the most common parasites in the world, infecting 1.2 billion people globally (N.R. de Silva, 2003). The spectrum of disease associated with A. lumbricoides infection is known as ascariasis, and morbidity assessed as disability-adjusted life years (DALYs) is approximately 10.5 million , Furthermore, morbidity with serious health consequences is observed in 122 million cases per year(M.S. Chan, 1997). Infection may occur when food is handled without removing or killing the eggs on the hands, clothes, hair, raw vegetables/fruit, or cooked food that is (re)infected by handlers, containers, etc. Bleach does not readily kill Ascaris eggs but it will remove their sticky film, to allow the eggs to be rinsed away. Ascaris eggs can be reduced by hot composting methods, but to completely kill them may require rubbingalcohol, iodine, specialized chemicals, cooking heat, or "unusually" hot composting (for example, over 50°C (120°F) for 24 hours .

Most diagnoses are made by identifying the appearance of the worm or eggs in feces. Due to the large quantity of eggs laid, physicianscan diagnose using only one or two fecal smears (Murray, 2005),While Fecal contamination is one of the most serious environmental health problems in poor countries, where 3million 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 (Kolsky, 1995). The most significant cause of iron-deficiency anemia in third world children is parasitic worms hookworms, whipworms, and round. Worms cause intestinal bleeding, which is not always noticeable in faeces, and is especially damaging to growing children (Calis, 2008). 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 (Dreyfuss, 2000).

Anaemia is often associated with parasitic disease such as malaria and hookworm infections (Anumudu, 2007 and Hotez, 2008). Hookworms contribute to anaemia because it induces iron deficiency by chronic intestinal blood loss. The two species of hookworms Ancylostomaduodenaleand Necatoramericanuscause 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 Trichurisand Ascarisinfections have been known to influence nutritional status (Anumudu, 2007).

II. SUBJECTS AND METHODS

A. Specimens

From March till August 2013, 36 samples were collected from patients and 20 healthy male who attended the clinics in AL-Sadder teaching Hospital and AL-Hakeem Hospital in AL-Najaf province, Stool samples were collected into clean, wide-mouth specimen bottles, from male patients and five ml of blood samples were also drawn from the same

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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 two parts; each of them was kept in deep freeze at -20C° till used for the determination of 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 haematological parameters Hb, PCV and RBCS.

B. Specimen Processing

Freshly voided stool specimens were processed and examined microscopically using X40 objective lens for intestinal parasites as described by (Paniker, 1989).). Ten X40 objective fields of the stool smears were examined before a slide was considered negative.

C. 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 analyser.

D. Ferritin ELISA

This test was intended to quantify the serum levels of ferritin through the immunoezymatic 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.) .

E. Procedure

- The components of the kit were equilibrated at the room temperature before use.
- 25 µ l of standard, controls and sample was added per well.
- 100 µ l of Biotinylated ferritin Antibody was added to each well. Wells were covered with a sealing tape and incubated for 30 minutes. The timer was started after the last sample addition.
- The micro plate was washed six times with 300 ml of wash buffer using bioeliser washer ELx 50 (bio kit, U.S.A.).
- 100 µ l of ferritin Enzyme Reagent was added per well and incubated for 30 minutes. The bio Elisa reader ELx 800 (bio kit, U.S.A.) was turned on and set up the program in advance. 6- The micro plate was washed as described above.
- 100 µ l of working substrate solution was added per well and incubated for about 15 minutes or until the optimal blue colour density develops.
- 50 µ l of stop solution was added to each well. The colour will change from blue to yellow.
- The absorbance on bio Elisa reader EL x 800 was read at a wave length of 450 nm immediately. Results were provided within 1 minute on the LCD display and printed out.

F. Statistical Analysis

Data were analyzed using the software packages Graphpad prism for Windows (5.04, Graphpad software Inc. USA), Data are presented as the mean ± standard error (SE). The comparison between the patients and healthy groups were analyzed by one-way ANOVA. A p-value < 0.05 was considered significant.

III. RESULTS

A. Relation Between Iron Of Ascariasis Patients And Healthy Group

The result of fig.1 shows comparison between ascariasis patients and healthy group where as significant decrease (P<0.05) of serum iron concentration in ascariasis patients 32.321 ± 0.221 Ug/dL as compared to healthy group 60. 237± 3.518 Ug/dl.

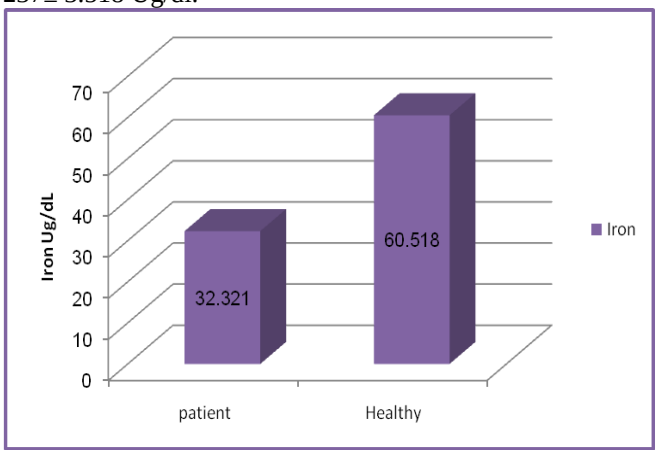

Fig.1. comparison between Iron level of ascariasis patients and Healthy group.

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

B. Relation Between Ferritin Of Ascariasis Patients And Healthy Group

The Result of fig.2 showed comparison between ascariasis patients and healthy group where as significant decrease(P<0.05) of serum ferritin concentration in ascariasis patients 13.436 ± 0.592 ng/ml in compared to healthy group 24.613 ± 1.120 ng/ml.

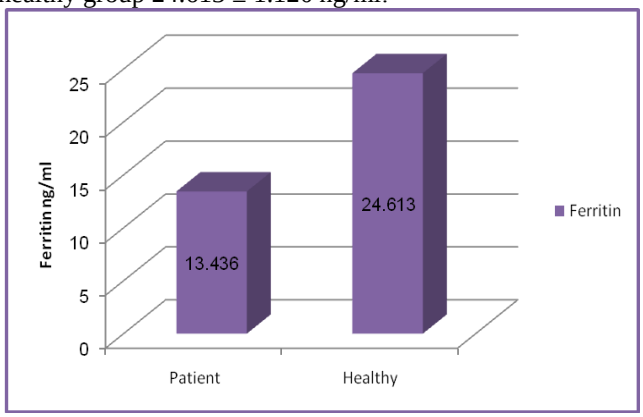

Fig.2. comparison between ferritin level of ascariasis patients and Healthy group.

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

C. Hematological Criteria

The result of fig.3 showed comparison between ascariasis patients and healthy group where as significant decrease (P<0.05) of Hb, PCV and RBCs count in ascariasis patients 9. 548 ± 0.581 gm/dL in compared to healthy group 13.548 ± 0.160 gm /dL,29.871 (%) in compared to healthy group 37.997(%), 3.755×10^6/ mm^3 in compared to healthy group 4.988×10^6/ mm^3, as seen in Table(3).

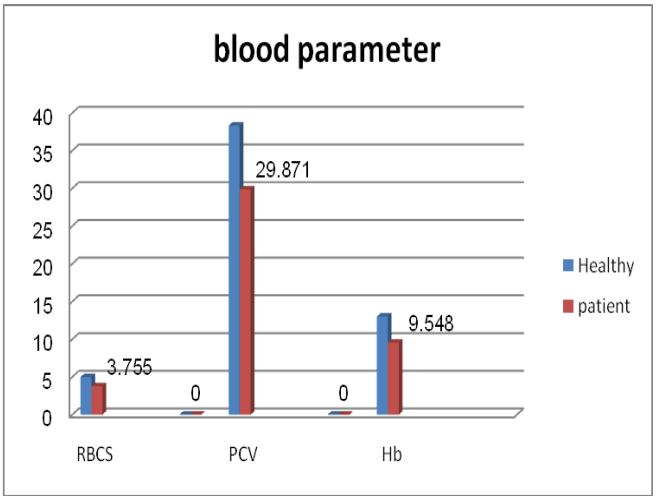

Fig.3. comparison between blood parameter (Hb, PCV and RBCS) of ascariasis patients and Healthy group.

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

IV. CONCLUSION AND DISCUSSION

The results revealed the serum iron, ferritin, count of red blood corpuscular; hemoglobin concentration and packed cell volume significantly decrease in A. lumbricoides infection patients compared to healthy group. The consuming of iron by A. lumbricoidesmay 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 (Olsen, 2003).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 A. lumbricoides infection compared to control group, this result maybe due to hemolysis of RBCs by A. lumbricoides 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 (Rehman, 2008 and Al-Qenaei, 2008).Orthe decreased of Hb, PCV and RBCs count caused by this parasite maybe due to a deficiency of iron, folic acid, and protein (McGregor, 1966).The relationship between parasitic infestation and anemia is a pathogeno-physiologic type (Kaeni, 2003).

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