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GIARDIASIS, HELICOBACTER PYLORI AND SERUM LEVELS OF SOME MICRONUTRIENTS

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Abstract
Giardiasis and *H. pylori* in upper gastrointestinal system absorbed some vitamins and mineral occurs. This study detected the serum levels of copper, zinc, vitamins B12 & B9 among giardiasis and/or *H. pylori* patients and compared the serum levels of these minerals and vitamins among them. A total of 95 patients suffered from acute diarrhea were subjected to questionnaire taking, stool analysis, iron/haematoxylin staining, *H.pylori* antigen detection and serum level detection of zinc, copper, vitamins B12 & B9. Patients were divided into G1 (n=13) for microscopic *Giardia +ve*, G2 (n=40) *H. pylori +ve*, G3 (n=6) both *Giardia* & *H.pylori +ve*, G4 (n=36) *Giardia* & *H. pylori –ve* (control). The mean age of patients was 45.7±17. On comparing variables among patients; residence, abdominal pain and flatulence were statistically significant ($P \leq 0.05$). Serum levels of zinc, vitamins B12 & B9 were within normal ranges, except copper level was higher than normal in all groups. G3 (co-infections) showed the lowest levels of zinc, vitamins B12 & B9. None of serum levels were statistically significant on comparing the four groups. Both giardiasis and *H. pylori* altered levels of micronutrients but during acute infection serum levels of zinc, vitamin B12 & B9 were still within normal ranges. Pattern of infection either chronic or acute were basic regarding micronutrients levels.
Keywords: Patients, Giardiasis, *H. pylori*, Copper, Zinc, Vitamins B12, & B9.

Introduction

Zinc and copper are crucial minerals for growth and worked against free radicals (Olivers *et al,* 2003). Zinc plays a role to help lymphocytes secretion and antibodies formation since deficiencies will affect lymphocytes (Culha and Sangun 2007). Copper strengths the action of haemopiotic system and maintains hemoglobin and iron absorption (Celiksoz *et al,* 2005).

Giardiasis decreased copper (Arbabi *et al,* 2015) and zinc levels (Demirci *et al,* 2003; Zarebavani *et al,* 2012). *Giardia* colonizes the upper GIT, trophozoite adherence damage the intestinal epithelium affecting absorption (Ghieth *et al,* 2018), where vitamin B12 & B9 were absorbed (Olivers *et al,* 2002).

Both vitamins are essential for keeping CNS and bone health, decreasing strokes by methylation of homocystine (Elstgeest *et al,* 2017; Spence. 2017). Giardiasis affected the vitamins (Askari *et al,* 2007; Zarebavani *et al,* 2012).

Helicobacter pylori is gram-negative pathogen affect acidity (Gao *et al,* 2020) and interfere with micronutrients digestion (Öztürk *et al,* 2015). *H. pylori* colonized the entire gastric epithelium, and has an important urease activity, that leads to the ammonia production in order to protect itself from gastric acidity (Dzierzanowska-Fangrat and Dzierzanowska, 2006). The accompanied dyspepsia and malabsorption negatively change vitamin B12 and folate levels were improved by eradication (Rasool *et al,* 2012).

This study was implicated with the existence of the two organisms among patients with acute diarrhea and correlated zinc, copper vitamin B12 & B9 levels among them.

Materials and Methods

Study design: A cross sectional study was done among 95 adult patients suffering from acute diarrhea and attending Outpatients clinics, Beni-Suef University Hospitals.

Samples collection and processing: Three stool samples were collected from all patients and divided into three parts, one for coprscopic examination using direct wet mount and formalin-ethyl acetate concentration with saline and Lugol's iodine to detect *Giardia* and other parasites using x10 & x40 objectives. The second part was preserved in SAF preservative for further iron/hematoxylin staining. The last part was preserved in -20°c for detection of *H. pylori* antigen. All patients were subjected to blood samples and serum separation for biochemical estimation of copper and zinc levels using colorimeter by the commercial kits (copper fluid auto-Dibrom PAESA method and zinc fluid mono-reagent kits, CF25911050, ZF0100050; centronic GmbH /Germany).

Serum levels of vitamin B 12 & B 9 were detected by human vitamin B12 ELISA kit and human folic acid ELISA kit supplied by Bioassay Technology Laboratory (Catalog Number E1544Hu & E1509Hu; England) using ELISA. According to kits, normal levels of zinc: 46-150µg/dl, copper: 70-155µg/dl, vitamin B12: 160-970pg/dl and vitamin B9: 1.5-17g/ml

Detection of *H. pylori* antigen in stool by ELISA: Stool samples were analyzed for *H. pylori* antigen using sandwich ELISA according to manufacturer's instructions, using EDI™ Fecal *H. pylori* Antigen ELISA kit, (Epitope Diagnostic, Inc, San Diego, USA).

Statistical analysis: Data were analyzed and processed using Statistical Package for Social Science, (SPSS) software version 20. Frequency and percentage were used for qualitative data. Quantitative data were presented by M±SD. Comparing groups as to mean of serum levels of vitamins and minerals was done using Anova test. *P* values of less than 0.05 were considered significant.

Results

Among 95 diarrheic patients with mean age of 45.7±17, microscopic giardiasis and staining was detected in 19 samples (20%), and *H. pylori* antigen was detected in 46 samples (48.4%). According to the microscopic examination of *Giardia* in stool samples and yielding of ELISA for *H. pylori* antigen detection, patients were divided into four groups; G1: including patients with +ve microscopic giardiasis and -ve *H. pylori* antigen detection (n=13), G2: +ve *H. pylori* antigen and -ve for microscopic giardiasis (n=40), G3: patients co-infections with giardiasis and *H. pylori* antigen (n=6), G4: patients free from both microscopic giardiasis and *H. pylori* antigen (n=36). The majority were female (53.6%), residence in urban areas (54.7%) and using tape water (74.7%). The commonest symptom was flatulence (28.4%) followed by abdominal pain and fatigue (26.3% & 23.1%, respectively). On comparing frequency of variables between groups, residence, abdominal pain and flatulence were statistically significant (*P* value ≥0.05). By using Anova test for comparing the levels of vitamins and minerals on the four groups, none was statistically significant.

Results

The results were given in tables (1 & 2) and figure (1).

Table 1: Distribution of variables among groups

Variables			G1(n=13)	G2 (n=40)	G3 (n=6)	G4 (n=36)	Total n=95	*P value*
Demography	Sex	Male	5 (38.5)	22 (55)	3 (50)	14(38.8)	44 (46.3)	0.27
		Female	8 (6.51)	18 (45)	3 (50)	22(61.1)	51 (53.6)	
	Age (M±SD) years		42.84 ± 12.74	45.23 ± 16.96	40.11 ± 13.24	43.56 ± 14.22	45.72 ± 17.25	0.55
	Residence	Rural	9 (69.2)	15 (37.5)	1(16.6)	18(50)	43(45.2)	0.05*
		Urban	4 (30.7)	25 (62.5)	5(83.3)	18(50)	52 (54.7)	
	Water source	tape	10 (76.9)	30 (75)	3(50)	28(3.6)	71(74.7)	0.55
		filtered	3(23)	10 (25)	3(50)	8(22.2)	24(25.2)	
Clinical manifestation		Fatigue	4(30.7)	7 (17.5)	5(83/3)	6(16.6)	22 (23.1)	0.06
		Abdominal pain	11(84)	4(10)	6(100)	4(11.1)	25(26.3)	0.00*
		Flatulence	12(92.3)	5(12.5)	6(100)	4(11.1)	27(28.4)	0.00*

*Significant $P \leq 0.05$.

Table 2: comparing of mean laboratory values between groups

Characteristics	G1 (n=13)	G2(n=40)	G3 (n=6)	G4 (n=36)	Total (n=95)	*P* value
Zinc	101.7 ± 48.6	86.9± 67.7	82.4±40.1	137.1±219.9	108.0± 145.7	0.508
Copper	191.5±75.6	234.5±168.4	251.9±176.0	210.0±53.8	234.1± 157.3	0.285
Vitamin B12	570.2±668.0	668.9±499.6	493.98±464.0	534.22±574.9	512.6± 53.1	0.239
Vitamin B 9	3.79±2.4	3.03± 1.5	2.347±1.2	3.15±1.9	3.13± 1.8	0.432

*Mean was presented as Mean±SD

Discussion

In the present study, all zinc serum levels were within normal ranges (46-150µg/dl), however G4 (free from both organisms) was the highest serum level (137.1µg/dl), followed by G1 & G2, (101.7µg/dl, 86.9 µg/dl, respectively). Co-infections G3 was the lowest zinc level one (82.4µg/dl).

Despite of *Giardia* infection in G1 & *H. pylori* in G2, serum levels of zinc were within normal ranges. This could be a compensatory mechanism by the body following infection, where immediate depletions of body stores of zinc in acute infection (Rodríguez, 2017). Thus, it was expected that zinc deficiency during prolonged chronic infections where zinc is depleted from the body. But, co-infections of *Giardia* and *H. pylori* aggrevated lowering of zinc levels than in GI & GII, as zinc levels were still within normal ranges, since they were not chronic. Zinc deficiency occurred in chronic giardiasis accompanied with malabsorption (Ertan *et al,* 2002; Culha and Sangun 2007).

In contrast, Abou-Shady *et al.* (2011) in Egypt showed significant lowering in zinc levels among *Giardia* infected patients. Others reported decrease in zinc levels and increase in copper (Olivares *et al,* 2003; Culha *et al,* 2007; Quihui *et al,* 2010). Difference could be attributed to dissimilarities between the studied population, nutritional deficiencies and/or disease chronicity.

As regard copper, all serum levels were much higher than normal ranges (70-155µg / dl), but co-infections G3 was the highest Cu level (251.9µg/dl), followed by G2, G4, & G1 (234.5µg/dl, 210µg/dl, 191.5µg/dl, respectively). Copper toxicity is used by the host as a defense method against infection, and elevation of copper levels was reported following infections (Fu *et al,* 2014; Garcia-Santamarina and Thiele, 2015). In man, copper is pledged to ceroplasmin protein with levels correlated to its concentration (Gaetke *et al,* 2014), controvertible to zinc which is not stored affluently within body and levels declination occurred in chronic infections.

Elevation of copper levels occurred in all groups, which means that, it was not specific to giardiasis or *H. pylori*. GIV (free from giardiasis and *H. pylori)*, showed elevation of cu levels, but it was not ensured if patients were free from organisms rather than *Giardia* and *H. pylori*, as some parasites and commensals were diagnosed as *E. histolyica*, *B. hominis*, *E. coli* and *Taenia* species (21.05%, 6.3%, 4.2%, 4.2%, respectively) .

In the present study, vitamin B12 & B9, levels were within normal ranges; but co-infections G3 showed the lowest serum levels than other groups (493. 98pg/dl & 2.34g/ ml, respectively). Zarebavani *et al.* (2012) found no difference among patients with and without giardiasis and vitamins B12& B9 serum levels. Askari *et al.* (2007) reported vitamin B12 deficiency and normal B9 in giardiasis.

In the present, the co-infection of giardiasis and *H. pylori* decreased vitamins levels but not below normal levels, which could be attributed to the pattern of acute diarrhea.

Conclusion

H. pylori and giardiasis were prevalent among patients with acute diarrhea. Copper, zinc, vitamins B12 & B9 levels were within normal ranges in acute diarrhea. Co-infection markedly alternated the micronutrients levels more than other groups.

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Ethical Standards: Protocol was approved by Faculty of Medicine, Beni-Suef University Research Ethics Committee in accordance with the Helsinki declaration, 1964. Samples collection was done after informing patients with the study aim and singed permission.

Conflict of interest: The authors declared that they neither have special interest nor received funds.

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Explanation of figure

Fig. 1 a: *E. coli* cyst, b: *Giardia* cyst, c: *Giardia* trophozoite, d: *Giardia* cyst, e: *E. histolytica* cyst, f: *Capillaria* egg (x40, a, b, e & f: Lugol's iodine stain. c, d: iron/haematoxylin stain).