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EVALUATION OF ROTA VIRUS AND INTESTINAL PROTOZOAL INFECTIONS IN CHILDREN IN BABYLON PROVINCE

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SUMMARY

During a period extending from November 2016 to April 2017, a total of (120) samples were collected of diarrheal infected children in the Babylon Teaching Hospital for gynecology and children, Al-Noor Pediatrics Hospital and many Primary health care centers, stool and blood samples were collected, a combined with information about each case include age, sex, duration of disease, and being hospitalized or not. Sixty of healthy infants and children of the same age group were also included as a control group. Primarily, Rota virus was detected in 24 (20%) of the total cases. diarrheic patients were investigated by apid chromatography test for rotavirus, 24 samples (20%) showed positive results to rotavirus, among them 15 (12.5%) Male while 9 (7.5%) Females, with significant differences in rates of infection according to gender, from (120) stool samples we found that (18) 15% were positive to intestinal protozoa in which (10) 8.3% show *Entamoeba histolytica* and (8) 6.6% show *Giardia lamblia.*

1. The highest percentage of infection was shown in children with age from (18-24) months it was 33.3% who depending on bottle feeding. The study showed high level of IL-6 in patients group in Compare with control group, The mean concentration of IL-6 recorded (123 ± 25 pg/ml), There was a significant differences ($p \leq 0.05$) between patients and control group (58 ± 24 pg/ml), also the results of Interleukin-17 was elevated too in patients group Mean 111 ± 17 pg/ml while it was 31 ± 9 in healthy control group.

2. From 120 stool samples we found that viral infection was 12.5% in male and 7.5% in female while it was (18) 10% for protozoal infection, also we confirm the infection with

Rotavirus was high in patient in rural (59%) more than urban area (41%), and the results investigated that vaccinated children with second dose more (83%) infected with Rotavirus rather than first dose (17%) while vaccinated children was (94%) and non-vaccinated children (6%). The viral particles purification profile was also demonstrated, by using RNA extraction assay and semi nested PCR technique, there was 2 genotype was detected P and G, Six genotypes were detected represent in G1 with (37.50%) the highest percentage follow by G2 with (16.66%) and the lowest percentage for G4 and G8 with(4.16%) for both. Five genotypes were detected represent in P[8] with (33.33%) the highest percentage follow by P[4] (20.83%) and the lowest percentage for P[9] with (4.16%). A mixed infections with more than one genotype G1 P[8] was observed and was found jointly in 9 samples from 24 positive samples, while observed G1 P[4] in 3 samples , G2 P[4] in 5 samples, G3 P[8] in single case, G9 P[8] and G4 P[8] in 2 samples.

Rotavirus

Rotaviruses were first discovered by electron microscopy in 1973 in Australia by thin-section electron microscopic examination of duodenal biopsies obtained from children with acute diarrhoea, Since then, rotavirus gastroenteritis was known as the major cause of sever dehydrating diarrhoea in children worldwide.[1]

Rotaviruses are responsible for significant gastrointestinal disease which transmitted by the fecal-oral route, primarily in children less than 5 years of age and the young of other mammalian species. Rotaviruses are 70nm icosahedral viruses that belong to the Reoviridae family. There are seven rotavirus groups (A, B, C, D, E, F, and G) but only three types (A, B, and C) infect humans, Group A rotaviruses are the most important from a public health stand point because these cause the majority of all rotavirus morbidity.[2]

Group-A rotaviruses are further classified into subgroups based on the specificity of epitopes that are also present on VP6. The majority of strains belong to either subgroup I or subgroup II, although some isolates carry both subgroup-I and subgroup-II epitopes and a few do not belong to either subgroups.[1]

Serotype specificity is determined by the outer capsid proteins VP4 and VP7, both of which independently induce neutralizing antibodies.[3]

After an incubation period of 1 to 3 days, rotavirus gastroenteritis begins with acute onset of

fever and vomiting followed 24 to 48 hours later by watery diarrhea.[4] The spectrum of rotavirus illness ranges from mild disease of limited duration to severe diarrhoea with vomiting and fever that can result in dehydration and shock, electrolyte imbalance and death.[5] Typically, there are 10 to 20 bowel movements per day. Symptoms generally persist for 3 to 8 days. Fever occurs in up to half of all infected children and is usually low grade, although up to one third of patients may have a temperature higher than 39°C. Vomiting is nonbilious and occurs in 80% to 90% of infected children. Vomiting is usually brief and lasts 24 hours or less in most children.

METHODS

Collection of samples

Stool samples

One hundred and twenty samples from children with diarrhea and from (60) healthy sample as control all children were under 5 years. The study was done in Hilla city the center of Babylon province from patients admitted to Babylon Teaching Hospital for gynecology and children, Al-Noor Pediatrics Hospital and many of Primary health care centers during a period extending from November 2016 to April 2017. Demographic data collection included name, gender, age, weight, immunization status and feeding pattern. Stool samples were collected in sterile screw cap containers for detection of Rota virus and intestinal protozoa, then storage in deep freeze (-20ºC), 10 ml of blood samples were also collected from patients for immunological and molecular study.

Stool examination for intestinal protozoa

The wet preparation was used to detect presence of intestinal protozoa by placing a drop of saline on a clean slide and placed a small amount of stool on the slide and mix with saline (0.85%) cover with a cover slip, also using of iodine solution to examine the nuclei of cysts and examine under the light microscope (10x and 40x objectives).

Ethical approval: verbal approval was taken from all patients and healthy children parents, who were enrolled in the study.

Rapid chromatography test for rotavirus

This test is done according to manufacture company (Abon Biopharm).

Serological markers

Interleukin 6

This test is done according to manufacture company (Elabscience).

RESULTS AND DISCUSSION

Percentage of Rota virus infections using rapid chromatography test

One hundred and twenty samples from children with diarrhea and from (60) healthy sample as control all children were under 5 years. The study was done in Hilla city the center of Babylon province from patients admitted to Babylon Teaching Hospital for gynecology and children, Al-Noor Pediatrics Hospital and many of Primary health care centers during a period extending from November 2016 to April 2017 only 24 samples (20%) showed positive results to rotavirus, among them 15 (12.5%) males and 9 (7.5%) females, significant differences in rates of infection between them as shown in table (3-1).

Table (3-1): Distribution of Rotavirus infection according to patients gender.

Gender	Samples No.	No. of positive rotavirus	percentage
Male	72	15	12.5 %
Female	48	9	7.5 %
Total	120	24	20 %

Our results show significant proportion of diarrhea is due to rotavirus (20%) and rota virus may be responsible for diarrhea in children under five years of age. This results was similar with results of Al-Rifai *et al* (2009) who found that percentage of Rotavirus was (18.5%) of 259 children under 5 years suffer of infectious diarrhea and disagreed with Al-Kelaby (2008) who found that (16.13%) from 62 cases were positive to rotavirus and Al-Jobory (2013) who also found that (47.65%) positive to rotavirus from 470 cases. Rota virus is transmitted from person to person through the fecal-oral route, this occurs when the viruses found in stool of infected child is swallowed by another child, in other words children become infected if they play with infected material.[9] We found that percentage of infection was highest in male (12.5%) that the female (7.5%), this due to male has susceptible and likely to be admitted to hospitals that female whether this difference is due to sex susceptibility to infection.

Table (3-2): Distribution of Rotavirus according age groups of patients.

Age (Months)	Sample	Positive	percentage
Up to 6	54	10	8.33 %
6 – 12	44	10	8.33 %
12 – 18	12	2	1.66 %
18 – 24	6	2	1.66 %
24 - 30	1	0	0
30 – 36	1	0	0
36 – 42	2	0	0
42 - 48	0	0	0
Total	120	24	20 %

From table (3-2) we found that most of infected children in our results were under 2 years age, with highest prevalence in those between (18-24) months and (up to 6) months also (6-12) (8.33%), this results agree with results of Magzoub *et al*, and Ansam (2017). Studies worldwide have reported that the most vulnerable age group to rotavirus infection is under 2 years of age with highst prevalence between (6-12) months of age (Junaid *et al*., 2011). And agreement with De Donne *et al.* (2013) who confirm that most of Rota virus cases were identified in the age group (0- 24 months), in particular in the age group (0-12 months).

The high percentage of infection in these ages return to feeding pattern, they depended on bottle feeding rather than breast feeding, figure (3-3) it agree with results of Rawaa *et al*, (2015) and Ansam (2017) maternal antibodies do play an important role in protection from rotavirus infection[9] other study suggested that breast feeding can prevent clinically severe rotavirus diarrhea, particularly when it constitutes the sole source of food and liquids in the child diet.[15]

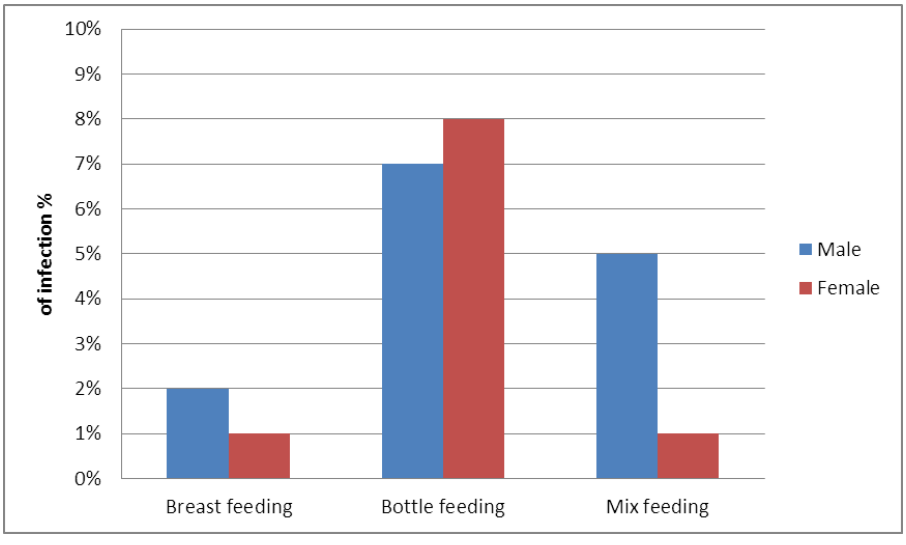

Figure (3-3): Distribution of rotavirus infection according to feeding pattern.

Figure (3-4) reveals the distribution of Rotavirus infection in urban and rural area, we found that the percentage of infection was higher (59%) in rural area rather than in urban area (42%). the results of our study was in agree with results of Rawaa *et al.(2015)* who investigated that Rotavirus infection was highly prevalence in children in rural area these due to unsanitary practices associated with the child development, socioeconomic status including level of mother education and residence consider a risk factor for transmission of rotavirus (Al-Jabiry, 2009). while Ansam (2017) found that no significant difference in infection with Rotavirus infection among patients reside urban and rural area.

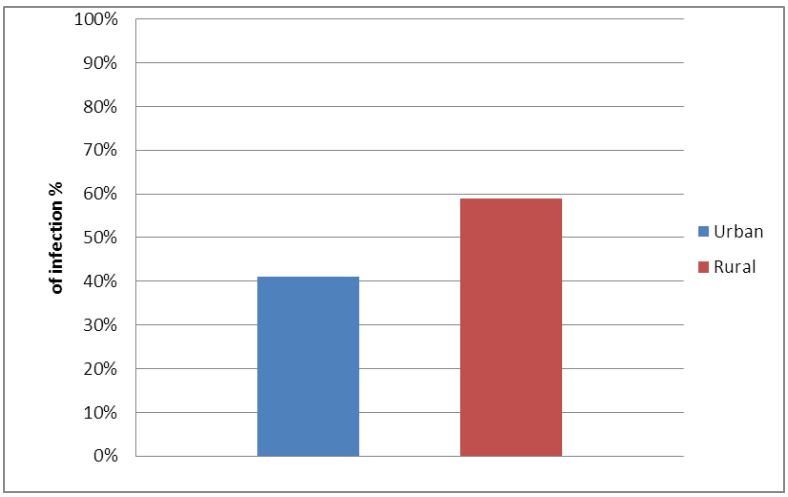

Figure (3-4): Distribution of Rotavirus infection according to residence area.

Rotavirus positively was found in vaccinated children (94%) figure (3-5) show higher than non-vaccinated children (6%). statistical analysis shown a significant difference between vaccinated and non-vaccinated children ($p \leq 0.05$) this results was dis agree with results of Preeti *et al.* (2016) who confirm that rota virus infection was significantly higher in non-vaccinated children than in vaccinated, and agree with other studies which confirm that the efficacy of rotavirus was lower ranging from 51% to 64%(Mahdi *et al.*, 2010). also we found that rotavirus infection was in vaccinated children with first dose of vaccine it was (17%) and in children with second dose of vaccine was (83%).

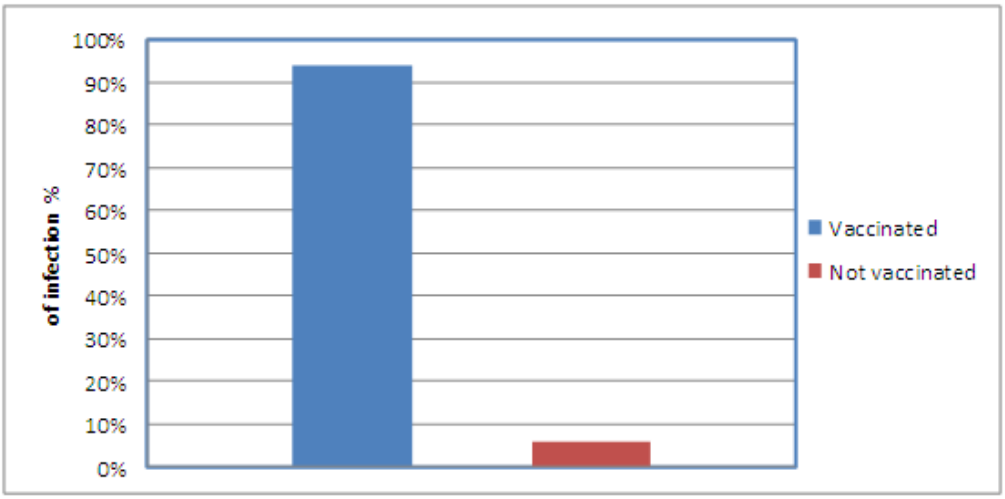

Figure (3-5): Distribution of Rotavirus infection according to vaccinated and non-vaccinated children.

The main concentration of Interleukin-6 and Interleukin-17 in serum of patients and control groups

The study showed high level of IL-6 in patients group in compare with control group, The mean concentration of IL-6 recorded (123 ± 25 pg/ml), with significant differences ($p \leq 0.05$) between patients and control group (58 ± 24 pg/ml) figure (3-7).

These results were in agreement with the results of Chen *et al.*(2014) who found that significante increase in level of serum IL-6 in children with rotavirus infection These finding may be due to that the infection with rotavirus induces cell mediating immunity (CMI) characterized by a high Th1 –cell response (John *et al*., 2000). Also Jiang *et al*. (2003) demonstrated an increased cytokines (IL-6) in children with acute rotavirus diarrhea compared with those in control children these results indicate that both the Th1 and Th2 type of cytokines are produced in young children with natural rotavirus infection. Also, the mean concentration of IL-17 in rota virus infected children showed an elevation which was (111 ± 17pg/ml) in comparison with rotavirus free children (31 ± 9 pg/ml) with significant difference ($p \leq 0.05$) between them. This results was in agree with results of Huaifu *et al*.(2015) who confirmed that the level of serum IL-17 in children with rotavirus enteritis were significantly increased. IL-17 is pro inflammatory cytokine that can caused the upregulation of chemokines and the invasion of inflammatory cells in tissues,[23] and the increasing in IL-17 level it may be due to increasing in IL-6, many studies indicates that an excessive level of IL-6 cytokine produced following viral infection promotes the development of IL-17 producing pathogenic helper T cell.[24]

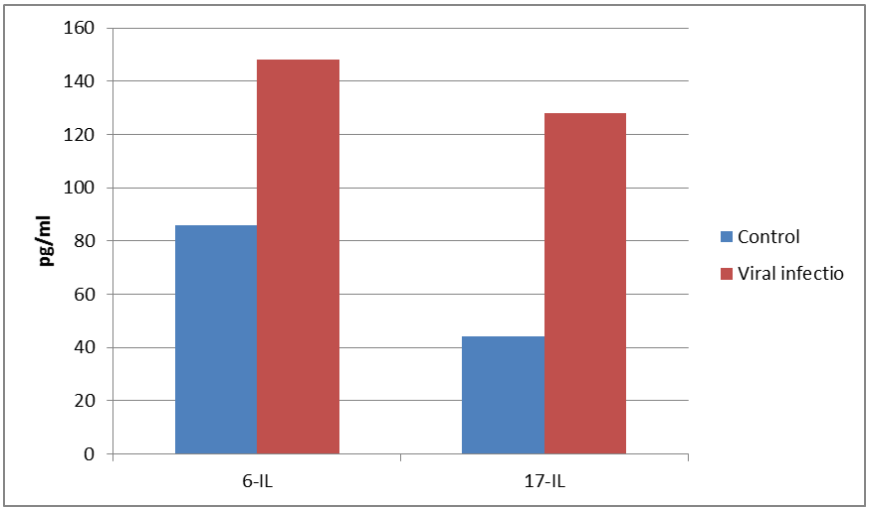

Figure (3-7): Levels of serum Interleukin-6 and Interleukin-17 in patients and control groups.

Table (3-3): Percentage of G genotypes of rotavirus.

G type	primer	No.	percentage
G1	aBT1	9	37.50 %
G2	aCT2	4	16.66 %
G3	G3-Aust	3	12.50 %
G9	G9 or mG9	2	8.33 %
G4	aDT4	1	4.16 %
G8	aAT8	1	4.16 %
Non-typeable	----------	4	16.66 %
Total		24	100 %

Among of 24 rotavirus positive sample, G was the most frequented detected (9 sample) with 37.5%, than followed by G2 with 16.66% while the lowest percentage was detected in G8 and G9 it was 4.16%, 8.33 respectively (table 3-3) this results was in agree with results of Riza and Atila (2014) who confirm that G9 was highest recorded (48.78) rotavirus PCR positive sample. other study confirm that G1 was the most prevalent genotype, representing more than 70% of the rotavirus infections (Zeller *et al.*, 2010) but our study was disagreement with study of Midgley *et al.,* (2014) who indicates that G9 has been the most predominant genotype identified among rotavirus strain, also showed an increase in the frequency G4 and G3 genotype while other study was investigated that G4 was the most predominate genotype of rotavirus infection.[28]

These differences in frequencies of rotavirus genotypes may be due to different in geographical region or selective effect of rotavirus vaccins or reassortment between the circulating strains.

Percentage of P genotypes of rotavirus

Table (3-4): Percentage of P genotypes of rotavirus.

P type	primer	No.	Percentage
P[8]	1T-1	8	33.33 %
P[4]	2T-1	5	20.83 %
P[6]	3T-1	3	12.50 %
P[10]	5T-1	2	8.33 %
P[9]	4T-1	1	4.16 %
Non-typeable	----------	5	20.83 %
Total		24	100 %

Table (3-4) show that the predominant P genotype was P[8] (33.33%) followed by P[4] with 20.83% than 12.50 for P[6] while 20.83% was non-typeable, while the lowest percentage recorded for P[9] it was 4.16%. the results of our study agreement with results of Herish *et Al.*,(2006) who investigated that P[8] genotype was most predominant and P[4] was the lowest. Also Abood *et al.*,(2013) confirmed that P[8] appear in highest percentage it was (61.4%) while P[6] was (5.7%). Durmaz *et al.*,(2014) found that more than 97% of rotavirus strains corresponded to P[8] genotype.

Percentage of genotype combination of G and P genotypes in rotavirus

Table (3-5): Percentage of genotype combination of G and P genotypes of rotavirus.

Genotype	Positive	Percentage
G1 P[8]	9	37.50 %
G2 P[4]	5	20.83 %
G9 P[8]	4	16.66 %
G1 P[4]	3	12.50 %
G4 P[8]	2	8.33 %
G3 P[8]	1	4.16 %
Total	24	100 %

Mixed infections with more than one genotype G1 P[8] was observed and was found in the present study 9 samples from 24 positive samples, while observed G1 P[4] in 3 samples, G2 P[4] in 5 samples, G3 P[8] in single case, each G9 P[8] in 2 samples finally G4 P[8] in 2 samples.

The results found the most frequent genotype were G1 P[8] it was 37.5% and the lowest frequent genotype were G3 P[8] it was 4.16%, this result agree with Mullick et al.,(2014) who reported the most common genotype as G1 P[8] and G9 P[8] Herish et al.,(2006) who found the most genotype combinations were G1 P[8], G2 P[4] and G1 P[6] it was (19.33,6.11

and 6.11)% respectively .other study confirmed that genotype G1 P[8] was the most common combinations followed by G2 P[4] and finally G2 P[8].[31]

2 3 4 6 8 L 11 12 13 14

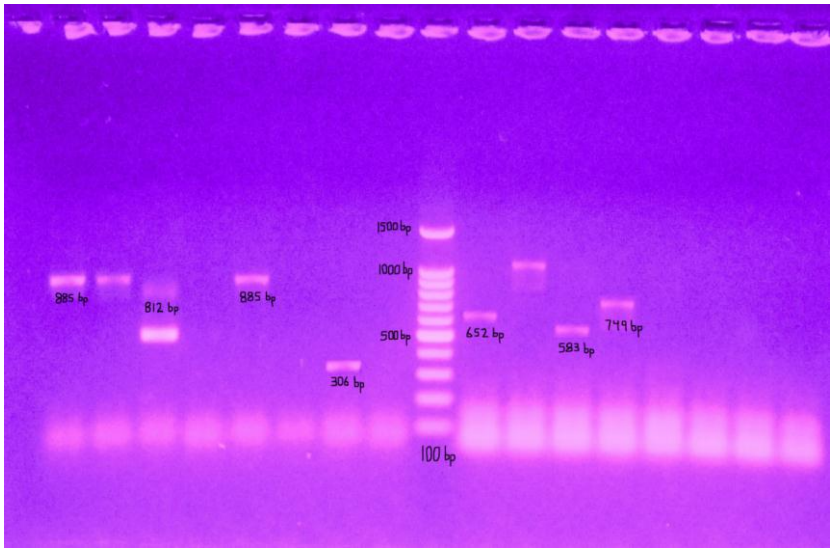

Figure (3-8): Gel electrophoresis of VP7 gene (G type) that the positive result represents 2,3,4,6,8,11,12,13 and 14 isolated from left and right the L:Ladder.

The Gel electrophoresis on agarose gel for the amplification product of the second phase with the presence of the primers (Beg9) and (End9) for the encoding of the seventh viral protein VP7 The letter L refers to the material indicating the basal lengths in the base pair The wells from 2 to 14 represents the product of amplification of the complementary DNA.

2 3 4 5 6 8 9 L

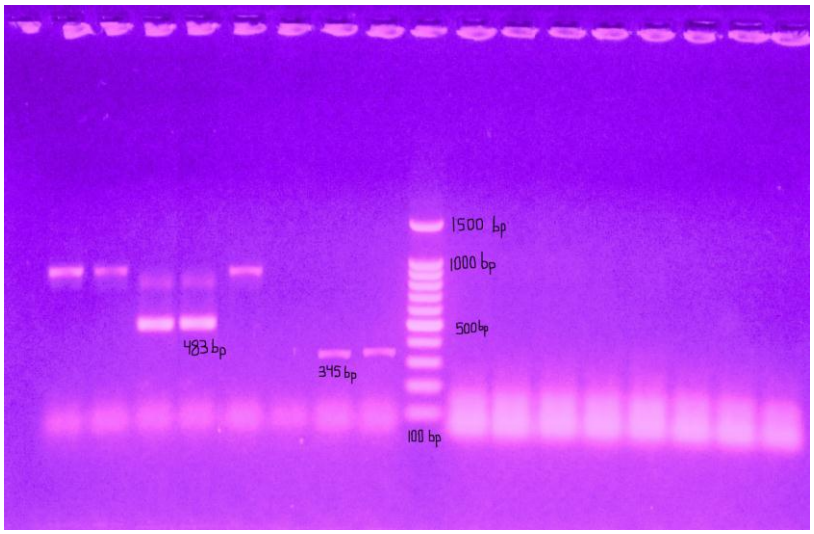

Figure (3-9): Gel electrophoresis of VP4 gene (P type) that the positive result represents 4,5,8 and 9 isolated from left of the L:Ladder.

The gel electrophoresis on agarose gel for the amplification product of the second phase with the presence of the primers (con3) and (con2) for the encoding of the fourth viral protein VP4 The letter L refers to the material indicating the basal lengths in the base pair The wells from 2 to 9 represents the product of amplification of the complementary DNA.

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