ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

CHRONIC IMMUNE THROMBOCYTOPENIA AND HELICOBACTER PYLORI INFECTION IN SUDANESE PATIENTS.

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Manuscript Info

Manuscript History:

Received: 14 January 2016
Final Accepted: 26 February 2016
Published Online: March 2016

Key words:
Idiopathic,Immune, Thrombocytopenia, H. Pylori, Anti-platelets antibodies .

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Abstract

Background/Aim: Immune thrombocytopenia (ITP) is an autoimmune disease mediated by anti-platelet auto-antibodies. There is growing evidence that the eradication of H. pylori effectively increases platelet count in a considerable proportion of H.pylori- infected ITP patients. The study aimed to determine sero-prevalence of H. pylori in chronic ITP patients and to compare the ITP characteristics in H.pylori infected and non-infected ITP patients.

Patients/Method: the study was done on 60 already diagnosed ITP patients of at least 6|12 duration. On all patients were done Complete blood count (CBC), blood cells morphology (thin film), IgG for H.pylori antibodies and anti-platelet antibodies by ELISA. Anti-lewis antibodies were done by a serological method. All patients were screened for hepatitis B, C and HIV by ICT. The initial platelets count at diagnosis was obtained from the patients note and bone marrow examination performed by consultant hematologist. Their ages ranged between 2-74 years, there were 21 males and 39 females. 57 patients already were underwent corticosteroids treatment.

Results: Anti H.pylori IgG, Anti-platelets, and anti-Lewis abs were detected in 63%, 58%, and 28%, respectively. There was a significant difference between platelets count at time of diagnosis and time of enrolling, P: < .00001. Moreover, a significant difference was found in platelets count at time of enrolling between H. pylori sero-positive and sero-negative ITP patients, P: 0.00006. In ITP treated patients (57/60) there was a significant rise in platelets count after compared to the initial count, P: 0.00001. In comparison of ITP characteristic between H.pylori sero-positive and sero-negative ITP patient; there was a significant difference in regard to anti-platelets antibodies status, P: 0.0001, platelets count at time of enrolling P: 0.00006, and corticosteroid response, P: 0.01.

Conclusion: Anti -H.pylori and anti-platelets antibodies were frequent in ITP patients. Reduction in the platelet count was observed in H.pylori sero-positive ITP patients than sero-negative ones. In the majority of H.pylori sero-positive ITP patients; thrombocytopenia still persists even in those patients who underwent corticosteroids treatment.

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Introduction:-

Immune thrombocytopenic purpura (ITP) is the most common autoimmune hematopoietic disorder, affecting peoples of different ages. The earlier name ('idiopathic' thrombocytopenic purpura) was previously used because, in most cases, the underlying cause was indefinite. Most recently, many etiological factors has grown significantly and the former name idiopathic is used infrequently (Ahn, 2010). ITP is induced by anti-platelet auto-antibodies that bind to platelets antigens, accelerating platelet destruction (Stasi, 2012). The autoantibody response largely targets platelet-surface glycoproteins of GPIIb/IIIa and GPIb. Although the etiology of ITP is doubtful, microorganisms such as HIV and hepatitis C virus are identified to contribute to its progress (Stasi, 2009), indicating that in a particular subset of ITP, infectious agents play an important role in the pathogenesis of the autoimmune response.

Helicobacter pylori (H. pylori), a gram-negative spiral -microaerophilic commensal-bacteria that colonizes the human stomach, is an etiological agent of gastritis, gastric and duodenal ulcer, and carcinoma of the stomach (Tan and Goh, 2012). H. pylori have been concerned in the development of various systemic disorders, including autoimmune diseases, cardiovascular disorders, and neurological disorders (Emilia et al., 2007). The strongest proof has been declared for ITP, with expert researches revealing that the disease improved after successful eradication of H. pylori. Many hypotheses have been postulated for the mechanisms of H. pylori infection in inducing ITP. One of these is the molecular mimicry, according to which H. pylori strain could induce antibody production in response to antigens that cross-react against various surface glycoproteins in the platelets (Franceschi et al., 2004). The support of this hypothesis emerges from recent data of Italian researchers, indicating that H. pylori cagA gene had significant higher prevalence in patients with ITP than in a control group (Emilia et al., 2007). Other presumed targets of molecular mimicry are Lewis antigens, which are expressed by H. pylori in a specific strain. Lewis antigens adsorb to platelets and might be as targets for anti-Lewis antibodies in some patients with genetic background (Gerhard et al., 2002). Other studies have revealed that some strains of H pylori bind von Willebrand factor (VWF) and induce glycoprotein Ib– and FcRIIa-dependent platelet aggregation in the presence of H. pylori antibodies (Byrne et al., 2003). Activation may advance platelet clearance and antigen presentation, which increases production of antibacterial antibodies. Both H. pylori infection and ITP are associated with a polarized Th1-type phenotype (Stasi et al. 2007, Guo et al., 2007). Accordingly, it may be speculated that H. pylori infection creates an immunological environment that facilitates the onset and/or persistence of ITP (McCrae, 2004). In conclusion there are data consistent with an association between H. pylori infection and ITP (Hashino et al., 2003; Hino et al., 2003; Jargue et al., 2001).

Several researchers have tried to recognize characteristics of H. pylori-associated ITP by comparing the clinical characteristics of ITP in H.pylori infected and non-infected patients, H. pylori infected ITP patients were significantly older than uninfected patients (Fujimura et al., 2005), but this is expected because prevalence of H. pylori infection increases with age increase (Suerbaum and Michetti, 2002). Multiple studies have failed to detect significant variations in any other demographic or clinical characteristic, including sex, platelet count, or response to therapy. In this study we investigated the sero-prevalence of H. pylori in ITP Sudanese patients; we compared the characteristic of ITP in H.pylori infected and non-infected ITP patients.

Methods:-

Ethical approval was obtained from the Research Ethics Committee at FMLS-Khartoum University. A cross sequential hospital based study was conducted in Hematology Clinic in Fedail Hospital - Khartoum state in a period between August 2013 and August 2015. The study included 60 already diagnosed ITP patients who were referred for follow-up. ITP diagnoses was based on the presence of idiopathic thrombocytopenia (<100 $x10^3$/µl) persistent for more than 6 months, with normal or increased megakaryocytes in the bone marrow (George et al., 1996). A written consent form was obtained; questionnaire was filled with demographic and clinical data for each enrolled patient. The initial platelets count at diagnosis was obtained from the patients note and bone marrow examination performed by consultant hematologist. The clinical data were obtained from patients' records in the Hospitals. Exclusion of other alternative causes of thrombocytopenia was performed as follow: Lypmphoproliverative disorders and others malignancy were excluded clinically, by blood count, and cell morphology of peripheral blood and bone marrow. SLE and other autoimmune diseases were excluded by Coomb's test. HIV, Hepatitis B, and Hepatitis C were excluded by screening rapid test. At time of enrolling venous blood was collected and following tests were performed:, Coomb's test, viral screening rapid test, complete blood count using Sysemx KX-21 blood

analyzer, anti-platelets and H.pylori IgG antibodies by using ELISA technique (Abcam Co., UK), and anti-lewis by serological method.

Statistical analysis:-

Data were collected in master sheet paper, analyzed by computer using Microsoft office excell; then presented in figures and tables. Fisher's exact test and simple t-test were used to test whether there is a significant difference between ITP characteristics in H.pylori infected and non-infected patients. The level of significance was set at ($p \leq 0.05$).

Results:-

Age and Sex:-

Previously diagnosed (60) ITP patients referred to Fedail Hospital for follow up, mean duration of diagnosis-24 months (range 6-48 months). 21 (35%) were males and 39(65%) were females, with ratio of 1:2, (table 2), six were children's (10%) and 54 were adults (90%) (Table: 2), their ages ranged between 2-74 years (mean ages -34 years). Platelets counts' mean at diagnosis -25 $x10^3$ cell/µl, (table: 1), bone marrow examination was normal and revealed adequate number of megakaryocytes in all enrolled patients. 57 of the total 60 enrolled ITP patients were treated for thrombocytopenia using corticosteroids, three patients who were recently diagnosed (mean duration of diagnosis-7 months) didn't received any treatment for ITP. At time of enrolling in the study complete blood count parameters revealed peripheral thrombocytopenia in 43 (72%) of ITP patients. All patients were screened for viral infections using immuno-chromatography test (ICT).

H.pylori sero-prevalence in ITP:-

Anti H.pylori IgG antibodies were detected by ELISA test in 38(63%) out of the 60 confirmed ITP patients (fig: 2).

Anti-platelets and Anti-Lewis antibodies in ITP:-

Anti-platelets antibodies were detected in 35/60 (58%) of enrolled ITP patients (figure: 2), all of them had co existing H.pylori Abs.

Anti-Lewis antibodies were positive in 17/60 (28%) of enrolled ITP patients (figure: 2), were co- detected with anti H.pylori antibodies in 12/38 (32%) of H.pylori sero-positive ITP patients, were also co-determined with anti-platelets antibodies in 11/35 (31%) of ITP patients.

Platelets count:-

Mean of platelets counts at time of diagnosis was 25 $x10^3$ cell/µl, there was significant difference with platelets count at time of enrolling (mean: 109 $x10^3$ cell/µl) after 24 months mean duration of diagnosis, P: < .00001. However, there was a significant difference between platelets count at time of enrolling in H.pylori sero-positive (mean: 50 $x10^3$ cell/µl), and sero-negative (mean: 145 $x10^3$ cell/µl) ITP patients, P: 0.00006). Furthermore, in ITP treated patients (57/60) there was a significant increasing difference (P: 0 .00001) between platelets count at time of diagnosis (mean: 25 $x10^3$ cell/µl) and at time of enrolling (mean: 111 $x10^3$ cell/µl), (table: 1, 2).

Comparison between ITP characteristic in H. pylori sero-positive & sero-negative patients:-

There were no significant differences in ITP characteristics regarding age, gender, bleeding type, duration of diagnosis, Lewis antibodies, and Mean Platelets Volume (MPV); P-values were > 0.05, while there was a significant difference in regard to platelets count P-values (0.00006), anti-platelets antibodies status P-values(0.0001), and the response to corticosteroid treatment, P-values(0.01), (table: 2).

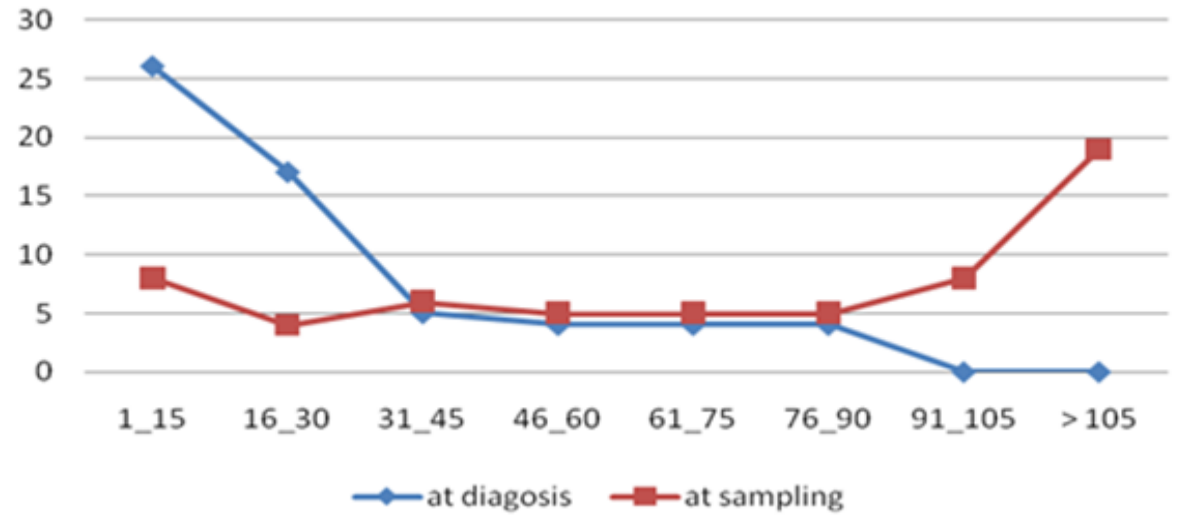

Fig. 1: Frequency Distribution of Platelets count $\times 10^3/\mu l$ at time of diagnosis and enrolling.

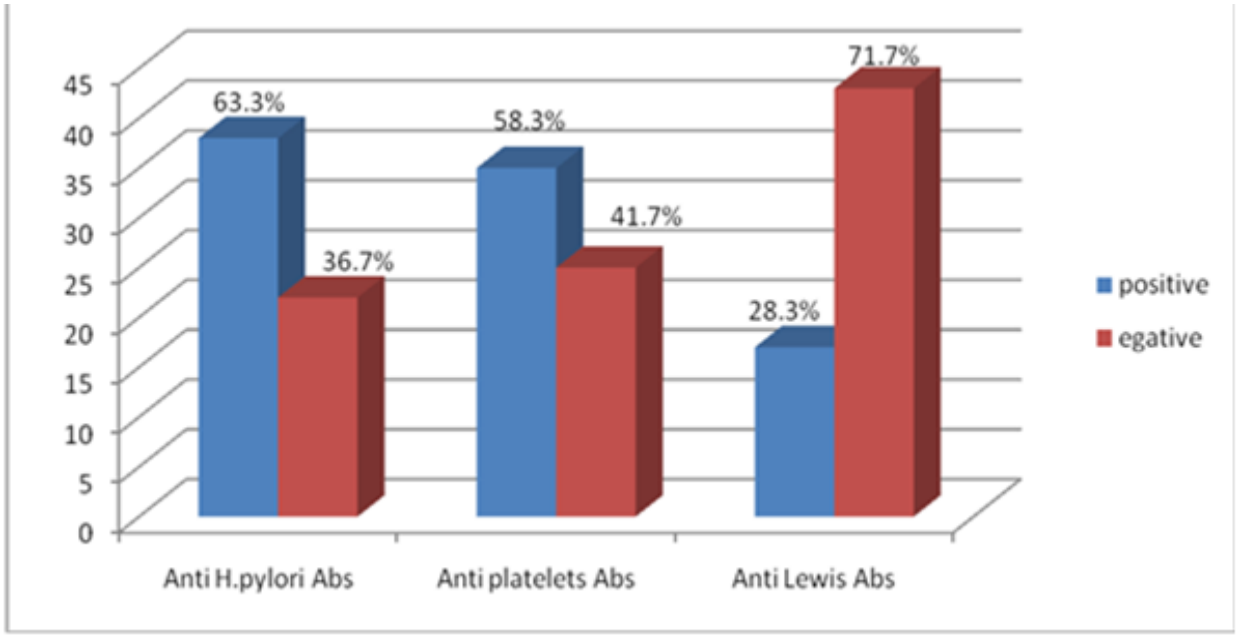

Fig. 2: Percent Frequency of anti. H.pylori, anti- Platelets, andti Lewis antibodies in ITP patients.

Table 1: Platelets mean in H. pylori sero-positive/sero-negative and treated/untreated ITP patients:-

		Number of patients	Platelets mean $\times 10^3/\mu l$ Mean at diagnosis	Mean at enrolling
Total enrolled ITP patients		60	25	109
H. pylori	+ve patients	38	25	14
	-ve patients	22	50	145
ITP treated patients	yes	57	25	111
	No	3	67	23

Table 2: Comparison of ITP characteristics in H. pylori sero-positive and sero-negative ITP patients:-

Characteristic		H. pylori(+ve) ITP	H. pylori(-ve) ITP	p.value
Gender	Males	12	9	0.57
	Female	26	13	
Ages	children's	4	2	0.67
	Adults	34	20	
Duration of diagnosis/month	over 24	12	8	0.78
	Below 24	26	14	
Bleeding type	spontaneous	12	7	1.0
	Superficial	26	15	
Platelets count at enrolling	over $100\times10^3/\mu l$	8	9	0.00006
	Below $100\times10^3/\mu l$	30	13	
ITP Treatment response	yes	8	12	0.01
	No	30	10	
Anti-platelets Abs	positive	35	0	0.0001
	Negative	3	22	
Anti-Lewis Abs	positive	12	5	0.6
	Negative	26	17	
MPV fl	Normal	17	13	0.8
	Increased	15	15	

Discussion:-

There are data consistent with an association between H. pylori infection and ITP (Hashino et al., 2003; Hino et al., 2003; Jargue et al., 2001).

However, due to limitation of self-fund we have failed to include controls group in order to investigate the association between ITP and H. pylori infection in case control study, for this reason we conducted a cross-sequential study to determine the sero-prevalence of H. pylori antibodies and anti-platelets antibodies among Sudanese ITP patients and to compare the characteristics of ITP in H. pylori- infected and non-infected patients.

The study included 60 previously diagnosed ITP patients with 24 month mean duration of diagnosis and 25 $x10^3$ cell/µl mean of platelets counts at diagnosis.

The prevalence of H. pylori infection in adult ITP patients was not found different from that reported in the general healthy population matched for age and geographical area (Liebman and Stasi, 2007). Most studies were conducted in Italy, where the H. pylori rate in the middle-aged adult general population is nearly 50%, (Russo et al., 1999) or Japan, where the prevalence of the infection is greater than 70% (Graham et al., 1994). Using serological tests, Michel et al recorded a low prevalence (29%) of H. pylori infection in 51 adult ITP patients of white French origin; the same rate of infection was found in control subjects (Michel et al., 2002). Determination of H. pylori prevalence among Sudanese population was carried out in small sample size - previous un-published – a study by Khalid and his colleagues in Khartoum University-2009, who were detected H. pylori prevalence of 56% among Sudanese. In

the study of Dino and his colleagues (Dino et al., 2011); investigations for H. pylori had been carried out in 22 ITP patients; 12 (54.5%) patients were positive, with similar mean platelet count at the time of the diagnosis in H. pylori positive and negative patients (9,583.3±3,987.6/µl versus 12,000±9,568.4/µl; p=0.74). In the present study, sero-prevalence of H. pylori IgG antibodies were detected in 63% of included ITP patients; indicating an existence of H. pylori infection in chronic ITP.

Mean of platelets counts at time of diagnosis was varied significantly with the mean at time of enrolling after 24 months mean duration of diagnosis. Furthermore, in ITP treated patients there was a significant difference between platelets count at time of diagnosis (mean: 25 $x10^3$/µl) and at time of enrolling (mean: 111 $x10^3$/µl). The explanation of this due to fact that 57 out of 60 enrolled patients were received corticosteroids treatment. However, a peripheral thrombocytopenia was still detected in 72% of ITP patients at time of enrolling in the study. This phenomenon is strengthening by the fact that the 3 ITP patients who were not received corticosteroids treatment and was positive for anti-H. pylori IgG, anti-platelets, and anti-Lewis antibodies; had a significant difference in platelets count between time of diagnosis and time of enrolling in the study (after mean duration of 7 months).
In H. pylori sero-positive and sero-negative ITP patients; the platelets count was not varied at time of diagnosis, 25 versus 50 $x10^3$/µl.; same finding observed by Dino and his colleagues ((Dino et al., 2011)). At time of enrolling in the study (after mean duration of 24 months); the platelets count varied significantly, mean: 14 versus 145 $x10^3$/µl, this could be of H. pylori infection inducing more cross reacting auto-antibodies against platelets antigens. In this study, anti- platelets antibodies were more frequently co-detected with anti-H. pylori IgG antibodies in 35/38 (92%) of H. pylori sero-positive ITP patients. It has become clear that H. pylori-associated ITP is a subset of ITP in which H. pylori infection is actively involved in the pathogenic process. In patients with H. pylori associated ITP, H. pylori eradication increases the platelet count in parallel with a suppression of anti-platelet autoantibody production, and results in the remission or even cure of the disease in many patients. Since eradicating H. pylori does not increase the platelet count in non-ITP subjects, the platelet recovery after successful H. pylori eradication is specific to ITP patients, and is likely to be mediated through the inhibition of an ongoing autoimmune response to platelets (Matsukawa et al., 2010). On the other hand, anti-Lewis antibodies also were co-detected with anti-H.pylori IgG antibodies in 12/38 (32%) of H. pylori sero-positive ITP patients in present study. In a study of Italian group, the prevalence of the H. pylori cagA gene was significantly higher in patients with ITP than in a control group (Emilia et al., 2007). Other putative targets of molecular mimicry are Lewis (Le) antigens, which are expressed by H. pylori in a strain-specific manner. Le antigens adsorb to platelets and might serve as targets for anti-Le antibodies in patients with an appropriate genetic background (Gerhard et al., 2002).

Another hypothesis suggests that molecular mimicry of cagA or Lewis antigens and platelet antigens may initiate the development of ITP, but with time continued platelet destruction and epitope spreading may lead to the development of chronic thrombocytopenia refractory to eradication of H. pylori infection (Cines, 2007).

Several authors have tried to identify characteristics of H. pylori-associated ITP by comparing the clinical features of adult ITP patients with or without H. pylori infection. ITP patients infected with H. pylori were significantly older than uninfected patients (Emmerich et al., 2007), but this is predictable because the prevalence of H. pylori infection increases with age in the general population (Craig et al., 2010).

Multiple studies have failed to detect significant differences in any other demographic or clinical characteristic, including sex, platelet count, or response to therapy. In this study, comparison between ITP characteristic in H.pylori sero-positive and sero-negative patients was analyzed, there were no significant differences in ITP characteristics in regard to age, gender, bleeding type, Lewis antibodies, and mean platelets volume (MPV). However, there was a significant difference in regard to platelets count, anti-platelets antibodies status, and the response to corticosteroid treatment. Of course, in H. pylori sero-positive ITP patients more frequent (92%) anti-platelets auto-antibodies were detected facilitating the peripheral platelets destruction and leading to reduction in platelets number.

Conclusions:-

Anti -H. pylori and anti-platelets antibodies were more frequent in ITP patients, more reduction in platelet count observed in H. pylori -infected ITP patients than non-infected one. In majority of H. pylori infected ITP patients; thrombocytopenia still persists even in those patients who underwent corticosteroids treatment. H. pylori should be investigated in all cases of chronic un-explained thrombocytopenia, if necessary; H. pylori treatment should be established in chronic thrombocytopenic patient who received corticosteroids or immunoglobulin treatment and not recovered; especially in adults' patients.

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