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*RESEARCH ARTICLE*

# IMMUNE THROMBOCYTOPENIA. EGYPTIAN EXPERIENCE WITH STUDY OF IL-17, TGFB, CYTOKINES IN CHRONIC AND PERSISTENT IMMUNE THROMBOCYTOPENIA PATIENTS.

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## *Abstract*

**Background:** Autoimmune diseases are characterized by various factors that contribute to a breakdown in self tolerance. In this study we review the demographic features of 150 immune thrombocytopenic Egyptian patients and for cases who were chronic and persistent with negative both autoimmune screen and virology for hepatitis B and C, we measured IL-17 and TGF-β by ELISA. **Results:** Our results revealed Chronic and persistent cases who fulfilled the criteria for cytokine assay were 45 cases with a mean (± SD) age of 31.60 ± 8.78 years. Thirty two patients were presented by skin manifestations (71.1%).Eight patients presented with mucous bleeding (17.8%) and five patients presented by combined skin and mucous membrane bleeding (11.1%). Comparison between the cases studied and control groups revealed statistically significant lower platelet count in cases rather than the control. While the two measured cytokines were statistically significant higher in cases rather than the control. Correlation between platelet count and the level of cytokines was statistically insignificant. All cases were under treatment by low dose corticosteroid in addition to another immunesuppression medication. **Conclusion:** the higher expression of Treg cytokines (IL-17 and TGF-B) may be explained by effect of immune suppression use or up regulation of their receptors on Treg cells which have resistance to their activity.



## 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. **[1]**

Many studies in recent years have shown that the abnormities of T lymphocyte play an important role in the pathogenesis of active ITP.**[2]**

These abnormalities include the increased number of the T helper 1(Th1) cells **[3],** the decreased number or defective suppressive function of regulatory T cells **[4],** and the platelet destruction by cytotoxicity T lymphocytes

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(CTL) **[5].** 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**.[6].**

ITP is associated with a Th1 type of T helper cytokine response which secretes IL-12, while that of type Th2 is down regulated. Treatment with a large dose of dexamethasone, the Th1-type cytokine spectrum returns to the dominant position after treatment with dexamethasone. Shifting the cytokine patterns from Th1 to Th2 may be a potential immunotherapy for ITP. **[7]**

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 remain uncertain in ITP. **[8]**

TGF-β partially suppresses T-cell proliferation, by inhibiting effector T-cell differentiation into Th1 or Th2 effector cells through multiple mechanisms. **[9]**

In this study we analyzed the demographic characters of 150 cases of ITP and then measured IL-17 and TGF-B in chronic and persistent cases to compare our results with previous studies.

**Patients and methods:-**

This study was approved by Research Ethical Committee of Beni-Suef university Hospital-Beni-Suef University. We reviewed all cases of immune thrombocytopenia admitted between Jan 2013 till Jan 2016. Chronic and persistent cases were selected for studying cytokins.All cases were patients attending the Clinical Haematology Clinic of Internal Medicine Department at Beni- Suef Hospital . The control subjects were workers of the clinic or relatives to the patients. Collection of samples from chronic and persistent cases was between Jan 2016 till July 2016. Collection of samples began after obtaining written consent. Inclusion criteria included age above 17 years and diagnosis of immune thrombocytopenia not less than 3 months. Cases of thrombocytopenia excluded if associated with HCV antibodies and/ or HBs antigen. Cases of splenomegaly, ANA and / or anti DNA, anticardiolipin or lupus anticoagulant positive cases and pregnant females 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 TGF-b and IL-17 were measured 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 non-parametric Mann-Whitney test. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. P-values less than 0.05 were considered as statistically significant.

## Results:-

We had 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-21 years) where the median duration was 2.5 years. The median platelet count at the time of diagnosis was 15,000/ $mm^3$ where 58 patients (38.8%) had a platelet count <10,000/ $mm^3$ Other demographic and laboratory data of patients are 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 1mg/kg/day PO), there was complete response (CR) in 40.3% and 59.7 % patients were nonresponsive to therapy. Patients who had failure of response to 1st line of therapy were given a 2nd line of therapy and data are seen in table II. Chronic and persistent cases who fulfilled the criteria for cytokine assay were 45 cases. Demographic data revealed that among cases 38 (84.4 %) of patients were females and 7 (15.6 %) of patients were males with a mean (± SD) age of 31.60 ± 8.78 years. In control (group II), 35 (77.8 %) of patients were females and 10 (22.2%) of patients were males with a mean (± SD) age of 29.29 ± 8.01 years (P value <0.001). Comparison between the two studied groups regarding age and sex showed statistically non-significant values (P-values 0.274 and 0.419 respectively). Thirty two patients were presented by skin manifestations (71.1%).Eight patients presented with mucous bleeding (17.8%) Five patients presented by combined skin and mucous membrane bleeding (11.1%).None of cases had splenomegaly, hepatomegaly or lymphadenopathy. Comparison between the two studied groups revealed statistically significant lower platelet count and haemoglobin in cases rather than the

control. While the two measured cytokines (IL-17, and TGF-b) were statistically significant higher in cases rather than the control as shown in table III. Correlation between platelet count and the level of cytokines is shown in table IV was statistically insignificant in cases.

## Discussion:-

This study aims to assess level of T cell cytokines (IL-17, TGF-b, ) in chronic and persistent ITP who were 45 cases comparing our results with other studies. Chronic ITP was reported in 32 patients (71.1%) while persistent ITP was reported in 13 patients (28.9%). All cytokine levels were statistically significant higher in cases rather than control. Regarding IL-17, it was found increased with T helper 17 in both recent and reactive ITP**.[10]** and After the treatment with immunosuppressive therapy, IL-17 was down-regulated**.[11]**

While in chronic ITP, Hunag et al found IL-17 to be same to that of normal controls. **[12]** while we found level to be higher and this prove the role of IL-17 in chronic ITP as well as acute and reactive form.

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

The TGF-β level in peripheral blood of newly-diagnosed patients was lower than that in normal controls, and increased after treatment and was significantly higher than that in newly diagnosed patients and the level where higher in those responded to treatment**.[13]**

The serum levels of TGFβ level was significantly decreased in Li W et al. However, after the treatment by immunosuppressive therapy, TGFβ was down-regulated**[14]** which is not consistent with our results.

Corticosteroids, rituximab, and thrombopoietin receptor agonists (A-TPOs) all increase levels of Tregs and TGF-β. The A-TPOs also increase Treg levels, which could explain why complete remission has been seen in some cases**.[15]**

## Conclusion:-

The higher expression of Treg cytokines (IL-17 and TGF-B) may be explained by effect of immune suppression use or up regulation of their receptors on Treg cells which have resistance to their activity.

**We declare no conflict of interest.**
**We are thankful to all workers in the haematology clinic of Beni-Suef university Hospital**.

| Item | Number of patients | % |
|---|---|---|
| Sex | 123 Females<br>27 males | 82<br>18 |
| Onset | 69 acute<br>66 chronic<br>15 persistent | 45<br>44<br>10 |
| ANA positivity | 21 | 13.8 |
| Anti DNA positivity | 3 | 1.8 |
| Antiphospholipid antibodies (APL) positivity | 7 | 4.8 |
| HBsAg positivity | zero | zero |
| Anti HCVab | 21 | 13.8 |
| H pyelori 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 1:-** Demographic and laboratory data of 150 ITP patients

| 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 pyelori therapy | 5 (3.3) | 20 |

**Table II:-** second line therapy and % of complete remission (CR)

| Item | Cases Group I) | | | | Control (Group II) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Median | Range | Mean | SD | Median | Range | P value |
| Hb g/dl | 11.47 | 2.12 | 11.60 | 6.10- 16.60 | 13.18 | 1.15 | 13.00 | 11-16 | < 0.001 |
| WBCs(x $10^9$/L) | 8.30 | 2.56 | 8.00 | 2.20- 14.30 | 7.28 | 1.71 | 7.10 | 3.20-10.5 | 0.069 |
| Plt(x $10^9$/L) | 50.38 | 33.12 | 67.00 | 3.00-.100.00 | 278.82 | 78.61 | 263.00 | 150 -410 | < 0.001 |
| TGF-B(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 |

**Table 3:-** shows the comparison between the two groups regarding laboratory parameters.

| Cytokine | Correlation coefficient | P value |
|---|---|---|
| TGF-B | -0.26 | 0.13 |
| IL-17 | 0.06 | 0.6 |

**Table 4:-** correlation between platelet count and cytokines

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