

## Effect of amoebiasis on serum electrolytes in children diarrhea

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Received 16/10/2004 ; accepted 26/10/2005

### الخلاصة

أجريت هذه الدراسة على 54 طفلا مصابا بالإسهال نتيجة الإصابة بطفيلي امبيوسيز وشملت الدراسة 22 طفلا غير مصابين بهذا الطفيلي كمجموعة سيطرة. قورنت نتائج كل فئة عمرية للمصابين مع نتائج نفس الفئة العمرية للأطفال غير المصابين، و أوضحت النتائج إن مستوى كل من البيليروبين الكلي والمباشر وغير مباشر في مصل الدم للأطفال المصابين كانت ذات مستوى معنوي أعلى من مجموعة السيطرة لنفس الفئة العمرية. بينما كانت مستويات الأليكتروليتات لكل من الصوديوم، الكالسيوم، البوتاسيوم، الكلور، والفسفور في مصل الدم للأطفال المصابين كانت أقل معنويا مما هي عليه في مجموعة السيطرة لنفس الفئة العمرية للأطفال غير المصابين. تشير هذه الدراسة إلى إن الإصابة بهذا الطفيلي تؤثر على وظائف الكبد والأمعاء مما يؤدي إلى حدوث بعض التغيرات البايوكيميائية في مصل دم المصابين.

### ABSTRACT

This study has been performed on 54 patients with Amoebiasis after they had diagnosed by microscopic examination and we have ignore other causes of diarrhea. The aim of this work is to study the effect of diarrhea caused by Amoebiasis in children on some of biochemical variables. The patients were divided into three groups according to the age, the control consist of 22 children with out Amoebiasis (healthy chilled). The results of each age group of patients were compared with the same age group of the control, also the results indicated that the level of serum total, direct and indirect bilirubin in patients with Amoebiasis was significantly higher than that for control for each age group. The results indicated that the level of serum sodium, potassium, chloride, calcium and phosphorous of the patients was significantly lower than that for control in each age group. This study indicates that the diarrhea caused by Amoebiasis affected some of the liver and the intestine functions, which led to some biochemical changes in serum.

### INTRODUCTION :

Amoebiasis is currently defined as infection with the protozoan parasite *Entamoeba histolytica*. Normally resident in the large intestine, amoebae occasionally penetrate the intestinal mucosa and may disseminate into other organs<sup>(1)</sup>. About 90% of infection are asymptomatic, and the remaining 10 percent produce a spectrum of clinical syndromes ranging from dysentery to abscesses of the liver or other organs<sup>(2,3)</sup>. the organism has two stages (cyst & trophozoite) and multiplies asexually by binary fission. No sexual stage exists, and humans are the only hosts<sup>(4)</sup>. cyst of diameter 10-16µm with four nuclei when mature, one nucleus when immature<sup>(2)</sup> and are resistant to environmental conditions<sup>(5)</sup>. Trophozoites with a single nucleus of diameter 20-40µm<sup>(2)</sup>, their cytoplasm consists of a clear ectoplasm and a densely granular endoplasm containing a spherical nucleus which has a small central karyosome and fine granular chromatin material. Trophozoites are rapidly killed by exposure to air or stomach acid, however, and therefore cannot cause infection<sup>(6)</sup>. The cyst which is resistant to gastric acidity and digestive enzymes excites in the ileum to form eight trophozoites<sup>(7)</sup> colonize the cecum first then move distally through the colon<sup>(8)</sup>.

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After encystation infectious cysts are shed in the stool, in some patients, the trophozoites invade either the bowel mucosa causing symptomatic colitis, or the bloodstream, causing distant abscesses of the liver, lung, or brain<sup>(9)</sup>. Liver abscesses are always preceded by intestinal colonization, which may be asymptomatic. Trophozoites invade veins from the gut to reach the liver through the portal venous system<sup>(10)</sup>. Extension of hepatic lesion may reach the peritoneum and the pericardium, less commonly, the lesion extends through the skin. Rarely, *E. histolytica* trophozoites cause brain abscesses<sup>(11)</sup>.

In mammals the maintenance of osmotic pressure and water distribution in the various body fluid compartment is primarily a function of the four major electrolytes, Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, and HCO<sup>3-</sup>. In addition to water homeostasis, these electrolytes play an important role in the maintenance of PH, regulation of proper heart and muscle function, involvement in oxidation–reduction (electron transfer) reactions, and participation in catalysis as cofactors for enzymes<sup>(12)</sup>. It become quite apparent that abnormal levels of electrolytes may be either the cause or the consequence of a variety of disorders and the determination of electrolytes is one of the most important functions of the clinical laboratory<sup>(13)</sup>.

Electrolytes are classified as either anions, negatively charged ions that move toward the anode, or cations, positively charged ions that move toward the cathode in an electrical field. The major electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, and HCO<sup>3-</sup>) occur primarily as free ions, where as significant amounts (>40%) of Ca<sup>2+</sup>, Mg<sup>2+</sup>, and trace elements occur associated with proteins such albumin<sup>(14)</sup>.

## MATERIALS AND METHODS:

The samples were obtained from children who presented to the Al-Mansur teaching hospital for children, Baghdad, the study includes 53 patients with Amoebiasis, and the patients were divided into three groups:

Group 1: included patients <6 years old.

Group 2: included patients from 6 to 10 years old.

Group 3: included patients from 11 to 15 years old.

Diagnosis was confirmed on the basis of microscopic examination of fresh stool specimens. The amount of fresh stool specimen in normal saline was used for finding of either trophozoites or cysts. Twenty two children (1 month to 15 years old) were used as control; they were divided into three groups as in the patients groups.

### Serum Bilirubin Test :

It depends on the transformation of bilirubin to violet red compound, which is called azobilirubin by reacting with azosulphanilic acid. The direct bilirubin directly reacts with the azosulphanilic acid in the aquatic solution, while measuring of indirect bilirubin requires dissolving in methanol. The normal value of total bilirubin is 0.3-1.2mg/100ml, for direct bilirubin is up to 0.5mg/100ml, for indirect bilirubin is up to 0.7mg/100ml<sup>(15)</sup>.

### Method:

	test	test control	direct	direct control
<b>dist.water</b>	1.8 ml	1.8 ml	1.8 ml	1.8 ml
<b>serum</b>	0.2 ml	0.2 ml	0.2 ml	0.2 ml
<b>diazo reagent</b>	0.5 ml	—	0.5 ml	—
<b>methanol</b>	2.5 ml	2.5 ml	—	—
<b>dist. Water</b>	—	—	2.5 ml	2.5 ml

All tubes were allowed to stand in the dark for 30 minutes then the absorbance of each tube was measured at 540nm.

$$\text{Serum total bilirubin mg/100ml} = \frac{\text{Test} - \text{Test control}}{\text{Standard}} \times 8$$

$$\text{Serum direct bilirubin mg/100ml} = \frac{\text{Direct bilirubin} - \text{Direct control}}{\text{Standard}} \times 8$$

$$\text{Serum indirect bilirubin mg/100ml} = \text{Serum total bilirubin} - \text{Serum direct bilirubin}$$

### Serum Sodium, Potassium, Chloride, Calcium, and Phosphorus Tests :

All these electrolytes are measured by flame photometer, the normal range for serum is 135-146Meq/l, 3.5-5.2Meq/l, 95-105Meq/l for sodium, potassium, chloride respectively<sup>(16)</sup> and 8.4-102mg/100ml, 2.7-4.5mg/100ml for calcium and phosphorus respectively<sup>(17)</sup>. The biostatistical method used for analysis is one-way ANOVA method.

## RESULTS AND DISCUSSION:

### Effect of Amoebiasis on Serum Sodium :

The results showed that the level of serum sodium was 139.12±1.72Meq/l in control group 1 compared with 118.42±0.56Meq/l in group 1 of patients with Amoebiasis. The level of serum sodium was 140.28±0.89Meq/l in control group 2 compared with 127±0.81Meq/l in group 2 of patients with Amoebiasis. The level of serum sodium was 140.57±1.17Meq/l in control group 3 compared with 131.25±1.19Meq/l in group 3 of patients as shown in (fig-1). By one-level ANOVA the level of Na in group of patients was significantly lower than that for control group for each age group (p<0.01). By one-way ANOVA the level of sodium in group 1 of patients was significantly lower than for group 2 of patients which was significantly lower than that for group 3 of patients (p<0.01). These results which indicate that the level of serum Na is decreased during Amoebiasis in agreement with the results reported by many publishers<sup>(18,19)</sup>.

The decrease of Na during Amoebiasis is because that *E. histolytica* exerts a lytic effect on tissue, light and electron microscopic studies have been interpreted as showing lyses of mucosal cell on contact with amoebae or alternatively diffuse mucosal damage before amoebic invasion<sup>(20)</sup>. Amoebiasis increased secretion and impaired absorption of Na, and since Na<sup>-</sup> glucose co-transport is affected in the intestine because of the decrease in glucose absorption, this leads to decrease the level of serum sodium.

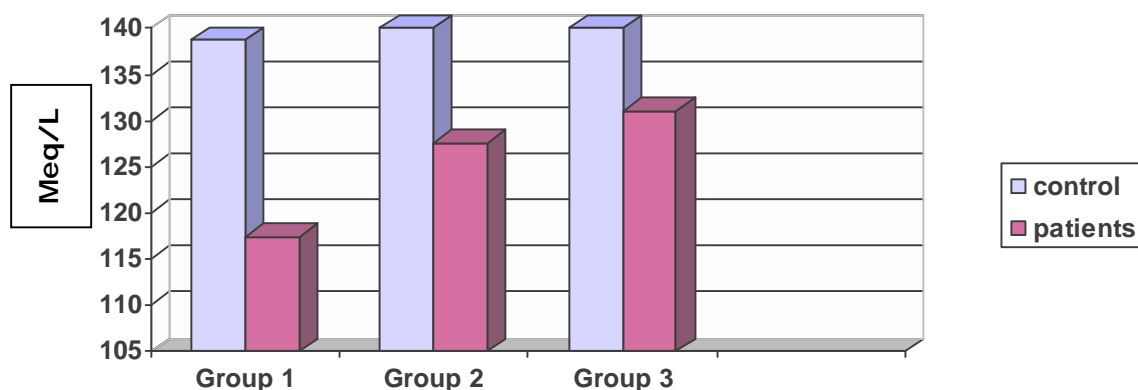


FIG 1 . EFFECT OF AMOEBIASIS ON SERUM SODIUM.

### Effect of Amoebiasis on serum potassium :

The level of serum  $K^+$  (fig-2) was  $3.95 \pm 0.11 \text{ Meq/l}$  in control group 1 compared with  $3.18 \pm 0.06 \text{ Meq/l}$  in group 1 of patients. The level of serum K was  $4.18 \pm 0.16 \text{ Meq/l}$  in control group 2 compared with  $3.18 \pm 0.13 \text{ Meq/l}$  in group 2 patients. The level of serum K was  $4.24 \pm 0.17 \text{ Meq/l}$  in control group 3 compared with  $3.0 \pm 0.03 \text{ Meq/l}$  in group 3 of patients. By one-way ANOVA the level of K in group of patients was significantly lower than for control group for each age group ( $p < 0.01$ ). While the difference in the level of K between the groups of patients was not significant. These results which indicate that the level of serum K is decreased in patients agreed with the results reported by Butler et al.<sup>(21)</sup>. In a study of the causes of death in diarrheal diseases they found that Amoebiasis is a cause of hypokalemia<sup>(14)</sup>.

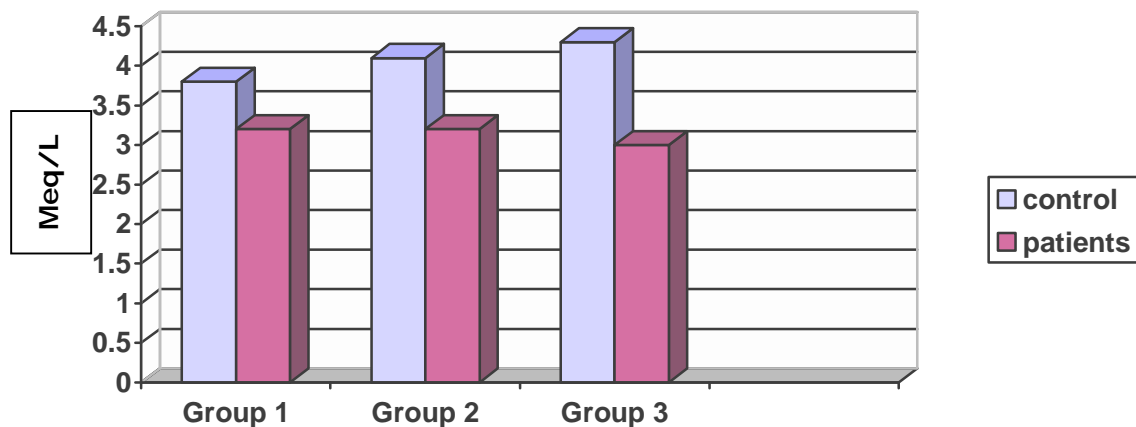


FIG 2 . EFFECT OF AMOEBIASIS ON SERUM POTASSIUM.

### Effect of Amoebiasis on serum chloride :

The results showed that the level of serum  $Cl^-$  was  $100.62 \pm 1.95 \text{ Meq/l}$  in control 1 compared with  $91.17 \pm 0.52 \text{ Meq/l}$  in group 1 of patients. The level of serum  $Cl^-$  was  $102.14 \pm 1.56 \text{ Meq/l}$  in control group 2 compared with  $91.42 \pm 1.25 \text{ Meq/l}$  in group 2 of patients. While it was  $101 \pm 1.11 \text{ Meq/l}$  in control group 3 compared with  $90.0 \pm 0.61 \text{ Meq/l}$  in group 3 of patients. By one-way ANOVA the level of  $Cl^-$  in group of patients was significantly lower than that for control for each age group ( $p < 0.01$ ). while it was not significant between the groups of patients as indicated in (fig-3).

The results indicate that the level of serum  $Cl^-$  is decreased during Amoebiasis are agree with that reported by Aucott & Ravdin<sup>(22)</sup>. The decrease of  $Cl^-$  during Amoebiasis is because of diarrhea, where patients, produce stools containing large amounts of  $Na^+$ ,  $Cl^-$ , and  $K^+$ , since in the intestine the absorption of Na promotes absorption of  $Cl^-$  through a Para cellular pathway and since Amoebiasis increased secretion and impaired absorption of  $Na^+$  in the intestine, this prevents absorption of  $Cl^-$  through a Para cellular pathway and since Amoebiasis causes secretory diarrhea.

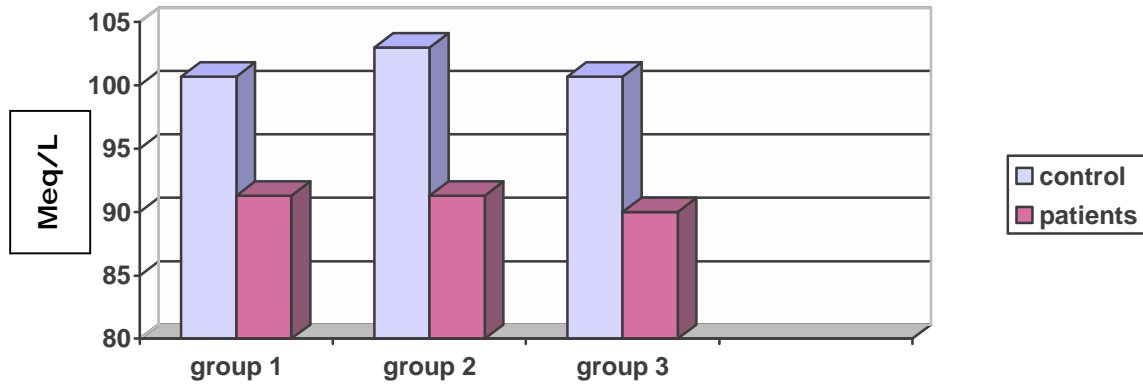


FIG 3 . EFFECT OF AMOEBIASIS ON SERUM CHLORIDE.

#### Effect of Amoebiasis on Serum Calcium :

The results showed that the level of serum calcium was  $9.86 \pm 0.14 \text{ mg}/100 \text{ ml}$  in control group 1 compared to  $8.04 \pm 0.03 \text{ mg}/100 \text{ ml}$  in group 1 of patients. The level of serum  $\text{Ca}^{+}$  was  $9.88 \pm 0.07 \text{ mg}/100 \text{ ml}$  in control group 2 compared with  $8.1 \pm 0.11 \text{ mg}/100 \text{ ml}$  in group 2 of patients while its level in control group 3 was  $9.82 \pm 0.05 \text{ mg}/100 \text{ ml}$  compared with  $7.95 \pm 0.07 \text{ mg}/100 \text{ ml}$  in group 3 of patients (Fig-4). One-way ANOVA analysis showed that the level of Ca in group of patients was significantly lower than that for control group for each age group ( $p < 0.01$ ), while the difference in the level of Ca between the groups of patients was not significant. The results which indicate that the level of serum Ca is decreased during Amoebiasis are agreed with the results reported by Iruksen, et al.<sup>(23)</sup>. The decrease of serum Ca during Amoebiasis is because of that, Ca is actively absorbed in the duodenum largely regulated by the active form of vitamin  $\text{D}_3$  which metabolized first by the liver and then by the kidney to form  $(1,25 [\text{OH}]_2 \text{D}_3)$ , calciferol stimulates the synthesis of Ca-binding protein and Ca-activated ATPase, all involved in active Ca transport and since liver function abnormalities are frequently present with intestinal Amoebiasis, so vitamin  $\text{D}_3$  is not metabolized by the liver, this decreased the absorption of Ca in the duodenum and leads to decrease the level of Ca in serum.

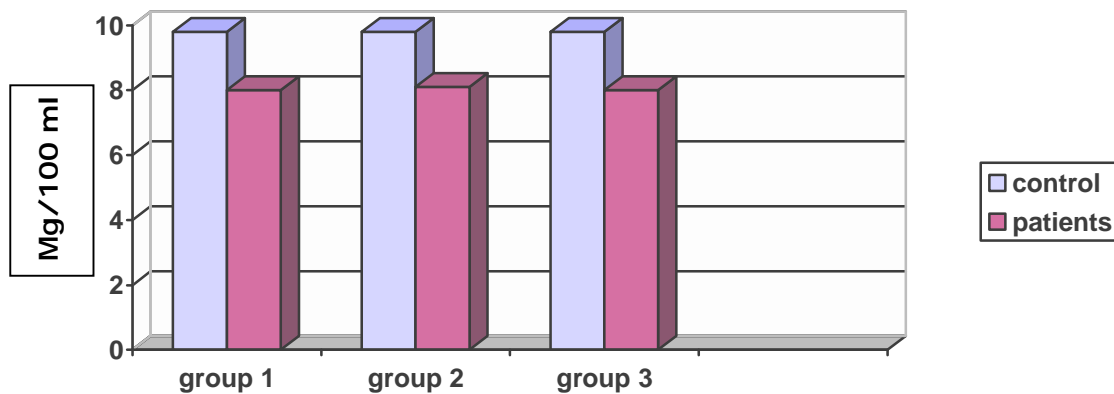


FIG 4 . EFFECT OF AMOEBIASIS ON SERUM CALCIUM.

### Effect of Amoebiasis on Serum Phosphorus :

The level of serum phosphorus was  $3.82 \pm 0.09 \text{ mg}/100 \text{ ml}$  in control group 1 compared with  $1.64 \pm 0.05 \text{ mg}/100 \text{ ml}$  in group 1 of patients with Amoebiasis, the level of serum P was  $3.28 \pm 0.27 \text{ mg}/100 \text{ ml}$  in control group 2 compared with  $1.34 \pm 0.09 \text{ mg}/100 \text{ ml}$  in group 2 of patients while in group 3 its level was  $3.68 \pm 0.15 \text{ mg}/100 \text{ ml}$  in control group 3 compared with  $1.57 \pm 0.1 \text{ mg}/100 \text{ ml}$  in group 3 of patients as in (Fig-5).

One-way ANOVA analysis showed that the level of P in group of patients with Amoebiasis was significantly lower than that for control group for each age group ( $p < 0.01$ ). While the level of P between the group of patients with Amoebiasis was not significant. The available references did not refer to the effect of Amoebiasis on the serum phosphorus, the decrease of serum P during Amoebiasis is due to that, *E. histolytica* exerts a lytic effect on tissue; amoebae appear to invade the colonic epithelium. Amoebiasis causes secretory diarrhea<sup>(24)</sup> which is characterized by a large volume of fecal output caused by abnormal fluid and electrolyte transport and this leads to decrease the level of phosphorus in serum.

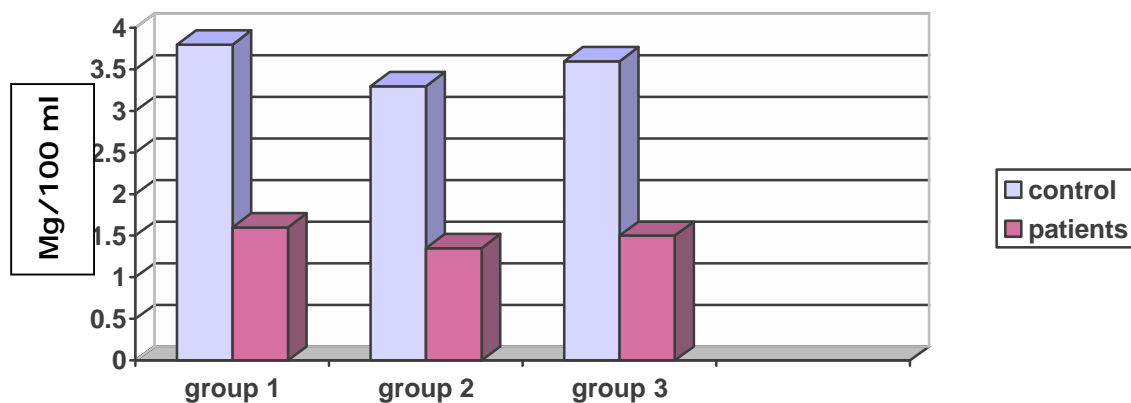


FIG 5 . EFFECT OF AMOEBIASIS ON SERUM PHOSPHORUS.

### Effect of Amoebiasis on serum Bilirubin :

The results of serum bilirubin was showed in (Fig-6), by one-way ANOVA the level of total bilirubin in group of patients was significant higher than that for control group for each age group ( $p < 0.01$ ) while the difference in the level of total bilirubin between the groups of patients was not significant. The direct bilirubin results were shown in (Fig-7), by one-way ANOVA, the level of bilirubin (direct) in group of patients was significantly higher than that for control group for each age group ( $p < 0.01$ ). While the difference in the level of bilirubin between the group of patients was not significant. The results of indirect bilirubin level in group of patients (fig-8) was significantly higher than that for control group for each age group ( $p < 0.01$ ). But the difference in the level of bilirubin indirect between the groups of patients was not significant. Our results were agreed with that obtained by Wanke et al.<sup>(25)</sup> the elevation of serum bilirubin during Amoebiasis is because of that amoebiasis is a cause of diarrhea and dysentery this leads to malabsorption which leads to chronic malabsorption for the bile acids which leads to increase the level of serum bilirubin<sup>(26)</sup>.

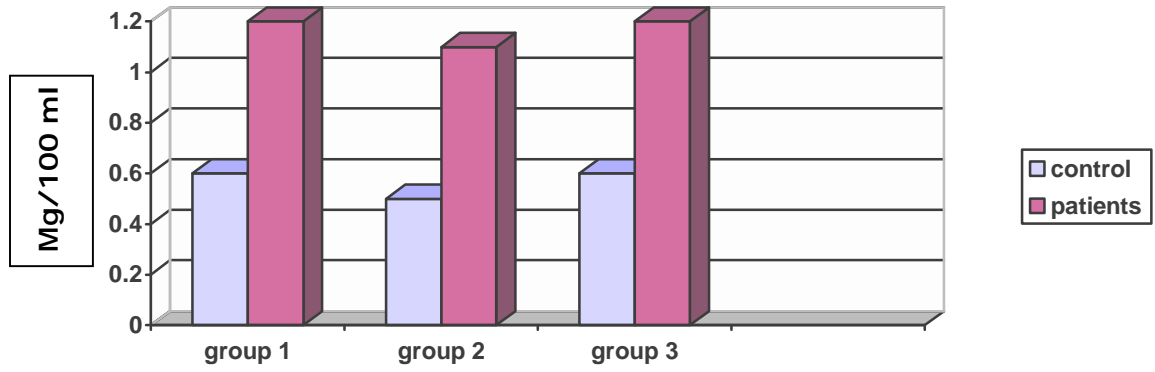


FIG 6 . EFFECT OF AMOEBIASIS ON SERUM TOTAL BILIRUBIN.

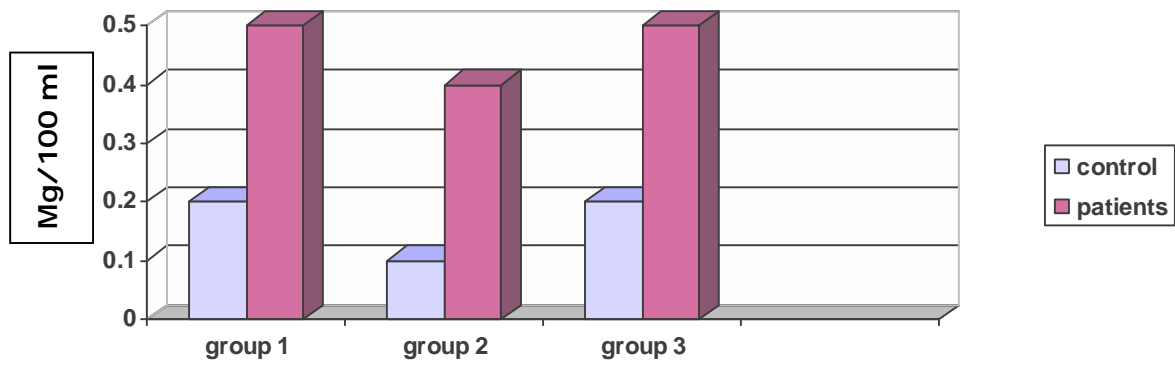


FIG 7 . EFFECT OF AMOEBIASIS ON SERUM DIRECT BILIRUBIN.

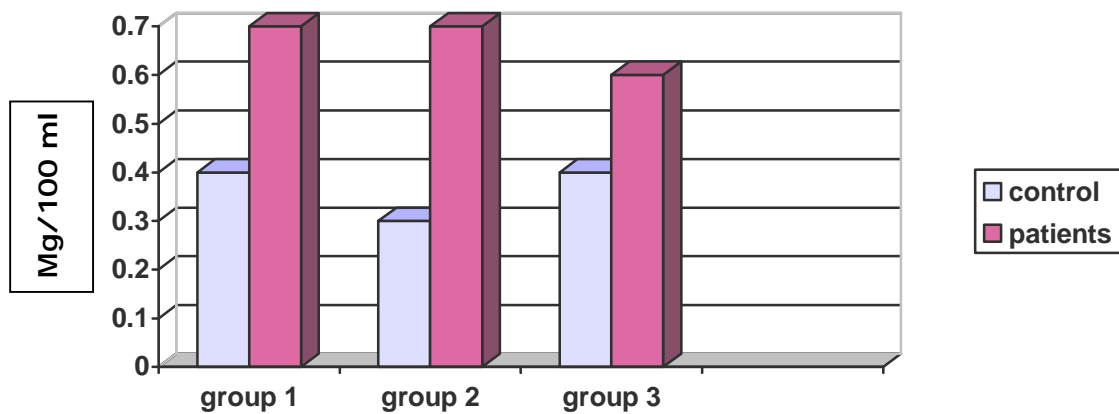


FIG 8 . EFFECT OF AMOEBIASIS ON SERUM INDIRECT BILIRUBIN.

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