ORIGINAL ARTICLE

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Chronic immune thrombocytopenia. Egyptian experience

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Abstract

Pathogenesis of thrombocytopenia is heterogeneous and resistance to treatment is a great challenge. In this study, we reviewed the demographic features of thrombocytopenic Egyptian patients. We also analyzed the role of T cell in chronic immune cases. IL-12, IL-35, IL-17, and TGF-β were measured by ELISA. The median age at the time of diagnosis was 30 years and its range was 14–70. The median platelet count at the time of diagnosis was 15×10^9/L. Regarding treatment and follow-up, there was an indication for treatment in 96% of patients. Of the 150 ITP patients who were given first-line therapy (corticosteroid 1 mg/kg/day PO), there was a complete response (CR) in 40.3% while 59.7% patients were nonresponsive to therapy. Forty-five chronic cases fulfilled the criteria for cytokine assay. Comparison between the case group and the control group revealed statistically significant lower platelet count in cases, while the four measured cytokines were statistically significant higher in cases rather than the control. Correlation between the platelet count and the level of cytokines was statistically insignificant. The remission rate in ITP on steroid as first-line therapy is less than 50%. The higher expression of IL-12 and IL-35 in chronic ITP is due to the persistently higher Th1 activity which explains the continuity of the disease, while the higher expression of Treg cytokines (IL-17 and TGF-β) may be explained by effect of immunosuppression or upregulation of the receptors on Treg cells.

Keywords IL-23 · IL-12 · IL-35 · TGF-β · Chronic ITP

Introduction

The pathophysiology of immune thrombocytopenia (ITP) is heterogeneous and complex. The presence of antibodies against platelet glycoproteins has traditionally been considered to play a central role (Consolini et al. 2016).

Many studies in recent years have shown that the abnormalities of T lymphocytes play an important role in the pathogenesis of active ITP (Yang et al. 2014).

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These abnormalities include the increased number of the T helper 1 (Th1) cells (Panitsas et al. 2004), the decreased number or defective suppressive function of regulatory T cells (Yu et al. 2008), and the platelet destruction by cytotoxicity T lymphocytes (CTL) (Zhao et al. 2008). Dysregulated T cells in patients with ITP may also enable the development of platelet autoantibodies, have a direct cytotoxic effect on platelets, and impair platelet production by megakaryocytes (Zhan et al. 2013).

ITP is associated with a Th1-type of T helper cytokine response which secretes IL-12, while that of type Th2 is downregulated. The Th1-type cytokine spectrum returns to the dominant position after treatment with a large dose of dexamethasone. Shifting the cytokine patterns from Th1 to Th2 may be a potential immunotherapy for ITP (Ma et al. 2008).

The imbalance of Th17/Treg toward Th17 cells has been shown to play an important role in the peripheral immune response. However, the role of Th17/Treg in the pathogenesis of ITP still remains uncertain in ITP (Yu et al. 2015).

IL-12 expressed by antigen presenting cells (APC) promotes the generation of pro-inflammatory Th1 cells that are involved in the progress of many autoimmune diseases including ITP (Zhan et al. 2014; Zundler and Neurath 2015).

IL-35 is expressed on Treg cells and is involved in the suppression of an immune response (Sakaguchi 2011; Egwuagu et al. 2015). IL-35 has been shown to possess the potency of inhibiting the CD4+ effector T cells and alleviate autoimmune diseases. (Yang et al. 2014).

TGF-β partially suppresses T cell proliferation, by inhibiting effector T cell differentiation into Th1 or Th2 effector cells through multiple mechanisms (Wan and Flavell 2008).

In this study, we analyzed the demographic characters of 150 cases of ITP and studied the role of T cells in chronic ITP.

Patients and methods

This study was approved by the Research Ethical Committee of Kasr Al Aini Hospital-Cairo University. We reviewed all cases with immune thrombocytopenia admitted between January 2013 and January 2016. Chronic and persistent cases were selected for studying the cytokines. All cases were patients attending the Clinical Hematology Clinic of Internal Medicine Department at Kasr Al Ainy Hospital. The control subjects were workers of the clinic or relatives of the patients. Collection of samples from chronic and persistent cases was between January 2016 and July 2016 after obtaining written consents. Inclusion criteria included age above 17 years and diagnosis of immune thrombocytopenia not less than 3 months. Cases of thrombocytopenia were excluded if they were associated with HCV antibodies and/or HB antigen. ANA and/or anti-DNA, anticardiolipin or lupus anticoagulant positive cases, pregnant females, and cases with splenomegaly were also excluded.

Forty-five cases of chronic and persistent ITP (group I) who fulfilled the criteria and 45 control (group II) were subjected to IL-12, TGF-β, IL-17, and IL-35 measurement using ELISA test.

Statistics

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 23. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data, and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the nonparametric Mann-Whitney test. For comparing categorical data, chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency was less than 5. P values less than 0.05 were considered as statistically significant.

Results

We investigated 150 ITP patients. The median age at the time of diagnosis was 30 years and its range was 14–70. Duration of disease ranged between 1 month and 21 years where the median duration was 2.5 years. The median platelet count at the time of diagnosis was 15×10^9/L where 58 patients (38.8%) had a platelet count $< 10 \times 10^9$/L. Other demographic and laboratory data of patients are shown in Table 1. Regarding treatment and follow-up, there was an indication for treatment in 96% of patients. Of the 150 ITP patients who were given first-line therapy (corticosteroid 1 mg/kg/day PO), there was complete response (CR) in 40.3% while 59.7% patients were nonresponsive to therapy. Patients who had failure of response to first line of therapy were given a second line of therapy and data are demonstrated in Table 2. Chronic and persistent cases that fulfilled the criteria for cytokine assay were 45 cases. Demographic data revealed that among cases, 38 (84.4%) were females and 7 (15.6%) were males with a mean (±SD) age of 31.60 ± 8.78 years. In the control group (group II), 35 patients were females (77.8%) and 10 were males (22.2%) with a mean (±SD) age of 29.29 ± 8.01 years ($P < 0.001$). Comparison between the two studied groups regarding age and sex showed statistically nonsignificant values ($P = 0.274$ and 0.419 respectively). Thirty-two patients presented with skin manifestations (71.1%). Eight patients presented with mucous bleeding (17.8%), and five patients presented with combined skin and mucous membrane bleeding (11.1%). None of the cases had splenomegaly, hepatomegaly, or lymphadenopathy.

Comparison between the two studied groups revealed statistically significant lower platelet count and hemoglobin in

Table 1 Demographic and laboratory data of 150 ITP patients

Item	Number of patients	%
Sex	123 females	82
	27 males	18
Onset	69 acute	45
	66 chronic	44
	15 persistent	10
ANA positivity	21	13.8
Anti-DNA positivity	3	1.8
Antiphospholipid antibody (APL) positivity	7	4.8
HBsAg positivity	Zero	Zero
Anti-HCVab	21	13.8
Helicobacter pylori positivity	24	15.7
Anti-EBV IgM with high titer	4	2.7
Anti-CMV IgM	Zero	Zero
Abnormal thyroid function	10	6.4
Disease onset related to pregnancy	18	12

Table 2 Second-line therapy and % of complete remission (CR)

Type of treatment	Number (%) of patients	CR %
Splenectomy	24 (16.1)	3
Rituximab	30 (20)	60
TPO agonists	5	100
Steroid and azathioprine	68(45)	64
Steroid and azathioprine and danazole	8 (4.8)	66
Vincristine	13 (8.1)	20
H. pylori therapy	5 (3.3)	20

Table 4 Correlation between platelet count and cytokines

Cytokine	Correlation coefficient	P value
IL-35	0.15	0.3
IL-12	−0.13	0.4
TGF-β	−0.26	0.13
IL-17	0.06	0.6

cases rather than the control. The four measured cytokines (IL-17, IL-35, IL-12, and TGF-β) were significantly higher in cases rather than the control (Table 3). The correlation between platelet count and the level of cytokines was statistically insignificant in cases (Table 4).

Discussion

The remission rate under first therapy by corticosteroid in our patients was 40% and median age for presentation was 30 years. Eighty percent of cases were female. Our young age of presentation is similar to those in Asia but lower than Europe as seen. We have higher female percentage while all following studies have no sex prevalence.

Sultan et al. studied 417 patients and mean age was 41 years, with no female predominance. Secondary causes were in 65% (Sultan et al. 2016).

Zulfiqar AA et al. studied 41 patients with mean age 77 years with no sex prevalence. Response to first-line therapy was 39% (Zulfiqar et al. 2016).

Grimaldi-Bensouda L et al. studied 143 patients and median age was 48 years. Twenty-five were secondary with ANA positive results and response to first line was 37% (Grimaldi-Bensouda et al. 2016).

Dayama A et al. studied 100 patients with mean age 18 years and found 21% were secondary to anticardiolipin Abs and response to primary therapy was 48% (Dayama et al. 2016).

Weide R et al. studied 400 patients and found mean age was 55 years and response to primary therapy was 72% (Weide et al. 2016).

Koylu A et al. studied 260 patients with mean age 43 years and found secondary causes were positive in 40% and response to primary therapy was 76% (Koylu et al. 2015).

On second-line response complete remission was found on TPO antagonist, over 60% on rituximab. This is because both increase the levels of Tregs and TGF-β. The A-TPOs also increase Breg levels, which could explain why complete remission has been seen in some cases (Perera and Garrido 2017).

We studied T cell cytokines in chronic primary ITP cases resistant to first-line therapy and found IL-12, TGF-β, IL-17, and IL-35 were statistically significant higher than the normal control. However, no correlation was found between platelet count and any of cytokines examined.

These results are consistent with Huang et al. 2011 who studied 30 patients with chronic ITP and 15 healthy volunteers. Serum IL-12 levels of chronic ITP patients were significantly higher than that of normal controls ($P < 0.01$). They concluded that the imbalance of T cell subsets in ITP patients was mainly associated with IL-12 (Zhan et al. 2014).

Also, *Li* et al. studied 46 patients with ITP as well as 22 healthy controls. They found higher levels of IL-12 in patients than in controls. A markedly higher level of IL-12 was found in patients with chronic ITP than in patients with acute ITP with negative correlation to platelet count (Li et al. 2015).

Table 3 The comparison between the two groups regarding laboratory parameters

Item	Cases (group I)				Control (group II)				P value
	Mean	SD	Median	Range	Mean	SD	Median	Range	
Hb (g/dl)	11.47	2.12	11.60	6.10–16.60	13.18	1.15	13.00	11–16	< 0.001
WBCs (× 10⁹/L)	8.30	2.56	8.00	2.20–14.30	7.28	1.71	7.10	3.20–10.5	0.069
Plt (× 10⁹/L)	50.38	33.12	67.00	3.00–.100.00	278.82	78.61	263.00	150–410	< 0.001
IL-12 (ng/ml)	26.5	9.4	23.7	11.9–49	5.12	2.74	3.8	1.1–12.3	< 0.001
IL-35 (ng/ml)	5.1	2.14	5.8	1–11.5	2.89	1.74	2.6	0.96–11.7	< 0.001
TGF-β (ng/ml)	0.77	0.2	0.8	0.33–1.2	0.2	0.19	0.15	0.02–1.15	0.000
IL-17 (ng/ml)	0.42	0.1	0.41	0.28–0.9	0.15	0.09	0.14	0.02–0.37	0.000

Other studies found that IL-17 increased with T helper 17 in both recent and reactive ITP (Ye et al. 2015; Li et al. 2016); however, IL-17 was downregulated after the treatment with immunosuppressive therapy (Dayama et al. 2016). This was not consistent with our results and *Huang* et al. who found IL-17 to be the same as normal controls (Huang et al. 2011).

Regarding the levels of serum IL-35, our results revealed that it was significantly higher in cases more than controls (P value < 0.001).

Our results were different from *Yang* et al. who studied 56 adult primary ITP patients. Significantly lower plasma IL-35 levels were found in active ITP patients compared with those in remission ($P = 0.017$) and the healthy controls ($P < 0.001$). In active ITP patients, the plasma IL-35 levels displayed a significantly positive correlation with platelet counts ($r = 0.5335$, $P < 0.0008$) (Yang et al. 2014).

The discrepancy between our results and *Yang* et al. could be attributed to the effect of treatment the patients received as many studies revealed that the Tregs are affected by corticosteroid intake. One of these studies was carried by *Li* et al. [23] who studied 38 patients with chronic ITP and 36 matched healthy controls. The plasma levels of IL-35 in chronic ITP patients and healthy controls were also determined. The levels of IL-35 were considerably lower in the patients than in the controls before high dose dexamethasone treatment ($P < 0.01$) and had increased significantly ($P < 0.01$) after the treatment. There were no significant differences in the levels of the above cytokines between the post treatment patients and the normal controls ($P > 0.05$).

Regarding the levels of serum transforming growth factor (TGF-β) levels, our results revealed that it was significantly higher in cases rather than controls (P value < 0.001).

Huang et al. found that TGF-β level in the peripheral blood of newly diagnosed patients was lower than that in normal controls, but increased after treatment and was significantly higher than that in newly diagnosed patients. The level of TGF-β was higher in those responded to treatment (Huang et al. 2015).

The serum levels of TGF-β level were significantly decreased in patients in *Li W* et al.'s study. However, after the treatment by immunosuppressive therapy, TGF-β was downregulated (Li et al. 2016) which is not consistent with our results.

Conclusion

The remission rate in ITP on steroid as first-line therapy is less than 50%. The higher expression of IL-12 and IL-35 in chronic ITP is due to the persistently higher Th1 activity which explains the continuity of the disease, while the higher expression of Treg cytokines (IL-17 and TGF-β) may be explained

by effect of immunosuppression or upregulation of the receptors on Treg cells.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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