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EVALUATION OF VERTICAL TRANSMISSION OF A HELICOBACTER PYLORI ANTIGEN

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ABSTRACT

The risk of *Helicobacter pylori* infection in infants cannot be ignored because it can cause various gastroduodenal diseases such as: chronic gastritis, peptic ulcer and may lead to gastric cancer. *Helicobacter pylori* infection can be associated with intrauterine growth restriction and growth reduction in older children. In this work we studied the possibility of vertical transmission of *H.pylori* circulating antigen from infected mothers to their newborns using western blot and ELISA techniques. Western blot analysis, demonstrated a single immunoreactive band with a molecular weight of 58 kDa in serum samples from *H. pylori* infected mothers and in umbilical cord samples. The *H. pylori* antigen was detected in 47% of mothers sera and in 41% of umbilical cord samples using ELISA technique. *H. pylori* antigen was transmitted vertically from mothers to their newborn (vertical transmission rate =82%). So, the infected mothers are considered a risk factor for the transmission of infection to their newborn and the determination of *H. pylori* antigen may act as an immunodiagnostic tool for the manifestation of *H. pylori* infection.

Key words: Vertical Transmission - Helicobacter pylori Antigen.

INTRODUCTION

Helicobacter pylori (*H.pylori*) is one of the most common infective agents worldwide. It is an etiological agent of gastritis, peptic, and duodenal ulcer disease, The infection with *H. pylori* is a recognized risk factor in the development of gastric mucosa -associated lymphoid tissue lymphoma and adenocarcinoma **[Queiroz & Luzza, (2006)].** It is a bacterium that colonizes the digestive tract of 50% of the world's population. Infection commonly occurs in early childhood **[Taylor et al., (2007)].** Diagnostic tests used to detect *H. pylori* may even be classified either invasive, which require endoscopy and biopsies (rapid urease test, histological detection or culture) or non-invasive (serology,C^{13}-urea breath test, faecal antigen test) **[Vaira & Vakil, (2001)].** The invasiveness and expense of direct observation of the organism have led to search for valid and reliable noninvasive alternatives. Sensitive and specific immunoassay method was developed for the detection of *H. pylori* antigen in human serum **[Attallah et al., (2004)].** The mechanisms of *H. pylori* transmission are: 1.person-to-person transmission is most commonly implicated with fecal/oral, oral/oral, or gastric/oral pathways **[Brown, (2000)]** 2. infection is associated with conditions of crowding and poor hygiene **[Webb et al., (1994)]** and 3.with intrafamilial clustering **[Konno et al., (2005)].** Also, the Helicobacter pylori infection may affect Fetal and neonatal growth and may cause iron deficiency anemia and failure to thrive in infancy **[Doroudchi et al., (2004)].** So knowledge of mode of transmission of *H. pylori* is important to prevent its spread. In this regard, the present study aims at studying the possibility of vertical transmission of *H.pylori* circulating antigen from infected mothers to their newborns (transplacental passage) via the detection of circulating *H.pylori* antigen in serum of mothers and their umbilical cord samples.

MATERIAL AND METHODS

Subjects

Serum samples were collected from 129 pregnant women and from the umbilical cord blood during delivery. Also, 30 serum samples from healthy individuals were included as negative controls.

SDS-PAGE and Western Blotting:

Serum samples from pregnant mothers and umbilical cord blood were separated by SDS-PAGE according to the method of **[Laemmli, (1970)]**. Resolved samples were then electrotransfered onto the nitrocellulose filter (0.45 μm pore size, Sigma) in protein transfer unit according to the method of **[Towbin et al., (1979)]**. The nitrocellulose filter was blocked using a blocking buffer composed of 2 % (w/v) bovine serum albumin (BSA) dissolved in 10 ml tris-buffered saline (TBS), pH 7.4, rinsed in TBS, and incubated with monospecific anti-*H. pylori* IgG antibody diluted in blocking buffer with constant shaking overnight. The blots were washed three times (15 min/wash) by TBS followed by 3 h incubation with anti-rabbit IgG alkaline phosphatase conjugate, and then diluted with TBS. The blots were then washed three times with TBS. The reaction was visualized by incubating the nitro cellulose filter soaked in premixed BCIP/NBT alkaline phosphatase substrate and stopped by distilled water after color development within 10 min.

Detection of *H. pylori* antigen using ELISA:

The *H.pylori* antigen was detected using ELISA technique according to **[Attallah et al., (2004)]**. Polystyrene microtiter plates were coated with 50 μl / well of each serum and umbilical cord samples diluted 1: 400 with coating buffer (pH 9.6) and incubated overnight at 4°C. 200 μl/well of 0.2% BSA in coating buffer (pH 9.6) were added then the plates were incubated for 1 hour at room temperature. After washing, 50 μl/well of 1:30 diluted antibody in PBS-Tween 20 (PBS-T20) were added and the plates were incubated at 37°C for 2 h. After washing 50 μl/well of anti-rabbit IgG alkaline phosphatase conjugate diluted to 1:250 in 0.2% (w/v) BSA in PBS-T20 were added. and the plates were again incubated for 1 h at 37°C. The amount of coupled conjugate was determined by incubation with p-nitrophenyl phosphate substrate. The reaction was stopped by NaOH and the absorbance was read at 490 nm using ∑ 960 microplate autoreader (Metreiteck, Axiom, Burstadt, Germany).

Statistical analysis:

All statistical analyses were done by a statistical software package "SPSS 12.0 for windows, SPSS Inc.). The levels of markers were analyzed by ANOVA and the paired *t* test, P values at a two-sided $P < 0.001$ were considered statistically significant. Spearman correlation test

(r) was also used to investigate the relation between each two variables among each group. The result of the t-values was then checked on student's-t-table to find out the significance level (P value).

RESULTS

Identification of *Helicobacter pylori* antigen using western blotting:

A specific *anti- H. pylori* antibody (supplied by A. M. Attallah) was used as an immunological probe for identification of target *H. pylori* antigen using western blot. The specific anti-*H. pylori* antibody reacted against *H. pylori* in infected serum and umbilical cord samples at molecular weight of 58 kDa (**Figure 1).**

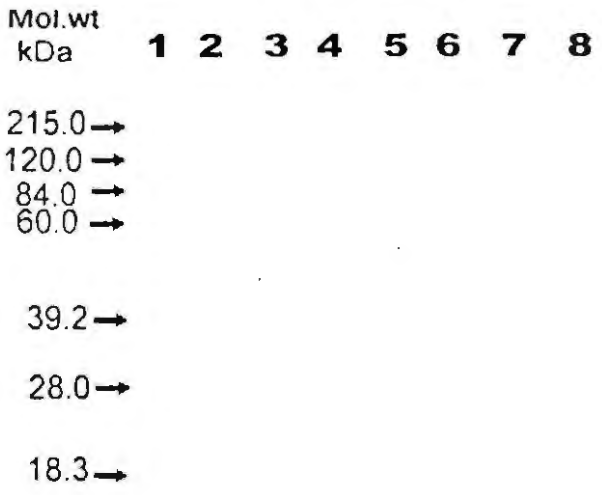

Fig.(1): Immunoblots of *helicobacter pylori* antigen (58 kDa) in sera and umbilical cord Samples of patients and non infected individuals using western blotting.

Lanes 1,2: healthy individuals. **Lane 5:** serum samples from non-infected mothers. **Lanes 3,7:** serum samples from infected mothers. **Lane 6**: umbilical cord samples from non-infected mothers. **Lanes 4,8**: umbilical cord samples from infected mothers.

Determination of the molecular weight of the reactive band using relative mobility:

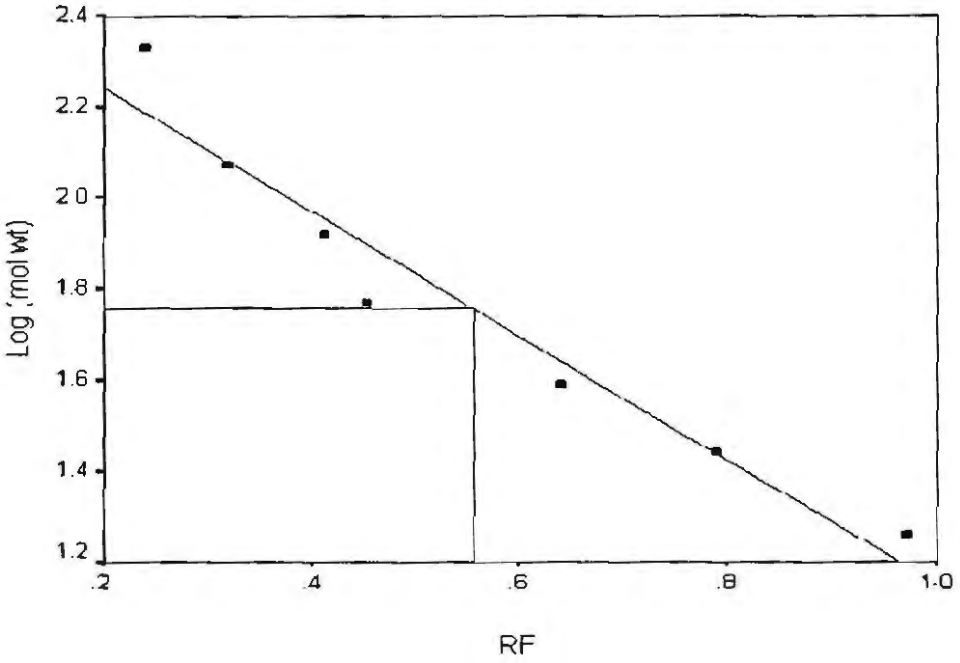

Fig.(2): Linear calibration of the standard molecular weights against their relative mobilities (R_F values). R_F of the reactive antigen is 0.55.

From **figure 2**, it is clear that the rate of flow of unknown antigen is 0.55, and the corresponding log Mol.Wt equals 1.76 which corresponds to molecular weight of 58-kDa.

Detection of *Helicobacter pylori* Antigen using ELISA technique:

The cut-off level of ELISA was set at 0.3 as calculated from the mean optical densities (at 490 nm) of serum from healthy individuals + 3 standard deviations.

129 serum samples of pregnant women, 61 (47%) were positive for *H. pylori* antigen and 68 (53%) were negative using ELISA. In cord samples there were 53 (41%). positive and 76 (59%) negative for *H. pylori* antigen, **(Table 1).**

Table (1): Detection of *H. pylori* antigen in serum from mothers and umbilical cord samples.

Samples	No. of negative	No. of positive
Mothers Sera n= (129) %	68 53%	61 47%
Umbilical Cord n= (129) %	76 59%	53 41%

The rate of vertical transmission of *H. pylori* antigen from infected pregnant mothers to their newborn through umbilical cord 82% since there are 50 positive fetuses born to 61 positive mothers. **(Table 2)**

Table (2): Detection of vertical transmission of *H. pylori* antigen from infected pregnant mothers to their newborns through umbilical cord using ELISA technique:

Serum samples	Vertical transmission through Umbilical Cord		Rate	
	Positive results	Negative results	Positive	Negative
Infected Mothers n= 61	n=50	n=11	82%	18%
Non-infected Mothers n=68	n=3	n=65	4.4%	95.6 %
Total n=129	n=53	n=76	41%	59%

There were 3 newborn positive for *H.pylori* antigen born to negative mothers.

The effect of age on the *H.pylori* infection in mothers and their babies:

The rate of *H. pylori* antigen infection did not differ greatly by age. So there was no statistical association between mothers infected by *H. pylori* antigen and their ages, (Table 3).

Table (3): The epidemiology data of *H. pylori* infection in mothers according to their ages:

Age range (year)	No. of Infected mothers	No. of Non infected mothers
16-20		
n=35	n=19	n=16
%	54.3 %	45.7 %
21-25		
n=46	n=21	n=25
%	45.7 %	54.3 %
26-30		
n=31	n=13	n=18
%	42 %	58 %
31-37		
n=17	n=8	n=9
%	47 %	53 %

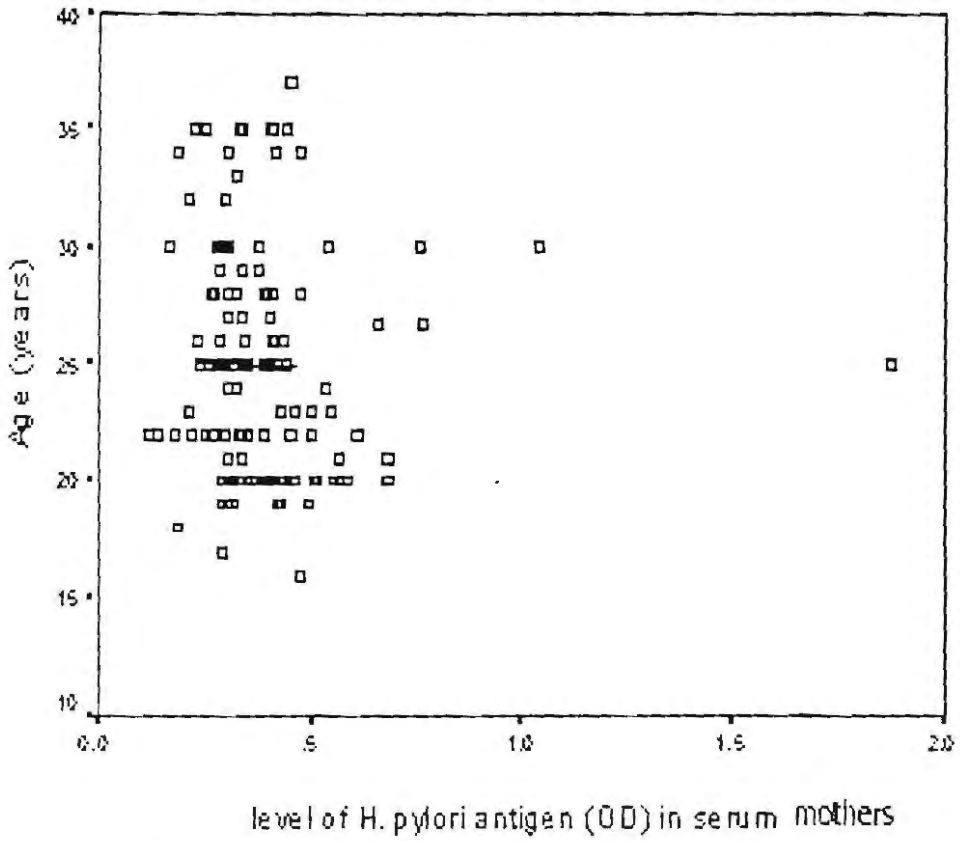

Fig. (3): Correlation between levels of serum of *H. pylori* antigen infected mothers and their ages (r=0.04, p=0.7).

No significant correlation was found between the levels of *H. pylori* antigen in sera of mothers and their ages (r=0.04; p=0.7) (Figure 3).In addition, there was no effect of age on vertical transmission of *H. pylori* antigen. (Table 4).

Table (4): Relation between age of infected mothers and vertical transmission of *H. pylori* antigen:

Age range (years)	No. of Positive mothers	Newborns		Rate	
		No. of Positive	No. of Negative	Positive	Negative
15-20	19	16	3	84 %	16 %
21-25	21	17	4	81 %	19 %
26-30	13	10	3	77 %	23 %
31-37	8	7	1	88 %	12 %

Correlation between levels of *H. pylori* antigen in serum samples of mothers and umbilical cord samples.

A significant correlation was evident between the levels of *H. pylori* antigen in sera of pregnant mothers and its levels in the umbilical cord samples ($r=0.419$; $P<0.0001$) (Figure 4).

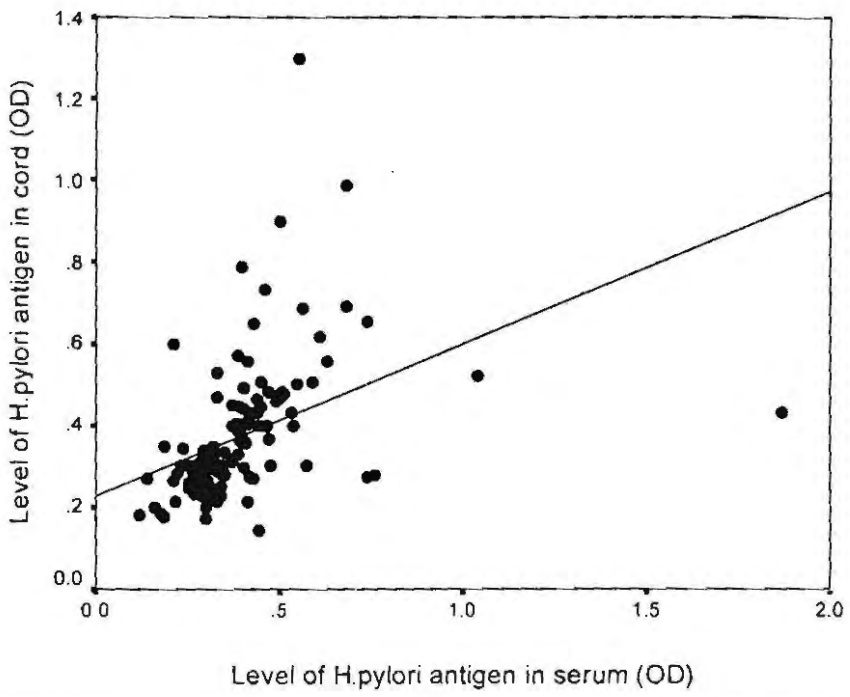

Fig.(4): Correlation between levels of *H. pylori* antigen (OD) in sera of mothers and umbilical cord samples (r=0.419; P<0.0001).

DISCUSSION

In the present work we studied the possibility of vertical transmission of *H.pylori* circulating antigen from infected mothers to their newborns (transplacental passage) via the detection of *H.pylori* antigen in sera of mothers and their umbilical cord.Although the present study is not the first report on the placental transfer of *H. Pylori* infection, it is the first one to detect *H. pylori* antigen in serum of pregnant mothers and umbilical cord samples.

The immunoblotting (Western blot) analysis, showed a molecular weight of 58 kDa for the single immunoreactive band in serum samples from *H. pylori* infected mothers and in umbilical cord samples. These results confirm those of **[Attallah *et al.*, (2004)]** who identified a specific 58-kDa antigen in *H. pylori* lysate and in serum samples from *H. pylori*-infected individuals by using monospecific antibody and western blot analyses.

Detection of serum *H. pylori* antigen by a reliable and useful method is not supposed by the detection of antibodies because the antibody detection tests are less useful in children below 10 years **[Okuda et al., (2002)]** and are not suitable for the follow-up examination of treated patients **[Kokkola et al., (2000)]**. In addition, the accuracy of the antibody tests is no longer adequate to justify their clinical use on clinical or economic grounds **[Vaira & Vakil (2001)]**. Furthermore *H. pylori* antigens can be detected in blood because soluble bacterial antigens of *H. pylori* on the stomach mucosa can be passively absorbed by pinocytosis and can be transferred into the blood through injured tight junctions and by the absorption of intestinal mucosal epithelial cells **[Cao et al., (1998)]**.

We used ELISA for the detection of *H. pylori* antigen in serum and umbilical cord samples collected from mothers at delivery; this method is suitable for the laboratory diagnosis and screening of large populations for *H. pylori* infection. This test has several potential advantages over other techniques for population studies. No expensive instrumentation or expertise is required to perform a standard ELISA. The advantages of the test include its ability to detect the infection with *H. pylori* and its usefulness with individuals for whom endoscopy is difficult to justify **[Garner & Cover (1996)]**.

By using ELISA technique we detected the positive infection of *H. pylori* antigen in 47% of 129 serum mothers samples and in 41% of 129 umbilical cord samples. and several authors evaluated *H. pylori antibody* in mothers with different detection rate (15.2% to 95.8%) **[Doroudchi et al., (2004); Weyermann et al., (2005) and Selda et al., (2008)]** compared to our rate equal (47%).

The evaluation rate in other studies may be higher than our detection rate; this may be due to that the presence of antibodies in the circulation for along time. They may be present in serum for months or years even in patients with remission or with change of the titers during the disease. Furthermore we found that, the vertical transmission of *H. pylori* from mothers to their newborns is very high with a rate of 82%. although **[Gold et al., (1997)]** found that the passive transplacental transfer of maternal anti-*H. Pylori* IgG occurred in 96% of the infants studied. **[Dursun et al., (1998)]** found that anti- *H. pylori* IgG was determined to be positive in 32 of 36 babies whose mothers were positive for anti- *H. pylori* IgG, wherease **[Doroudchi et al., (2004)]**found that more than 82% of seropositive mothers transferred *H. pylori* -specific

IgG antibodies to their fetuses. In the study of **[Selda et al., (2008)]** *H. pylori* IgG levels were measured at birth and they reported that the seropositivity for *H. pylori* was 95.8% in maternal sera and infant sera.

In our study, there was no correlation between the level of *H. pylori* antigen in sera of mothers and their ages (r=0.04; P=0.7).a finding which is in agreement with the results of **[gold et al., (1997)]** who found that there was no statistical association between maternal *H. pylori* seropositivity and maternal age. On the other hand, **[Goodman et al., (2004)]** found a prevalence of increased *H. pylori* antibody with age.

Also, we evaluated *H. pylori* infection in mothers according to the type of delivery. The mean ± SD values of *H. pylori* antigen in serum of mothers delivered by cesarean delivery was 0.38 ±0.13 and the mean ± SD values of *H. pylori* antigen in serum of mothers delivered by normal vaginal was 0.39±0.20. The relative risk of infection in mothers delivered by vaginal delivery was not significantly different from that delivered by caesarean section (P>0.05); this is in good agreement with the results of **[Doroudchi *et al.*, (2004)]**. Furthermore the relative risk of infection in children born by vaginal delivery was not significantly different from that in children born by caesarean delivery (P>0.05).The mean ± SD value of *H. pylori* antigen in cord samples of newborn delivered by cesarean delivery was 0.34±0.15 and the mean ± SD value of *H. pylori* antigen in cord samples of newborn delivered by vaginal delivery was 0.38±.17. In conclusion the *H. pylori* antigen was detected in 47% of serum of mothers and in 41% of serum of their newborn. Therefore, the results of the present study confirm that *H. pylori* antigen was transmitted vertically from mothers to their newborn (vertical transmission rate =82%) and indicate that *H. pylori* antigen crosses the umbilical cord and transmitted to newborn. Therefore, the infected mothers are considered a risk factor for transmission of the antigen leading to the infection of their newborns.

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دراسات كيميائية مناعية عن الإنتقال الرأسى لأحد أنتيجينات بكتيريا الهيليكوباكتر بيلورى

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قد أثبتت الدراسات أن ميكروب الهليكوباكتر بيلورى يصيب ٥٠ ٪ على الاقل من السكان. وقد أثبتت الدراسات الحديثة أيضا أن له دور في الإصابة بسرطان المعدة. ونظرا لأن الإصابة بهذا النوع من البكتريا يتم في سن مبكرة حيث تنتقل العدوى عن طريق الفم بواسطة الأكل أو الشرب الملوث إلى المعدة ويظل كامنا فيها حتى ما بعد سن البلوغ ولذلك فإنه من الأفضل تشخيص هذا النوع من البكتريا في مرحلة مبكرة من العمر. أيضا خطورة الإصابة بالهليكوباكتر بيلورى فى الأطفال لا يمكن إهمالها حيث وجد أن الإصابة بالهليكوباكتر بيلورى مصحوبة بإعاقة نمو الطفل داخل الرحم ونقص الحديد وحدوث الأنيميا وسوء التغذية ووجد أيضا أن الإصابة بالهليكوباكتر بيلورى فى الطفولة ربما يزيد من التعرض للإصابة بسرطان المعدة فيما بعد خلال فترة حياته. ومن خلال هذه الدراسة تم التعرف على أنتيجين الهيليكوباكتر بيلورى فى عينات سيرم الدم المأخوذة من الأمهات الحوامل ووجد أن٤٧% منهم مصابين وأيضا وجدالميكروب فى عينات الحبل السري أثناء الولادة بنسبة٤١%. وقد تم تحديد الوزن الجزيئى لهذا الانتيجين وهو٥٨ كيلو دالتون في كل من عينات السيرم للأمهات وعينات من الحبل السرى للأطفال حديثى الولادة. كما توصلت الدراسة إلى أن أنتيجين الهيليكوباكتر بيلورى ينتقل رأسيا من الأمهات الى أطفالهم بنسبة ٨٢% وبذلك تمثل الأمهات المصابة عامل خطر فى أنتقال الإصابة إلى أطفالهم عن طريق الحبل السرى ولهذا فان بالكشف المبكر عن هذا الأنتيجين فى الأطفال يساعد على حمايتهم وعلاجهم المبكر من هذا المرض الخطير.