BIOCHEMICAL STUDY FOR PATIENT WITH MAIN PROTOZOA DIARRHEAL AGENTS IN BABYLON PROVINCE, IRAQ

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Abstract
The current study during period was conducted from 1/September 2018 till 1/February 2019 examination of 60 stool samples (20 samples watery diarrhea for patient with intestinal protozoa, 20 samples watery diarrhea for patient without intestinal protozoa and 20 semi solid stool samples for healthy persons, It was examined by direct smear, Ziehl–Neelsen method (acid fast stain ) by using light microscope and Rapid test (chromatography immune assay For make sure that presence injury of those parasites or not,With measured of Severity of injury, The samples was taken from (adult 20-40 years male and female),The tests were carried out in the Advanced Parasitology Laboratory - Department of Biology - college of Science / University of Babylon. The current study. The samples was filtrated by tow stapes, At the beginning by filter paper then by sterile syringe filter ,The filtrated liquid examined to determine activity of amylase and lipase enzymes, The result showed that there are different between activity of two enzyme in stool samples with intestinal parasite and the other samples (healthy, diarrhea without parasite),And the results showed that there are A positive relationship between the level of activity of the studied enzymes and the severity of intestinal parasites.

Keywords: Intestinal parasites; Amylase enzyme, Lipase enzyme, pancreatic enzymes

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Introduction
Parasites are the main causes of human suffering, which may lead to ending his life as well as the oldest causes of diseases of diarrhea which known by the man from the past centuries and even our time [1]. The parasite included in medical parasitology are protozoa, helminthes and some arthropods[2].

Intestinal protozoa consist of four major parasitic groups in the human intestine ,The first three groups live live in cavity of human intestine it's extracellular parasites like Giardia lamblia, While the fourth is Sporozoa which living in the cells of the wrist wall it's intracellular parasites, The Coccidia is a group of parasite that are spread in animals and have a particular importance of veterans .This group spend all her life in the hidden walls of the guest except for a period where it is in the transition of the infective stage to the other host [3]

Acute injuries can sometimes develop into chronic infections that may last for several years with episodes of diarrhea or soft stool, as well as other symptoms including weakness, headache, muscle pain and poor absorption The parasitic infection caused iron deficiency and folic acid caused by mis-absorption due to damage to the intestinal mucosa [4].Some children fail to grow, itching, yellow bagitis and pancreatitis Which effect on level of human Pancreatic enzymes[5]

Materials and Methods
In current study 60 stool samples were collected from adult persons(20-40)years during the period from 1/ September/2018 to 1/January/2019, This samples were collected in sterile Plastic containers with a tight lid to keep samples moisture and prevent dry,. And transported to the Advanced Parasitology Laboratory at the Department of Biology, University of Babylon under cooling .Then examined this samples by direct smear,staining by Ziehl–Neelsen stain and Rapid test (chromatography immune assay)To ascertain the presence of these parasites or not. Then samples were Filtered at the beginning with the filter paper and then with the PTFE Sterile Syringe filter,(This method we have modified and used for the first time and can be adopted to purify and sterilize stool.

samples out to get fluids free of impurities and germs to be ready for biochemical tests). Thus, the sample is ready for the biochemical tests (amylase and lipase testing). (These kits were designed to measure enzyme activity in the serum and sometimes in urine, but we performed a simple adjustment in the stool sample to be suitable this test)

**Kit of amylase test** composed of Vial R1(working Reagent), as a figure (1)
**Manual procedure**: - 1 ml was taken from reagent in plastic tube bring to 37°C, 25µl was added from specimens to reagent (in the plastic tube), mixed, start the timer after 1 minute in the water bath, then Initial absorbance was recorded at 405 nm, it recorded again every 1 minute during 3 minutes [6].

![Kit of amylase test component](image)

**Figure (1): component of amylase test**

**Kit of lipase test** composed of:-
1- Vial R1 Enzymes-substrate (Lyophilisate)
2- Vial R2 Buffer
3- Vial R3 Start reagent
4- Vial R4 Lipase calibrator
5- Vial R5 Diluent for lipase calibrator. As a figure (2)

**Reagent preparation**:-
**First**, an equal amount of R1 was added to R2 and gently stirred and then left 10 minutes at room temperature,
**Second** The R4 was carefully opened and 3 ml of R5 was added and left to fully blend with the gage gently and let it settle down before use
(Quek shirking is avoided to avoid bubbles). While R3 was ready for use without any additions.
**Manual procedure**: - For this test three tubes were provided: - (Blank, calibrator, assay)
**Blank**: composed of 1000µL (R1+R2) Placed in a test tube with 20 µL of Distilled water, left for 4 minutes in the water bath at 37 °C, Then added 350µL of R3, strongly blended and then returned to the water bath left for 3 minutes. Finally measured absorbance every minute in three minutes at 550 nm.

![Kit of lipase test component](image)

**Figure (2): component of lipase test**

Finally measured absorbance every minute in three minutes at 550 nm.
**Calibrator**: composed of 1000µL (R1+R2) Placed in a test tube with 20 µL of (R4+R5), left for 4 minutes in the water bath at 37 °C. Then added 350µL of R3, strongly blended and then returned to the water bath left for 3 minutes Finally measured absorbance every minute in three minutes at 550 nm.
**Assay:** composed of 1000 µL (R1+R2) placed in a test tube with 20 µL of specimen, left for 4 minutes in the water bath at 37° C, then added 350 µL of R3, strongly blended and then returned to the water bath left for 3 minutes. Finally, measured absorbance every minute in three minutes at 550 nm [6].

**Calculation:** the result was calculated as follow:

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\text{Lipase activity} = \left( \frac{\Delta \text{Abs/min}_{\text{Assay}} - \Delta \text{Abs/min}_{\text{Blank}}}{\Delta \text{Abs/min}_{\text{calibrator}} - \Delta \text{Abs/min}_{\text{Blank}}} \right) \times \text{Calibrator concentration (251 IU/L)}
\]

\[
\mu \text{Kat/L} = \text{IU/L} \div 60
\]

**Estimation of the severity (Density)**

The severity of infection was measured by counting the number of parasites found in the infected person's stool sample using the red and white blood cell counts. The output is multiplied by the size of the sample [7].

**Calculation:**

Number of parasite per m³ = Number of parasites per square × volume of sample in same square (1/10 m³) = n × (1 × 10) m³ [7].

**Result**

Mean of activity for amylase enzyme may vary between healthy persons and patients with intestinal parasites; was (51.377) µkat/l in patients with intestinal parasite. It was (30.03) µkat/l in patients diarrhea without parasite, while it was (32.04) µkat/l in healthy persons as figure (3).

![Activity of amylase enzyme](image)

**Figure (3): Activity of amylase enzyme**

Also, activity of lipase enzyme was vary, Mean of its activity was (11.73) µkat/l, (3.4) µkat/l in patient with diarrhea without parasite. While it was (2.27) µkat/l in healthy persons as figure (4).

![Activity of lipase enzyme](image)

**Figure (4): Activity of lipase enzyme**

Also, there was a positive relationship between the severity of intestinal parasites and the Activity of amylase enzyme in persons with three parasites. Where the highest activity of amylase enzyme was (65.52) µkat/l in persons with higher severity of infection as figure (5).
The results showed that there are also a positive relationship between the severity of intestinal parasites and the Activity of lipase enzyme in persons with three intestinal parasites. Where the highest activity of lipase enzyme was (16.6)µkat/l in persons with higher severity of infection as figure(6).

**Figure(6):relationship between the severity of infection and the Activity of lipase enzyme**

Acute injuries of intestinal parasite can sometimes develop into chronic infections that may last for several years with episodes of diarrhea or soft stool, as well as other symptoms including weakness, headache, muscle pain and poor absorption. The parasitic infection caused iron deficiency and folic acid caused by mis-absorption due to damage to the intestinal mucosa. Some children fail to grow, itching, yellow bagitis and pancreatitis which effect on level of human Pancreatic enzymes.

The present study showed that there were significant differences in the level of amylase enzyme in the patients by watery diarrhea with intestinal protozoa. The highest mean level of amylase in patient with intestinal protozoa was 51.37µkat/L. While mean level of the enzyme in healthy was 23.04 µkat/L. Also there was a positive relationship between the severity of intestinal parasites and the Activity of amylase enzyme in persons with three parasites. Where the highest activity of amylase enzyme was (65.52)µkat/l in persons with higher severity of infection as figure(4-16). Previous results showed that activity of Amylase enzyme was fixed and did not change in patients with *G. lamblia* in the duodenal juice. That difference between the current results and the results of the few researches contests may be due to the difference in type of examined samples, experiment conditions, nature of enzymes used and parasite itself.

**Discussion**

Acute injuries of intestinal parasite can sometimes develop into chronic infections that may last for several years with episodes of diarrhea or soft stool, as well as other symptoms including weakness, headache, muscle pain and poor absorption. The parasitic infection caused iron deficiency and folic acid caused by mis-absorption due to damage to the intestinal mucosa. Some children fail to grow, itching, yellow bagitis and pancreatitis which effect on level of human Pancreatic enzymes.

The present study showed that there were significant differences in the level of amylase enzyme in the patients by watery diarrhea with intestinal protozoa. The highest mean level of amylase in patient with intestinal protozoa was 51.37µkat/L. While mean level of the enzyme in healthy was 23.04 µkat/L. Also there was a positive relationship between the severity of intestinal parasites and the Activity of amylase enzyme in persons with three parasites. Where the highest activity of amylase enzyme was (65.52)µkat/l in persons with higher severity of infection as figure(4-16). Previous results showed that activity of Amylase enzyme was fixed and did not change in patients with *G. lamblia* in the duodenal juice. That difference between the current results and the results of the few researches contests may be due to the difference in type of examined samples, experiment conditions, nature of enzymes used and parasite itself.
Conclusions

Intestinal parasites effect in level of activity of pancreatic enzymes by reducing the absorption in the intestine.

References