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Relationship between gastroenteritis caused by *Helicobacter pylori* and *Giardia lamblia* in malnourished children

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ABSTRACT

Malnutrition remains one of the most common causes of morbidity and mortality among children through the world. Approximately 9%of children below 5years of age suffer from wasting (weight for height below -2 standard deviations (<-2SD) of the national center for health statistics (NCHS)/WHO reference values) and are at risk of death or sever impairment of growth and physiological development .*Giardia lamblia* and *Helicobacter pylori* are two intestinal pathogens sharing the same mode of infection .They are known to infect the gastrointestinal tract of humans early in life and to be very prevalent in endemic areas throughout life. A case control selective study were done on patients with malnutrition attending Salah Al-deen hospital in Tikrit city included 100 children with malnutrition (60 males and 40 females), their age range was 2-60 months the study also included 100 healthy children (as control group) to evaluate the co-infection of *H. pylori* and *G. lamblia* infection among malnourished children. Each malnourished and control group were sent for general stool examination and for detection of *Giardia lamblia* trophozoite or cyst. Stool sample also tested for detection of *Helicobacter pylori* antigens. The study revealed that the frequency of *G. lamblia* and *H. pylori* in malnourished children was 14% and 40% respectively and significantly higher than in control group (5% and 20% respectively). The study also found that 34% of malnutrition children were infected with *H. pylori* , 8% were infected with *G. lamblia* and 6% were coinfected with the two microorganisms. The study showed that the highest rate of *Giardia lamblia* and *Helecobacter pylori* infection was occurred in malnourished children within the age group 25-36 months and most of them from rural area. The present study showed that 64% of study cases were with mild malnutrition, 22% moderate and 14% were with severe malnutrition. The aim of this study is to evaluate the co-infection of *H. pylori* and *G. lamblia* infection among malnutrition children in Tikrit City. And the conclusion of the study is that the frequency of giardiasis and *H. pylori* infection in malnourished cases are more than control.

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Introduction:

Malnutrition remains one of the most common causes of morbidity and mortality among children through the world. Approximately 9%of children below 5years of age suffer from wasting (weight for height below -2 standard deviations (<-2SD) of the national center for health statistics (NCHS)/WHO reference values) and are at risk of death or sever impairment of growth and physiological development [(1)].

Malnutrition is responsible, directly or indirectly, for 54% of the 10.8 million deaths per year in children under five and contributes to every second death (53%) associated with infectious diseases among children under five years of age in developing countries [(2)]. Infection causes energy loss on the part of the individual, which reduces productivity on the community level and perpetuates the alarming spiral of malnutrition, infection, disease, and poverty[(3)]. *H. pylori* is a gram-negative bacterium that is estimated to infect approximately half of the world population. It colonizes the gastric mucosa of its human host where it may give rise to symptoms such as recurrent peptic ulcers and chronic gastritis, and has also been associated with gastric cancer.[(4)]One of the bacterial causes of Malnutrition is *H. pylori* which may impair the absorption of many nutrients and then compromise the nutritional status of infected individuals causing the emergence of different clinical manifestations[(4)]. *Helicobacter pylori* infection is frequently acquired during infancy and lasts along adult life. The prevalence of hypochlorhydria predisposes to gastrointestinal infections, diarrhoea and malabsorption syndromes. Also the parasitic infection may be cause malnutrition like the protozoan parasite *G. intestinalis* (syn. *G. lamblia, G. duodenalis*) is the causative agent of giardiasis in a wide range of vertebrates, including humans. The parasite is estimated to cause 280 million cases of human giardiasis per year [(5)]. The disease is characterized by bouts of diarrhea, bloating, flatulence and malnutrition, and is especially troublesome in children living in low-

income countries where stunted growth and poor cognitive function have been correlated with the disease [6]. Parasitic infections in children (below 5 years of age) are problematic because of negative lifelong health consequences and can contribute to malnutrition resulting in growth retardation [7].

In low- income countries co-infections involving several different pathogens commonly ocurr[7]. several recent, cross sectional studies from different locations,have reported apotential association between *G.Lamblia* and *H. pylori*[8]. Both organisms colonize the gastrointestinal tract in their human hosts within aclose proximity and both organisms are known to infect children at a high rate in low –income countries[9].

Materials and methods

The study cases (malnourished cases) were taken from the general Pediatric ward and Rehabilitation ward for malnutrition cases.

A case control hospital based selective study were done on 100 children with malnutrition attending Salah Al-deen hospital in Tikrit city, during the period from 1st of February to the last of August 2019 aged from 2 months-60months. A comparable group of similar number of cases of apparently healthy children were taken as a control with normal weight for height

Study acceptance taken from the parents and Salah Al –deen hospital lab manager . Each case (malnourished cases) were assessed by a prepared questionnaire that include (name, age, sex, residence,……etc).

The anthropometric measurements were measured for each case included in the study (malnourished cases).These include Wt/age, Ht/age, OFC/age, Wt/Ht.

Each malnourished case were sent for general stool examination. The stool was taken immediately in to a clean container and sent for the lab and examined immediately (within 20 minutes) under light microscopy by experience lab person looking for *Giardia lamblia* trophozoite or cyst. The method of preparation of the sample for examination and process of the test were done by Zinc sulfate concentration method. We mixed one gram feces in 10ml of 33% zinc sulfate

solution and let it like during 10 minutes before the observation of 10 microscopic fields with X40.[10]

All samples were tested for *Helicobacter pylori*. This faecal monoclonal antigen test has high sensitivity, specificity, and accuracy in children, 91_96%, 95_96% and94_96% respectively. The test kit used in this research manufactured in United Kingdom by Cam Tech medical company. It is a rapid one step test for the detection of *H.pylori* antigen in stool samples[11]

We allow the test device and samples to reach room temperature 15_30c prior to testing.Using the applicator stick of the provided sample diluent vial, transfer a small portion (5 mm diameter) of stool specimen in to the sample diluent. Shake gently in order to unstuck and facilitate the sample dispertion. Hold the vial and add 4 drops to the sample in the test device then see the result.[11]

1.Negative only one green band appears in the strip.

2.Positive 2 bands appear (green and red).

3.Invalid no colored bands appear or only one band appear.

Results

The total number of patients in this study was 200 children. They were divided into two groups, malnutrition group included 100 patients and control group included 100 well-nourished children .Their age group from 2months to 60 months.

4.1. General characteristics of the studied groups

This case control study included 100 children with malnutrition (60 males and 40 females), their age range was 3-48 months (mean: 17.12±8.2 month), the study also included 100 healthy children (as control group) with the same characteristics of patient. Further features of patients and control were mentioned in Table (1)

Table (1) :General characteristics of the studied groups

Variables		Malnutrition children		Healthy control		P. value
		No.	%	No.	%	
Age groups (months)	2-12	46	46	44	44	NS*
	13-24	34	34	33	33	NS
	25-36	12	12	13	13	NS
	37-60	8	8	10	10	NS
	Total	100	100	100	100	NS
	(Mean±SD)	17.12±8.2		17.34±8.1		NS
Gender	Males	60	60	59	59	NS
	Females	40	40	41	41	
Residence	Rural	62	62	60	60	NS
	Urban	38	38	40	40	
Educational level of mother	Illiterate	52	52	45	45	N..S
	Read and write	14	14	15	15	
	Primary school	18	18	20	20	
	Secondary school	11	11	14	14	
	Higher	5	5	6	6	

* P. value >0.05 = non-significant (NS)

. Frequency of *Giardia lamblia* and *Helicobacter pylori* in malnutrition and control children

The present study revealed that the frequency of *G. lamblia* and *H. pylori* in malnutrition children was 14% and 40% respectively and significantly higher than in control group (5% and 20% respectively), as shown in Table (2) . The study also found that 34% of malnutrition children were infected with *H. pylori* alone, 8% were infected with *G. lamblia* alone and 6% were coinfected with the two microorganisms (Figure (1).

Table (2): . Frequency of *G. lamblia* and *H. pylori* in malnutrition and control children

		patients		Control		X^2	P. value
		No.	%	No.	%		
G. lamblia	positive	14	14	5	5	4.9	0.02 S*
	negative	86	86	95	95		
H. pylori	positive	40	40	20	20	13.2	0.0001 HS**
	negative	60	60	80	80		

* P. value: ≥ 0.01 and ≤ 0.05: significant (S)

** P. value <0.01: highly significant (HS)

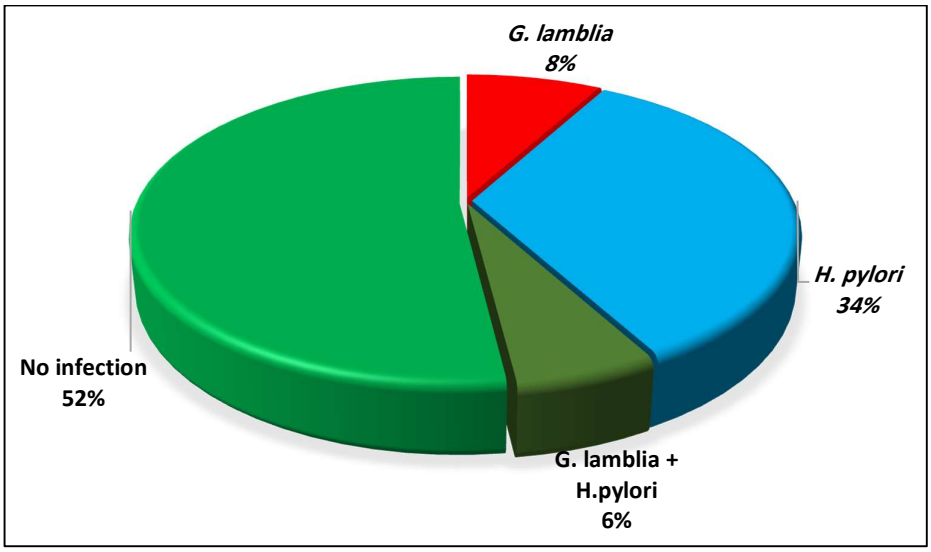

Figure (1): Coinfection of *G. lamblia* and *H. pylori* in malnourished children

In the present study, 37.5% of children with severe malnutrition were infected with *H. pylori* alone, 25% were coinfected with *G. lamblia* plus *H. pylori* and 18.75% were infected with *G. lamblia* alone. The study found that 45.45% of children with moderate malnutrition infected with *H. pylori* alone, 9.09% infected with *G. lamblia* and 4.55% coinfected with the tow microorganism, while 64.52% of children with mild malnutrition were without infection by these microorganisms (Table 3).

Table (1): Distribution of study cases according to *G. lamblia* and *H. pylori* infection and type of malnutrition

Infection	No.	Type of malnutrition					
		Mild		Moderate		Severe	
		No.	%	No.	%	No.	%
G. lamblia	8	3	4.84	2	9.09	3	18.75
H. pylori	34	18	29.03	10	45.45	6	37.5
Coinfection	6	1	1.61	1	4.55	4	25
No infection	52	40	64.52	9	40.91	3	18.75
Total	100	62	100	22	100	16	100
X^2		67.1		11.8		18.33	
P. value		0.0001 HS		0.008 HS		0.0001 NS	

. Relation of *G. lamblia* and *H. pylori* with age of children.

. Distribution of *G. lamblia* among malnourished cases and the control group in regard to age.

The study showed that the highest rate of *G. lamblia* infection (50%) was occurred in malnourished children within the age group 25-36 month, followed by 25% in the age group 37-60 month (Table 4).

Table (2): Distribution of *G. lamblia* among malnourished cases and the control group in regard to age.

Age groups (months)	Malnourished children					Control group				
	G. lamblia (+)		*G. lamblia* (-)		Total	*G. lamblia* (+)		*G. lamblia* (-)		Total
	No	%	No	%		No	%	No	%	
2-12	1	2.17	45	97.83	46	1	2.27	43	97.73	44
13-24	5	14.71	29	85.29	34	2	6.06	31	93.94	33
25-36	6	50	6	50	12	2	15.38	11	84.62	13
37-60	2	25	6	75	8	0	0	10	100	10
Total	14	14	86	86	100	5	5	95	95	100
X^2	19.07					4.2				
P. value	0.0001 HS					0.2 NS				

. Distribution of *H. pylori* among malnourished cases and the control group in regard to age.

The study showed that the highest rate of *H. pylori* infection (83.33%) was occurred in malnourished children within the age group 25-36 month, followed by 62.5% in the age group 13-24 month while 23.8% of *H. pylori* infection in control group were occurred in the 25-36 month, (Table 5).

Table (3): Distribution of *H. pylori* among malnourished cases and the control group in regard to age.

Age groups (months)	Malnourished children					Control group				
	H. pylori (+)		*H. pylori* (-)		Total	*H. pylori* (+)		*H. pylori* (-)		Total
	No	%	No	%		No	%	No	%	
2-12	13	28.26	33	71.74	46	9	20.45	35	79.55	44
13-24	12	35.29	22	64.71	34	7	21.21	26	78.79	33
25-36	10	83.33	2	16.67	12	3	23.08	10	76.92	13
37-60	5	62.5	3	37.5	8	1	10	9	90	10
Total	40	40	60	60	100	20	20	80	80	100
X^2	12.4					0.7				
P. value	0.002 HS					0.3 NS				

. Relation of *G. lamblia* and *H. pylori* infection with gender

. Distribution of *G. lamblia* infection according to patients gender

The study revealed that 20% of female malnourished children was infected with *G. lamblia* compared with 11.11% of male malnourished children (Table 6).

Table (4): Distribution of *G. lamblia* infection according to patients gender

Gender	Malnourished children					Control group				
	G. lamblia (+)		*G. lamblia* (-)		Total	*G. lamblia* (+)		*G. lamblia* (-)		Total
	No	%	No	%		No	%	No	%	
Males	6	11.11	54	88.89	60	4	6.78	58	93.22	59
Females	8	20	32	80	40	1	2.44	40	97.56	41
Total	14	14	86	86	100	5	5	95	95	100
X^2	1.99					0.8				
P. value	0.15 NS					0.3 NS				

. Distribution of *H. pylori* infection according to patients gender

The study revealed that 43.33% of male malnourished children was infected with *H. pylori* compared with 35% of female malnourished children (Table 4.7).

Table (5): Distribution of *H. pylori* infection according to patients gender

Gender	Malnourished children					Control group				
	H. pylori (+)		*H. pylori* (-)		Total	*H. pylori* (+)		*H. pylori* (-)		Total
	No	%	No	%		No	%	No	%	
Males	26	43.33	34	56.67	60	12	20.34	47	79.66	59
Females	14	35	26	65	40	8	19.51	33	80.49	41
Total	40	40	60	100	100	20	20	80	80	100
X^2	0.6					0.1				
P. value	0.4 NS					0.9 NS				

. Residence distribution of *G. lamblia* and *H. pylori* infection

. Residence distribution of *G. lamblia* infection in malnourished children.

In this study, 26.32% of rural malnourished children were infected with *G. lamblia* compared with 6.45% of malnourished children from urban area with highly significant differences (P<0.01). While 7.5% of rural control children were infected with the parasite (Table 8).

Table (6): Residence distribution of *G. lamblia* infection in malnourished children

Residence	Malnourished children					Control group				
	G. lamblia (+)		*G. lamblia* (-)		Total	*G. lamblia* (+)		*G. lamblia* (-)		Total
	No	%	No	%		No	%	No	%	
Urban	4	6.45	58	93.55	62	2	3.33	57	96.67	60
Rural	10	26.32	28	73.68	38	3	7.5	38	92.5	40
Total	14	14	86	86	100	5	5	95	95	100
X^2	7.72					0.7				
P. value	0.005 HS					0.3 NS				

. Residence distribution of *H. pylori* infection in malnourished children

In this study, 52.63% of rural malnourished children were infected with *H. pylori* compared with 32.26% of malnourished children from urban area with significant differences (P<0.05). While 27.5% of rural control children were infected with *H. pylori* (Table 9).

Table (7): Residence distribution of *H. pylori* infection in malnourished children

Residence	Malnourished children					Control group				
	H. pylori (+)		*H. pylori* (-)		Total	*H. pylori* (+)		*H. pylori* (-)		Total
	No	%	No	%		No	%	No	%	
urban	20	32.26	42	67.74	62	9	15	51	85	60
Rural	20	52.63	18	47.37	38	11	27.5	29	72.5	40
Total	40	40	60	100	100	20	20	80	80	100
X^2	4.7					2.3				
P. value	0.04 S*					0.1 NS				

* P. value: ≥ 0.01 and ≤ 0.05: significant (S)

. Type of malnutrition

. Distribution of study cases according to type of malnutrition by Water low classification.

As shown in Figure (2), the classification of malnutrition types was done by applying Water low method.

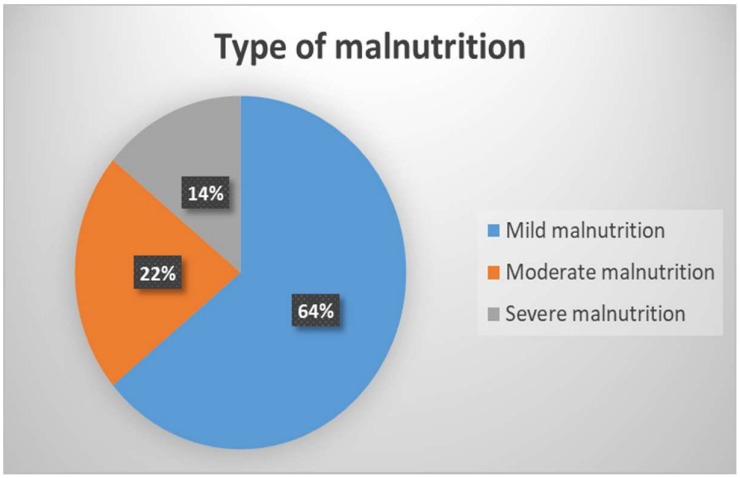

Figure (2): Distribution of study cases according to type of malnutrition by Water low classification.

4.7.2. Distribution of study cases (males and females) according to type of malnutrition by Water low classification.

As shown in Table (10), males comprise 53.13%, 77.27% and 64.29% of cases with mild, moderate and severe malnutrition respectively which were more than females.

Table (8): Distribution of study cases (males and females) according to type of malnutrition by Water low classification

Type of malnutrition	Males		Females		Total		P. value
	No.	%	No.	%	No.	%	
Mild	34	53.13	30	46.87	64	100	NS
Moderate	17	77.27	5	22.73	22	100	S
Severe	9	64.29	5	35.71	14	100	NS

Discussion

Malnutrition is responsible, directly or indirectly, for 54% of the 10.8 million deaths per year in children under five and contributes to every second death (53%) associated with infectious diseases among children under five years of age in developing countries[2].

Most of the study cases aged 3-12 months of age. This is goes with the study done in Iraq by Erzaiq et al [12]. the reason is that most of the cases where in the first year of life and this age group is the most valnurable group for malnutrition ,either because delayed feeding or some families not start feeding till after the first year of life . The proportion of male children who were malnourished was significantly higher than that of girls. This is similar to the observations in other studies done by Ndemwa et al in kenya [13]. It may be due to differences in in feeding and care practices of the two genders. Most of the malnourished cases from rural area and this goes with study done by Endris *et al*[14] this is due to poor health consciousness, low socioeconomic state and bad hygiene in rural areas This finding could be attributed to the fact that most families in the villages were large and experienced general food shortages.

Most of the study cases mothers were illiterate and low educational level. The reason why most of the cases have illiterate mothers is that due to the wrong way of preparation of milk or food or the way of sterilization[15]. Low literacy of parents can result in poor understanding of their children's health-related problems and has been found to be associated with malnutrition of children under the age of five years. Uneducated parents are less likely to clearly explain their child's symptoms to the physician, and this can act as a barrier in their child receiving the best possible care [15].

The frequency of giardiasis in malnourished cases are more than control because they are immunocompramiced and this supported by study done by Botero-Garcés *et al* [16] who described *Giardia*

lamblia as one of the important causes of malnutrition. This is due to the fact that *Giardia lamblia* infection affect the small intestine and causes total and subtotal villous atrophy and this will leads to malabsorption of several nutrient which is manifested as malnutrition signs and abdominal distention and stool features of malabsorption [12]. The frequency of *H. Pylori* in malnutrition also more than control and this goes with Franceschi *et al*[17] study who reported that *H. pylori* could be associated with childhood malnutrition in developing countries both because of increased susceptibility to enteric infections caused by hypochlorhydria and because of malabsorption of nutrients . Achlorhydria and hypochlorhydria are associated with an increased risk of giardiasis [17].

This case control study included 100 children with malnutrition (60 males and 40 females), their age range was 3-48 months (mean: 17.12±8.2 month), the study also included 100 healthy children (as control group) with the same characteristics of patients. The preset study revealed that the frequency of *G. lamblia* and *H. pylori* in malnourished children was 14% and 40% respectively and significantly higher than in control group (5% and 20% respectively), The study also found that 34% of malnourished children were infected with *H. pylori* alone, 8% were infected with *G. lamblia* alone and 6% were coinfected with the two microorganisms so that the high occurrence rate of *H. pylori* in patients with positive giardiasis supported the theory that conditions for *Giardia* survival are heightened by the bacterium *H. pylori.* A much higher rate of co-infection was observed in Iran by Shafie *et al* [18] who found that all Giardia positive patients were infected with *H. pylori*. Studies differ in explaining which organism was agonist in the presence of the other, Júlio *et al* [19]. reported that the presence of *H. pylori* infection was a risk factor for giardiasis. This can be explained by the fact that *H. pylori* create favorable conditions for the sustenance of Giardia colonization Abou *et al* [20] in Alexandria reported that giardiasis was

one of the most common enteroprotozoal diseases associated with *H. pylori* . Our result also supported with recent study done in Egypt by El-Badry *at el* [21]who reported that the coinfection between *H. pylori* and giardiasis was 52.5%. Another study done by Kazemian *at el* [22] in Iran who revealed that the acidity of stomach can be important. Also, pH increment would be concentrated once as a risk factor for contraction to parasites, especially those that can transmit through digestive tract like *Giardia lamblia.*

There was a significant increase in coinfection (both *G. lamblia* and *H. pylori*) in regard to severity of malnutrition this may be due to that malnutrition is one of the most common causes of immudeficiency in which immudeficiency increases with increase severity of malnutrition and this leads to that patient with sever malnutrition liable for several infections with different microorganisms like *G. lamblia* and *H. pylori* [8]. The causes behind immudeficiency in malnutrition are hyperproteinemia and hypogammaglobulenemia due to decrease in protein intake which affect the immunity of the patient. Odema in the other hand act as culture media for different microorganisms [8]. Skin abration especialy in napkin area facilitate the interance of several microorganisms (fungi or bacteria) as the skin is the first immune mechanism in the body. On the other hand poor intake of diet and poor sanitation which is the leading cause for malnutrition that make the malnourished patient liable for infection[20].

The highest rate of *G. lamblia* infection in malnourished cases(50%) was occurred within the age group 25-36 month followed by(25%)in the age group 13-24 month this could indicate that infection transmission occurs during intermediate childhood, perhaps when children normally play in very close contact [16]. The highest rate of *H. pylori* infection (83.33%) was occurred in malnourished children within the age group 25-36 month, followed by 62.5% in the age group 13-24 month while 23.8% of *H. pylori* infection in control group were occurred in the 25-36

month. It has been suggested that in developing countries the infection is usually acquired before five years of age.another study done by Rothenbach *et al*[23] who studied a group of Turkish children living in Germany and found that *H. pylori* infection was acquired mainly between the first and second years of life The frequency of *H. pylori* infection increased with increase in age becouse sources of infections would be limited person to person from caretakers, family members, or nursery attendants. As age increase, exposure to various infection sources increases hence the ultimate rate of infection.

Females with giardiasis showed higher frequency than males in contrast to *H.pylori* infection which showed males have higher frequency than females,however , in our study there were no statistical significant relation between giardiasis and *H.pylori* infection with gender .This agree with study done by Ramos *et al* [24] in southern Ethiopia which revealed that Gender was not a risk factor for harboring intestinal parasitic infections .

This study shows that *G. intestinalis* was the most frequently identified intestinal parasite in rural locations and this agree with recent studies done by Nunes *et al* [25]. Due to poor sanitation, contact with contaminated water supply, low level of education and malnutrition. We found that malnourished children with *H. pylori* infection live in rural (52.62%) more than urban (32.26%) Our results support the findings of prior studies that have also described the urban– rural differences in health in Gambia [26]. People living in urban areas are provided with better access to health services, education and other social support systems which are either not available or not easily accessible to residents in rural areas.[26]

The present study showed that 64% of study cases were with mild malnutrition, 22% moderate and 14% were with severe malnutrition Most of the study cases have mild malnutrition this may be due to that most of the patients admitted for other reasons like diarrhea or chest infection actually so that the weight is mildly affected [12].

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