RESULTS

During the period from June 2009 to November 2009 diarrheic stool samples of 100 children were examined by microscopic stool examination, and the cases enrolled in our study were selected randomly according to the results of microscopic stool examination, then the study was conducted on a total of 40 case in addition to 10 cases of other parasites , then these cases were divided into three groups of these Group I comprised 20 case (***G.lamblia*** positive group), Group II comprised 20 case (***G.lamblia*** negative group) while Group III comprised 10 cases (five cases were positive for ***E. histolyica*** and five cases were positive for ***C. parvum***) and this group is included only in this study to show if any cross reaction will occur with the specific primer of ***G.lamblia*** during the performance of real time PCR technique.

Table (2): Comparison between results of examination of stool samples in diagnosis of giardiasis in Group I, Group II and Group III.

Examined cases	Direct smear				Iodine stained smear				formol ether concentration technique			
	Positive		Negative		Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Group I	5	25	15	75	8	40	12	60	20	100	0	0
Group II	0	0	20	100	0	0	20	100	0	0	20	100
Group III	0	0	10	100	0	0	10	100	0	0	10	100
Total	5	10	45	90	8	16	42	84	20	40	30	60

Table (2): Shows comparison between results of direct stool examination by three methods (direct smear, iodine stained smear and formol ether concentration technique) in Group I, Group II and Group III, which were as follow: in Group I there were five + ve cases (25%) detected by direct smear, eight +ve cases (40%) were detected by iodine stained smear (five +ve cases were previously detected by direct smear plus three more cases were detected only by iodine stained smear), and 20 +ve case were detected by formol ether concentration technique (eight cases were previously detected by direct smear and iodine stained smear plus 12 more case were detected only by formol ether concentration technique).

While in Group II and Group III all these cases were –ve by the three methods .

- Out of total 50 case, there were five +ve cases for ***G.lamblia*** detected by direct smear (10%), and eight cases were detected by iodine stained smear (16%), then 20 case were detected by formol ether concentration technique (40%) (all these 20 +ve cases were related to Group I only).

Figure (8): Comparison between results of examination of stool samples in diagnosis of giardiasis in Group I, Group II and Group III .

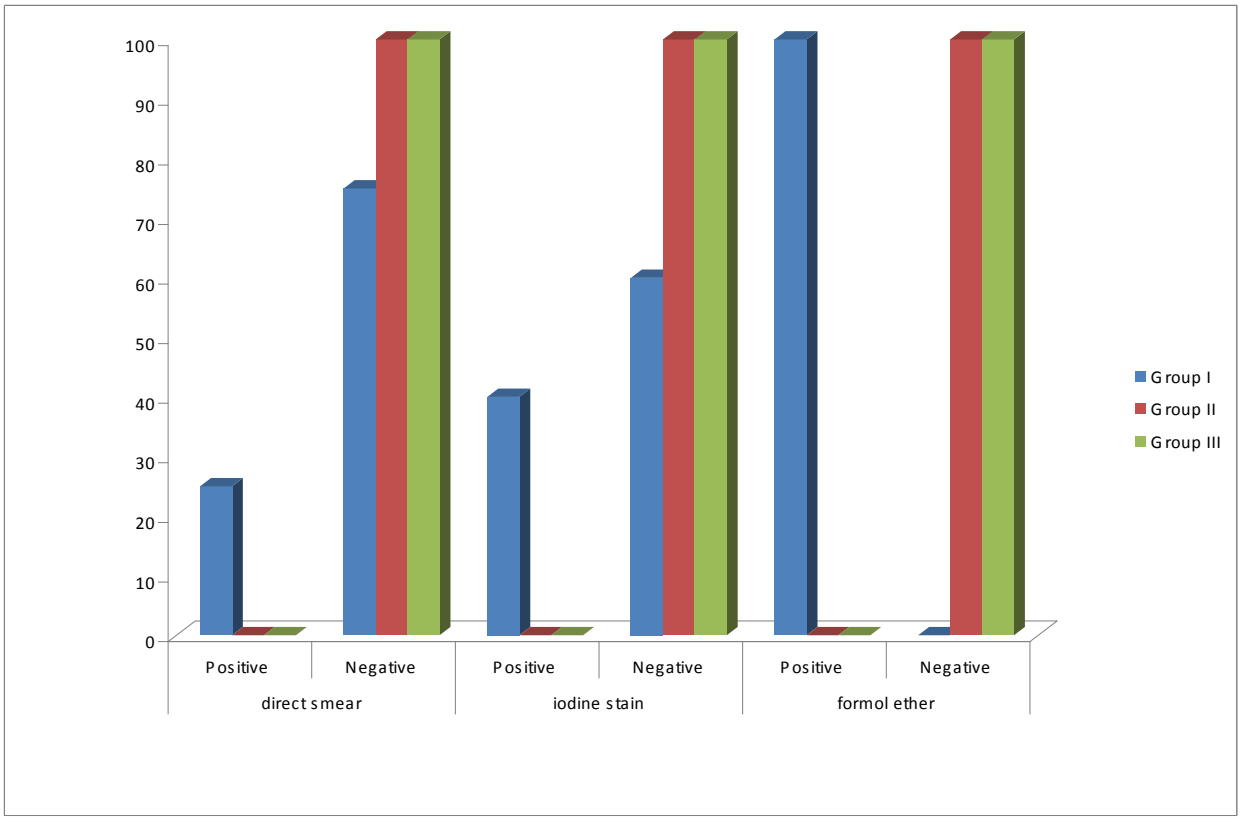


Table (3): Age distribution in Group I and Group II as detected by formol ether concentration technique .

Group / Age	Group I		Group II		Total	X^2	P
	No.	%	No.	%			
1-5	11	55	10	50	21	5.7	> 0.05
6-10	7	35	4	20	11		
11-15	2	10	6	30	8		
Total	20	100	20	100	40		

Table (3): Shows Age distribution in Group I and Group II, as the age of members of these two groups ranged from 1-15 years and it is as follows: in Group I, there were 11 case (55%) out of 20 examined case with age group (1-5 years), seven cases (35%) with age group (6-10 years) ,while there were two cases (10%) with age group (11-15 years), while in Group II, there were 10 cases (50 %) out of 20 examined case with age group (1-5 years), four cases (20%) with age group (6-10 years) while there were six cases (30%) with age group (11-15 years).

So in both groups children with age group (1-5 years) their number was 21 out of 40 examined cases, children with age group(6-10 years) their number was 11, while children with age group (11-15 year) their number was eight.

- Giardiasis was more common in children with age group between 1-5 years**.** The overall relations were statistically insignificant (p value > 0.05).

***Figure (9)*:** Age distribution in Group I and Group II as detected by formol ether concentration technique .

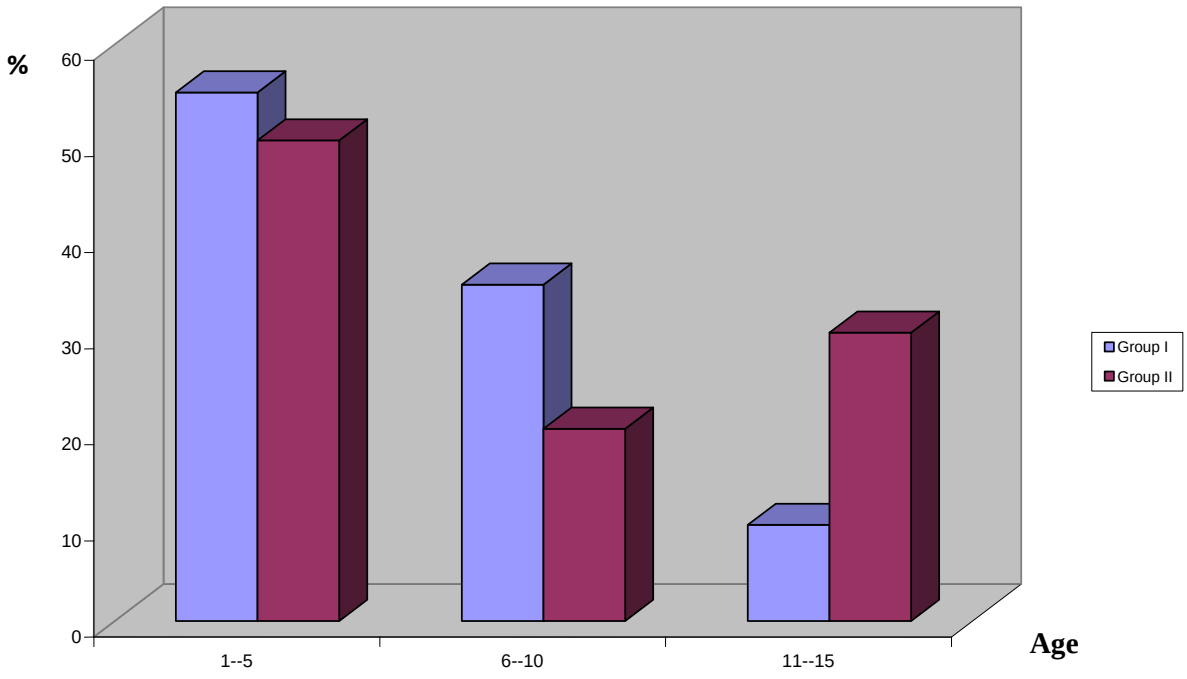


Table (4): Sex distribution in Group I and Group II as detected by formol ether concentration technique .

Group / Sex	Group I		Group II		Total	X^2	p
	No.	%	No.	%			
Male	12	60	13	65	25	0.1	>0.05
Female	8	40	7	35	15		
Total	20	100	20	100	40		

Table (4): shows sex distribution in Group I and Group II and it is as follows in Group I the number of male was 12 (60%) out of 20 examined case, while the number of female was eight (40%), while in Group II the number of male was 13 (65%) out of 20 examined case, while the number of female was seven (35%) out of 20 examined case.

So in both groups the number of male was 25 out of 40 examined cases while the number of female was 15 out of 40 examined cases.

- The number of males was higher than females. The overall relations were statistically insignificant (p value > 0.05).

Figure (10): Sex distribution in Group I and Group II as detected by formol ether concentration technique .

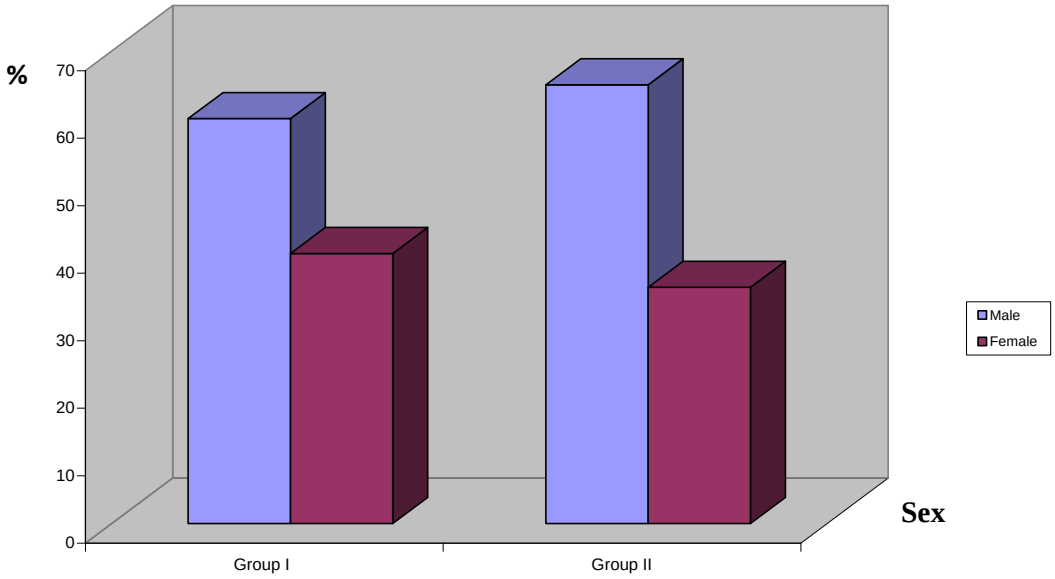


Table (5): Prevalence of giardiasis in Group I and Group II in correlation to residence as detected by formol ether concentration technique .

Group / Residence	Group I		Group II		Total	X^2	p
	No.	%	No.	%			
Rural	13	65	14	70	27	0.1	>0.05
Urban	7	35	6	30	13		
Total	20	100	20	100	40		

Table (5): shows the prevalence of giardiasis in Group I and Group II in correlation to residence and it is as follows: in Group I there were 13 cases (65%) out of 20 examined cases from rural areas, while there were seven cases (35%) from urban areas. In Group II there were 14 case (70%) out of 20 examined case from rural areas while there were six cases (30%) from urban areas.

So in both groups there were 27 cases out of 40 examined cases from rural areas while 13 cases from urban areas.

- The number of children from rural areas was higher than those from urban areas. The overall relations were statistically insignificant (p value > 0.05).

Figure (11): prevalence of giardiasis in Group I and Group II in correlation to residence as detected by formol ether concentration technique.

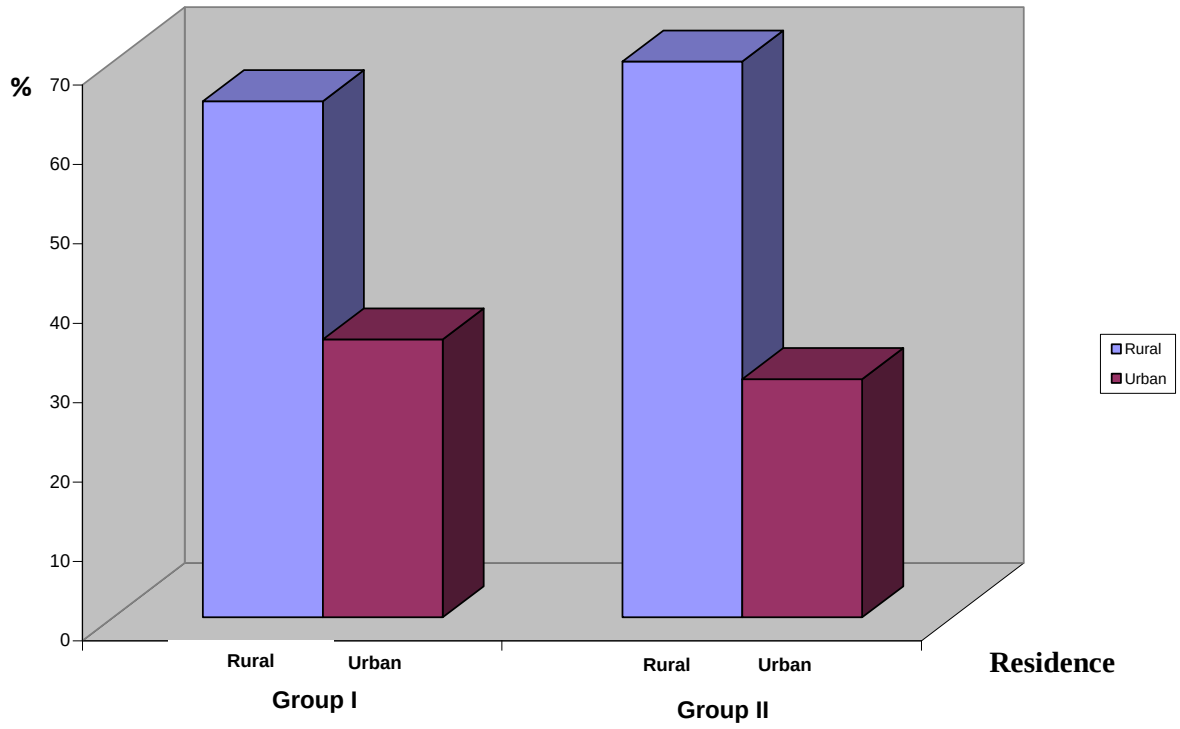


Table (6): Clinical presentation in Group I and Group II as detected by formol ether concentration technique.

Group / Clinical presentation	Group I (n=20)		Group II (n=20)		Total	X^2	p
	No.	%	No.	%			
Abdominal pain	11	55	10	50	21	0.5	> 0.05
Abdominal distension	10	50	9	45	19		
Loss of weight & failure to thrive	9	45	6	30	15		
Loss of appetite	5	25	3	15	8		
Vomiting	3	15	2	10	5		

Table (6): Shows the clinical presentation in Group I and Group II and it is as follows:

In Group I there were 11 case (55%) out of 20 examined case presented with abdominal pain, 10 cases (50%) with abdominal distention, 9 cases (45%) with loss of body weight and failure to thrive, 5 cases (25%) with loss of appetite and three cases (15%) presented with vomiting.

In Group II there were 10 caseS (50%) out of 20 examined case presented with abdominal pain, nine cases (45%) with abdominal distention, six cases (30%) with loss of body weight and failure to thrive, three cases (15%) with loss of appetite and two cases (10%) with vomiting.

So in both groups there were 21 case presented with abdominal pain, 19case with abdominal distention, 15 case with loss of body weight and failure to thrive, eight cases with loss of appetite and five cases presented with vomiting .

- Abdominal pain was the most common presenting symptom among children included in this study. The overall relations were statistically insignificant (p value > 0.05).

Figure (12): Clinical presentation in Group I and Group II as detected by formol ether concentration technique .

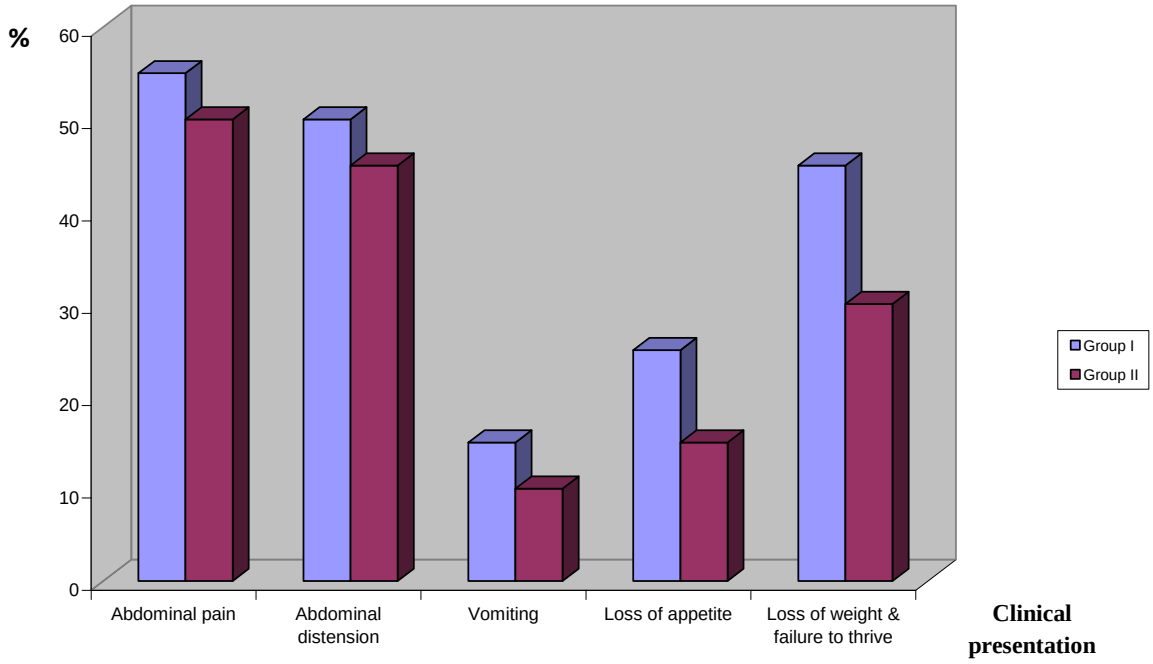


Table (7): Severity of diarrhea in Group I and Group II as detected by formol ether concentration technique .

Group / Severity	Group I		Group II		Total	X^2	p
	No.	%	No.	%			
Mild	4	20	5	25	9	0.4	>0.05
Moderate	4	20	5	25	9		
Severe	12	60	10	50	22		
Total	20	100	20	100	40		

Table (7): shows the severity of diarrhea in Group I and Group II as detected by formol ether concentration technique .

- The severity of diarrhea is determined according to the number of motion/day (***Gurwith et al., 1997)*** :
  - Mild : up to 5 motion/day.
  - moderate : 6-10 motion/day.
  - severe : more than 10 motion /day.

It is as follows: in Group I there were four cases (20%) out of 20 examined case presented with mild diarrhea, also there were four cases (20%) with moderate diarrhea but there were 12 case (60%) presented with severe diarrhea.

While in Group II there were five cases (25%) out of 20 examined case presented with mild diarrhea, also there were five cases (25%) with mild diarrhea and 10 cases (50%) presented with severe diarrhea.

So in both groups there were nine cases out of 40 examined cases presented with mild diarrhea, also there were nine cases with moderate diarrhea and 22 case with severe diarrhea.

- Severe diarrhea was more common than mild and moderate diarrhea among children included in this study. The overall relations were statistically insignificant (p value > 0.05).

Figure (13): Severity of diarrhea in Group I and Group II as detected by formol ether concentration technique .

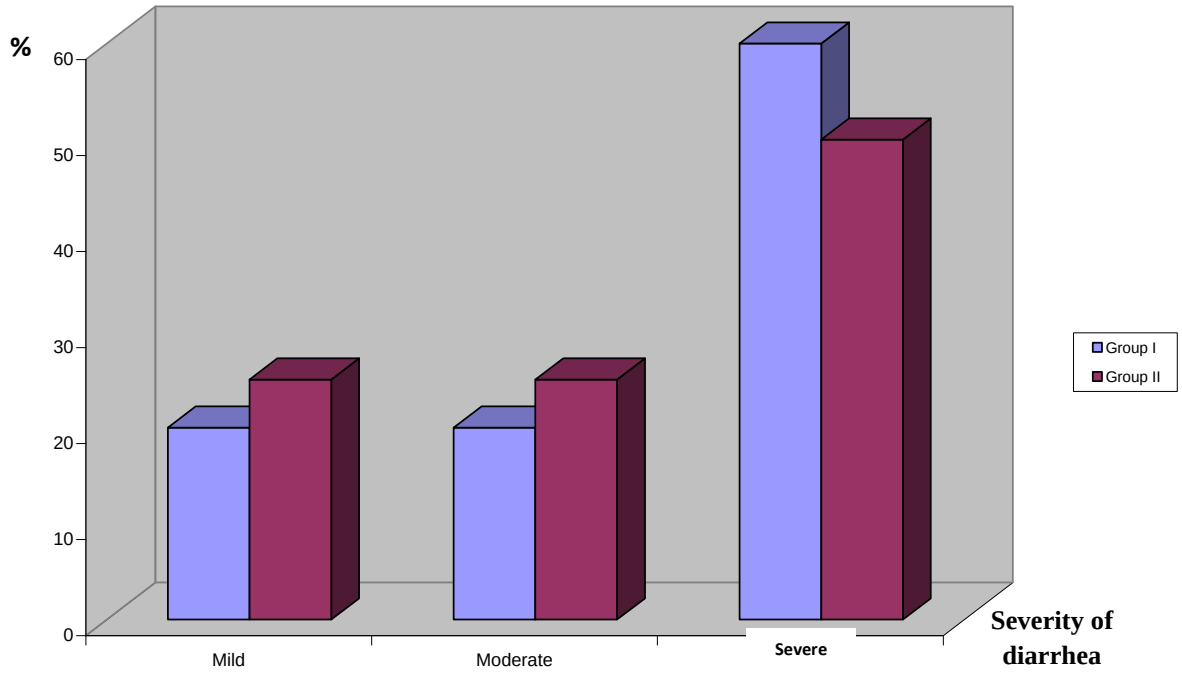


Table (8): Comparison between results of examination of stool samples using formol ether concentration technique and PCR in Group I, Group II and Group III .

Examined cases	Formol ether				PCR			
	Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%
Group I	20	100	0	0	20	100	0	0
Group II	0	0	20	100	6	30	14	70
Group III	0	0	10	100	0	0	10	100
Total	20	40	30	60	26	52	24	48

Table (8): Shows comparison between results of direct stool examination by formol ether concentration technique and molecular stool examination by PCR in Group I, Group II and Group III which were as follows: in Group I, there was 20 +ve case detected by formol ether concentration technique (eight cases were previously detected by direct smear and iodine stained smear plus 12 more case were detected only by formol ether concentration technique), also PCR detected all these above 20 case of Group I .

While in Group II all cases were -ve by formol ether concentration technique, but PCR detected six +ve cases (30%). while in group III all cases were -ve by formol ether concentration technique and PCR.

- Out of total 50 case, there were 20 +ve case were detected by formol ether concentration technique (40%) [all these cases related to Group I only]. While there were 26 +ve case detected by PCR (52%) [20 case related to Group I, while six cases related to Group II but there was any case related to Group III].

Figure (14):Comparison between results of examination of stool samples using formol ether concentration technique and PCR in Group I, Group II and Group III.

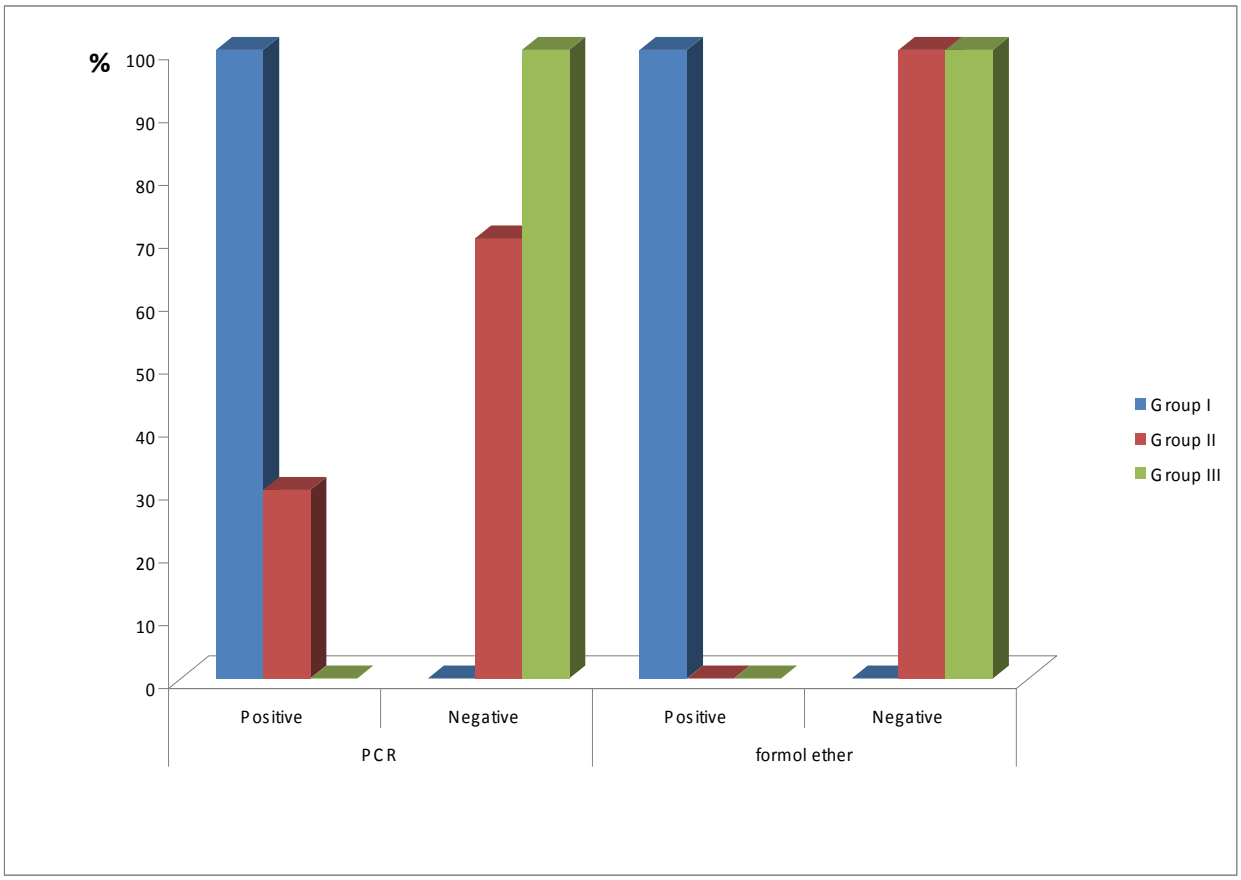


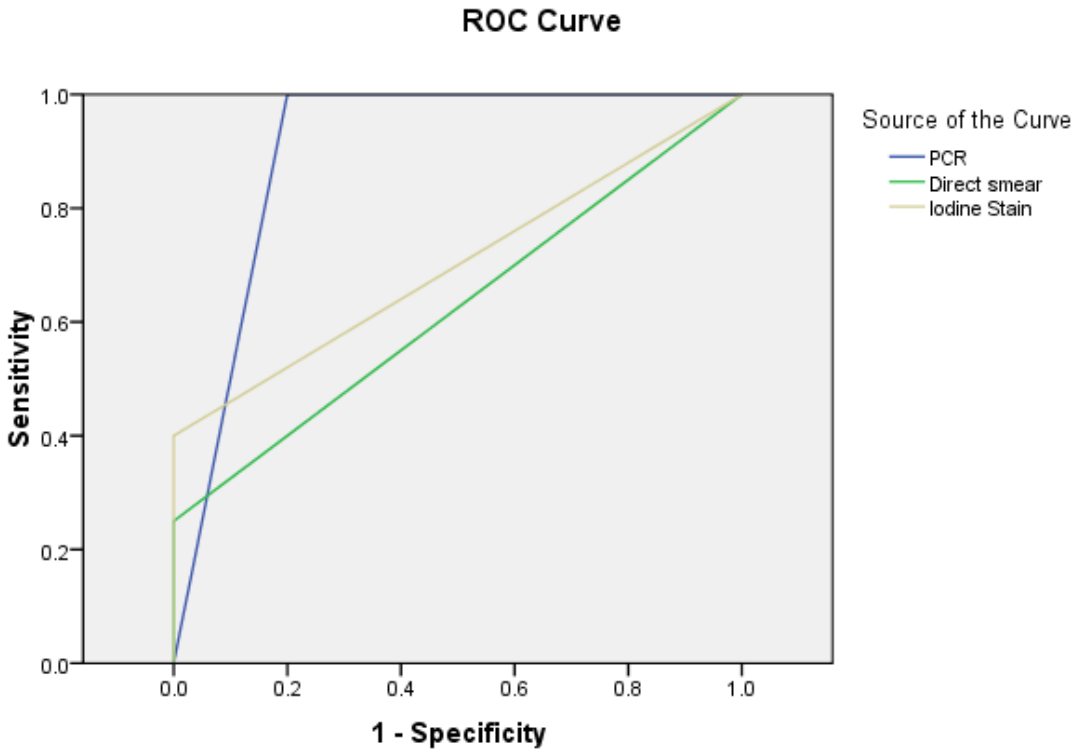
Table (9): Sensitivity and Specificity of direct smear, iodine stained smear and PCR according to formol ether concentration technique.

Screening test / Results	Direct smear	Iodine stained smear	PCR
Sensitivity	25%	40%	100%
Specificity	100%	100%	80%
PPV	100%	100%	76.9%
NPV	66.6%	71.5%	100%

PPV= +ve predictive values. NPV= -ve predictive values.

Table (9): Shows sensitivity and specificity of direct smear, iodine stained smear and PCR according to formol ether concentration technique. It was found that the sensitivity of direct smear, iodine stained smear and PCR was (25%), (40%) and (100.0%) respectively. On the other hand, the specificity of direct smear, iodine stained smear and PCR was (100.0%), (100.0%) and (80%) respectively. PPV of direct smear, iodine stained smear and PCR were (100.0%), (100.0%) and (76.9%) respectively. While NPV of direct smear, iodine stained smear and PCR were (66.6%), (71.5%) and (100.0%) respectively.

Receiver operating characteristic curve (ROC curve) **Fig. (15)** for comparison of the different methods:



Area Under the Curve	
Test Result Variable(s)	**Area**
PCR	0.900
Iodine Stained smear	0.700
Direct smear	0.625

The more the area under the curve the better the test

Data analysis of the results of real time PCR:

By using Light Cycler Software Version 4.05. and It was as follows:

- detection of +ve and -ve samples by using **melting curves** which differeniate between +ve and -ve samples by the difference in the melting temperature, as the flurescence emmited below 85° c represented -ve samples and indicated primer dimmer emission, while the flurescence emmited above 85° c and less than 90°c represented +ve samples with different peaks according to the concentration of the amplified product (**Fig.16** and **Fig.17**).

Amplification curves:

Real-Time PCR focuses on the exponential phase because it provides the most precise and accurate data for quantitation, within the exponential phase, the real-time PCR instrument calculates two values which are the threshold line which is the level of detection at which the reaction reaches a fluorescent intensity above the base line and the Cycle Threshold (CT) which is the PCR cycle at which the sample reaches threshold line (**fig. 18**).

The sigmoid shape of the amplification curve (in which the number of cycles is plotted against fluorescence), shows that the difference in the concentration of the initial ***G.lamblia*** DNA template, appeared as difference in the cycle threshold (CT), so the early CT, the more DNA concentration so the early amplification curve (**Fig. 19** and **Fig. 20**).