Journal of
AIDS & Clinical Research
ISSN: 2155-6113

Faranoush et al., J AIDS Clin Res 2013, 4:5
DOI: 10.4172/2155-6113.1000209

Research Article Open Access

Role of *H. pylori* Infection (Serology, PCR) in Chronic Idiopathic Thrombocytopenic Purpura in an Endemic Country: A Case Control Study, Tehran, IRAN

Mohammad Faranoush[1], Samileh Noorbakhsh[2],*, Azim Mehrvar[3] and zardokht Tabatabae[4]

[1]*Associate Professor in Pediatric Hematology & Oncology, Research Center of Pediatric Infectious Diseases Tehran University of Medical Sciences Tehran, Iran*
[2]*Full Professor in Pediatric Infectious Disease, Research Center of Pediatric Infectious Diseases Tehran University of Medical Sciences Tehran, Iran*
[3]*Assistant Professor in Pediatric Hematology & Oncology, Research Center of Pediatric Infectious Diseases Tehran University of Medical Sciences Tehran, Iran*
[4]*Microbiology, Research Center of Pediatric Infectious Diseases Tehran University of Medical Sciences, Tehran, Iran*

Abstract

Background: A practical guideline for detection and managements of some common infectious agents in cases with chronic ITP (Idiopathic Thrombocytopenic Purpura) is so important.

Objectives: to investigate the role of *H. pylori* infection in children with chronic ITP in an endemic area.

Materials and methods: A case control study done in pediatric ward Rasul Hospital, Tehran, Iran (2009-2010). 51 chronic ITP cases and 25 controls were assessed. *H. pylori* IgG & IgA ELISA (LDN -Germany) assesses in all cases and controls. All cases undergoing Bone Marrow Aspiration. *H. pylori* -PCR evaluated (QIAquickP® QIAGEN; Germany). P-value <0.05 was considered statistically significant.

Results: cases were between 1- 20 years (mean 13.3.5 ± 7.6 y). Platelet count varied between 5000-1330000 (mean 63621 ± 37369.9) Positive *H. pylori*- IgA observed in 70% (36/51) of cases and 4% (1/25) of controls; p-value=0.00. *H. pylori* (IgG) was not significantly difference between cases and controls. [51% (26/51) vs. 32% (8/25), p-value=0.09] .Poor agreement observed between *H. pylori* –IgA and *H. pylori* - IgG antibodies and severity of thrombocytopenia in ITP cases (Kappa=-0.11; 0.04). Positive PCR results was % 5.9% (3/51) in ITP cases without significant difference in age between positive and negative PCR results (mean age 9.3 ± 9.7 years vs. 13.5 ± 7.52 years; p-value =0.3) Poor agreement between positive PCR and positivity of IgA (actual agreement=47.062%; p-value =0.5; Kappa=- 0.04), and IgG antibodies (actual agreement=40.91%; p-value =0.6; Kappa=- 0.04 respectively) were observed in ITP cases.

Conclusion: We concluded that *H. pylori* infection (serologically) is high in young Iranian population. In chronic ITP, the H. pylori infection can be considered as an additional disorder which aggravates the main disease. The management of mild-to-moderate chronic ITP in Iranian patients, especially those with a recent onset of disease, should include an investigation for and eradication of infection with *H. pylori*.

Keywords: *H. pylori*; ITP (Idiopathic Thrombocytopenic Purpura); *H. pylori* IgG; IgA; PCR

Introduction

Idiopathic Thrombocytopenic Purpura (ITP) is defined as a characteristic rash associated with an abnormally low platelet count of unknown cause. In opinion of **James** and other authors Idiopathic Thrombocytopenic Purpura (ITP) defined as isolated thrombocytopenia with no clinically apparent associated conditions or other causes of thrombocytopenia. Exclusion of recognized alternative etiologies of thrombocytopenia was the basis for the idiopathic thrombocytopenic Purpura [1]. HIV; hepatitis C infections and *H. pylori* infection should be considered an alternative disorder [1-5].

Scandellari et al. showed a cross-reaction of an *H. pylori* urease B monoclonal antibody with platelet glycoprotein IIIa and suggested that the immune response to UreB may be involved in the pathogenesis of ITP [2]. The possible role of *H. pylori* infection in the development of ITP had studied in some systemic reviews [3-5]. Arnold et al. showed an overall platelet response in more than 50% of the patients successfully treated for the infection and increased response rates in countries with a high prevalence of *H. pylori* infection in background populations, i.e. in patients with less severe degrees of thrombocytopenia and in those with shorter disease duration. [1]

Figura et al. [5] reported that the cure of *H. pylori* infection totally corrects the thrombocytopenia in certain patients. In other patients with ITP, the infection can be considered as an additional disorder which aggravates the main disease, while in a third group of patients the eradication of *H. pylori* appears to have no effect on the course of thrombocytopenia [5]. *H. pylori* is a gram negative bacterium and considered the etiologic agent of some gastrointestinal and extra gastrointestinal as a class I carcinogen by the World Health Organization. Colonization of H. pylori has been found in dental plaques, saliva, tonsils, and sinus mucosa. *H. pylori* infection varies among countries and often within a country [6]. ITP in adult patients may be associated with serum antibodies. There are geographical disparities of both the frequency of *H. pylori* infection among patients with ITP and the frequency of platelet count responses following eradication of *H. pylori* infection, and these two frequencies correlate with each other [1]. There

***Corresponding author:** Samileh Noorbakhsh, Research Center of Pediatric Infectious Diseases, 4th floor Rasoul Hospital, Niayesh Street, Satarkhan Avenue, Tehran, 14455 Islamic Republic of Iran, Tel: 00982166525328; Fax: 00982166516049; E-mail: Samileh_noorbakhsh@yahoo.com, noorbakhgsh@tums.ac.ir

Received March 11, 2013; **Accepted** May 10, 2013; **Published** May 18, 2013

Citation: Faranoush M, Noorbakhsh S, Mehrvar A, Tabatabae Z (2013) Role of *H. pylori* Infection (Serology, PCR) in Chronic Idiopathic Thrombocytopenic Purpura in an Endemic Country: A Case Control Study, Tehran, IRAN. J AIDS Clin Res 4: 209. doi:10.4172/2155-6113.1000209

exist an inconsistency among previous studies which has prevented broad acceptance of the association of H. pylori infection with ITP. Moreover, the etiologic factors of ITP in the Iranian population are not well understood [7-12]. The etiologic factors of ITP in Iranian population are not well understood. *H. pylori* infection in the Iranian population is high [13-18]. Positive serum *H. pylori*- IgA reported in 15% and positive *H. pylori* – IgG in 11% of children with rhino sinusitis >2 weeks (mean age 4.2 years)

Except 1 study in Iranian adults, the relationship between *H. pylori* infection and ITP was not explained until yet. Rostami et al. reported the eradication of **H. pylori** on platelet recovery in patients with chronic ITP [19]. Providing the practical guidelines for detection and managements of some common infectious organisms like as *H. pylori* infection in ITP cases is so important in our country.

This case control study in children in Iran was conducted in order to investigate the role of *H. pylori* infection in cases with chronic ITP.

Material and Methods

A cross sectional study performed in the Department of pediatrics, Rasul Hospital, Tehran, Iran (2009 – 2010) The study was approved by the ethical Committee in Research Center of Pediatric Infectious Disease in Tehran University of Medical Sciences. We studied 51 consecutive Iranian patients with chronic ITP and 25 normal controls.

Initially a questionnaire was completed by an authorized physician, followed by a complete clinical examination. All cases and controls were examined by an internist for other concomitant disorders (immune deficiencies state; diabetes mellitus, renal/ heart failure; etc).Blood samples were taken for routine blood tests as well as serologic tests before BMA. Blood samples (2 ml) were centrifuged and transferred to our research laboratory. The serum was stored at -20°C until the serologic examination was performed. Specific *H. pylori* antibodies (IgG & IgA) in all cases and controls were assessed by ELISA. The commercial kits (Chemicon-Germany) were used and the results were interpreted as suggested by the manufacturer. Results were calculated quantitatively.

Bone Marrow Aspiration (BMA) had done in all studied cases. BMA samples placed in sterile tubes. Samples were centrifuged and homogenized, then preserved in -80°C. A PCR template Purification Kit (Roche; Germany) was used. The binding column tube was transferred to a new 1.5 ml tube. The integrity of DNA was assessed by gel electrophoresis (1% agarose). *H. pylori*- DNA was evaluated qualitatively by specific PCR primer kits (QIA quick P® QIAGEN; Germany). Diagnostic kits included a ready to use PCR mix Kit, positive and negative controls and other qualified reagents along with a protocol for detecting as low as 10 copies/ml of the *H. pylori* genome.

Statistical analysis

The student's *t* test was used to determine significant differences in means for continuous variables and chi-square for comparing categorical data in cases and controls. P-value s less than 0.05 were considered statistically significant.

The agreement between serologic test and PCR was assessed by the calculation of Kappa statistic. Landis and Koch suggested that a kappa greater than 0.75 represents excellent agreement beyond chance, a kappa below 0.40 represents poor agreement, and a Kappa of 0.40 to 0.75 represents intermediate to good agreement.

Results

Demographic results

45% (23) of cases were male and 55% (28) female. Ages varied between 1 to 20 years; mean 13.3.5 ± 7.6 years (Figure 1). 37.3% (19/51) of studies cases was young (< 10 years) and 63% (32 /51) was old (>10 years).

Mean age of ITP cases in males (11.6 ± 8 years) was insignificant (p -value =0.1) with female (14.6 ± 7.2 years). Platelet count varied between 5000-1330000; mean 63621 ± 37369.9. Platelet count was not different between male (62365 ± 40000) and female 164653 ± 35741) p-value =0.8.

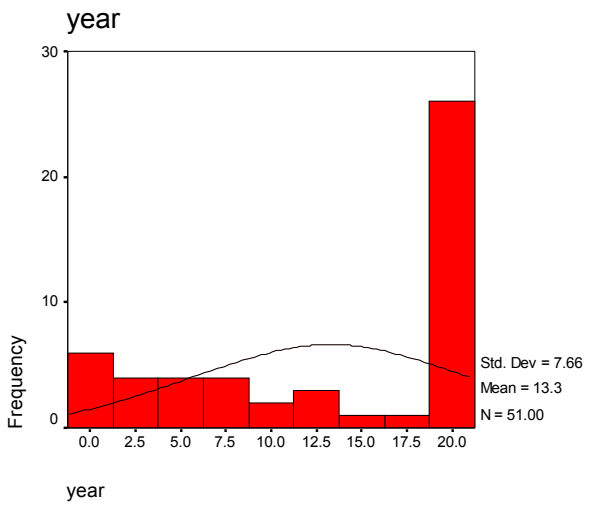
Sever ITP (PLT<20 × 109/L 0000) detected in 11.6% (6/51); Moderate ITP (PLT<20-80 × 109/L 000) in 54.9% (28/51); and Mild ITP (>80 × 109/L) in 33.3% (17/51) of cases. Serologic results: Positive *H. pylori*- IgA observed in 70% (36/51) of cases and 4% (1/25) of controls. Serum level for *H. pylori*- IgA in ITP cases was between 0.2-85 mg percent, mean 14 ± 18.

The mean level of *H.pylori*-IgA in female cases was higher than male cases. (18.4 ± 21.9 vs. 8.7 ± 10 mg%; p -value=.055). *H.pylori*-IgA positively was significantly higher in cases. [70% vs. 4% of controls, p-value=0.00]. Serum *H.pylori*- IgG level was between 0.2-119 mg%; mean 17.2 ± 25.3.The mean level of *H.pylori*- IgG had not significant differences between male and female (15.2 ± 30 vs. 18.8 ± 19.9mg%; p -value=0.6). *H. pylori* (IgG) was not significantly difference between cases and controls [51% (26/51) vs. 32% (8/25), p -value=0.09]. Poor agreement observed between *H. pylori* –IgA and *H. pylori* - IgG antibodies with severe ITP (Kappa=-0.11; 0.04) (Tables 1 and 2).

PCR results

Positive *H. pylori*–PCR in BMA was % 5.9% (3/51. The mean age was not different between ITP cases with positive and negative PCR results (mean age 9.3 ± 9.7 years vs. 13.5 ± 7.52 years; p-value=0.3).

Poor agreement observed between positive PCR IgA (actual agreement=47.062%; p-value=0.5; Kappa=-0.04); and IgG antibodies (actual agreement=40.91%; p-value=0.6; Kappa=-0.04, respectively) (Tables 3-5).



Mean age of ITP cases in males (11.6 ± 8 years) was insignificant (p -value =0.1) with female (14.6 ± 7.2 years).

Figure 1: Age distribution of ITP cases

	Observer 2			
	+	-		
+	14	8	22	73.33%
-	6	2	8	26.67%
	20	10	30	
	Actual agreement=		53.33%	
	Chance agreement=		57.78%	
	Kappa statistic=		**-0.11**	

Table 1: Correlation between positive IgA and severe ITP (PLT count <80000).

		Observer 2			
		+	-		
Observer 1	+	9	4	13	43.33%
	-	11	6	17	56.67%
		20	10	30	
		Actual agreement=		50.00%	
		Chance agreement=		47.78%	
		Kappa statistic=		**0.04**	

Table 2: Correlation between positive IgG and sever ITP.

		Observer 2			
		+	-		
Observer 1	+	3	23	26	59.09%
	-	3	15	18	40.91%
		6	38	44	
		Actual agreement=		40.91%	
		Chance agreement=		43.39%	
		Kappa statistic=		**-0.04**	

Table 3: Agreement between positive PCR IgA and IgG antibody.

Poor agreement (actual agreement=37.2%; p-value=0.6; Kappa=-0.06) detected between positive *H. pylori* –PCR and severity of ITP (PLT count <80000) (Table 6).

Discussion

We studied 51 chronic ITP cases aged between 1 to 20 years (mean13.3.5 ± 7.6 years), 63% of cases was older than 10 years. 54.9% of cases had Moderate thrombocytopenia (20000-80000), severe thrombocytopenia (PLT<200000) detected only in 11.6%.

H. pylori –DNA was positive in Bone marrow aspiration of 5.9% (3/51) cases with mean age 10 years, without difference in mean age of cases with positive and negative results (p-value =0.3). 70% (36/51) of cases had Positive serum *H.pylori*- IgA in compare with 4% (1/25) of controls (p-value =0.00).

Positive serum *H. pylori* -IgG observed in 51% of cases in compare with 32% in controls without significant difference (p-value =0.09). Severity of thrombocytopenia in ITP cases had poor agreement with positive H. pylori –IgA (Kappa index=-0.11); positive *H. pylori* - IgG antibodies (Kappa index= 0.04); and positive *H. pylori* –DNA (PCR) in BMA (Kappa index =-0.06). Positive *H. pylori*–DNA obtained in BMA of 5.9% ITP cases (mean age= 10years) was less frequent than 3 previous Iranian studies.

Chronic and persistent infection (positive-PCR) might be found in parts of upper respiratory tract (nasal polyp, adenoid tissues) for longer period. *H. pylori* infection was detectable in adenoid tissues of 15% children before 8 years which is lower than Khadem et al. study on adults cases [14-16]. Saffari et al. studied *H. pylori* antibodies in population in Shiraz (south of Iran) [14]. Seroprevalence to *H. pylori* infection is high in Iranian population [14-18]. Initial infection probably occurs at an early age, its prevalence increases with age. The infection will increase to 30 % in 2nd and 53.5% after 4th decade of life [16]. 28.3% of persons between 20-40 years; 32% of population between 41-80 years had positive *H. pylori*-IgG. But recent *H. pylori* infection (positive IgA) observed in 4% of controls are lower than previous studies (16.7%, 53.5% respectively).Higher age of cases might explain these differences. [17] Here, we found previous HP Infection (Positive IgG= 32%) in controls which is very close to previous studies in normal Iranian population [14-18]. Although *H. pylori* infection varies between countries and often within a country, higher age for cases in Farhadi et al. study is the probable cause for this higher infection in compare with studied children [20].

In countries such as Japan and Italy, where most studies of *H. pylori* eradication in ITP have been performed, testing for *H. pylori* infection has been recommended. HP eradication was successful in 87% (62/71) of adult cases with ITP who completed the eradication [5]. In proven cases eradication therapy is recommended as the initial treatment in *H. pylori*-infected patients [5,6]. Rostami et al. study [19] defined 48% (30/62) of HP-eradicated patients showed an ITP response, none (0%) of HP-negative ITP patient had improved. The ITP response persisted for 48 weeks in 93% (28/30) of the responders. The ITP responders had a shorter disease duration than none responders, p-value = 0.002 [19].

Conclusion

We concluded that *H. pylori* infection (serologically) is high in young Iranian population. In chronic ITP, the *H. pylori* infection can be considered as an additional disorder which aggravates the main disease. The management of mild-to-moderate chronic ITP in Iranian patients, especially those with a recent onset of disease, should include an investigation for and eradication of infection with *H. pylori*.

Acknowledgments

This study was supported by the Research Center of Pediatric Infectious Diseases. We thank "Research Center of Cellular and Molecular Biology" at the Tehran University of Medical Sciences.

		Observer 2			
		+	-		
Observer 1	+	1	25	26	50.98%
	-	2	23	25	49.02%
		3	48	51	
		Actual agreement=		47.06%	
		Chance agreement=		49.13%	
		Kappa statistic=		**-0.04**	

Table 4: Agreement between positive PCR and IgG antibody.

		Observer 2			
		+	-		
Observer 1	+	3	0	3	5.88%
	-	32	16	48	94.12%
		35	16	51	
		Actual agreement=		37.25%	
		Chance agreement=		33.56%	
		Kappa statistic=		**0.06**	

Table 5: Agreement detected between positive *H. pylori* –DNA (PCR) in BMA and severe ITP.

Total	Severity		
	2.00	1.00	
3	0	3	1.00 PCR HP
48	16	32	2.00 YLO
51	16	35	Total

Table 6: PCR- HP Severity Cross tabulation Count

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