# CXCL10 (TH1) Chemokine Expression in Patients with Immune Thrombocytopenia

*Thesis*

*Submitted for Partial Fulfillment of M.Sc. Degree*

*In Clinical and Chemical Pathology*

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*Ain Shams University*

*2012*

# دراسة ظهور سي اكس سي ال ١٠ كيموكايين (Th1) فى مرضى نقص صفائح الدم المناعى

رسالة

توطئة للحصول على درجة الماجستير

فى الباثولوجيا الاكلينكية والكيميائية


مقدمة من

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# Acknowledgment

*First of all, I would like to express my deep gratitude to* **ALLAH** *for his care and generosity throughout my life.*

*I would like to express my sincere appreciation to* **Prof. Nahela Ahmed Shalaby,** *Professor of Clinical and Chemical Pathology, Ain Shams University for her keen supervision and guidance and her overwhelming support that has been of great help throughout this work.*

*I am very thankful to* **Dr. Mona Ahmed Ismail,** *Assistant Professor of Clinical and Chemical Pathology, Ain Shams University for her great support & effort throughout the whole work.*

*I would also like to express my great thanks to* **Dr. Doaa Ahmed Gamal Eissa,** *Lecturer of Clinical and Chemical Pathology, Ain Shams University for the great effort she has done in this work and for helping me through it.*

*Finally, I would like to esxpress my deep thanks to* ***my beloved family,*** *for always being there for me.*

***Petra Wadie***

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# List of Abbreviations

| Abb. | Full term |
|---|---|
| ***AA*** | *Arachidonic Acid* |
| ***ABO*** | *Abo blood grouping system* |
| ***ACA*** | *Adrenal cortical Autoantibodies* |
| ***AD*** | *Addison disease.* |
| ***ADP*** | *adenosine diphosphate* |
| ***AITD*** | *Autoimmune Thyroid Disorders* |
| ***AITP*** | *Autoimmune Thrombocytopenic Purpura* |
| ***ANA*** | *Antinuclear Antibodies* |
| ***APS*** | *Autoimmune Polyglandular Syndrome* |
| ***ATIII*** | *Antithrombin III* |
| ***B.M*** | *Bone Marrow* |
| ***cAMP*** | *Cyclic Adenosine Monophosphate* |
| ***CBC*** | *Complete blood count* |
| ***CLL*** | *Chronic Lymphocytic Leukemia* |
| ***CMV*** | *Cytomegalovirus* |
| ***COPD*** | *Chronic Obstructive pulmonary Disease* |
| ***COX-1*** | *Cyclooxygenase -1* |
| ***CSF*** | *Cerebrospinal fluid* |
| ***CV*** | *Coefficients of Variation* |
| ***DIC*** | *Disseminated Intravascular Coagulation* |
| ***DITP*** | *Drug-induced Thrombocytopenia* |
| ***DMS*** | *Demarcation Membranes System* |
| ***DTS*** | *Dense Tubular System* |
| ***EDTA*** | *Ethylene diaminetetra acetic acid* |
| ***ELISA*** | *Enzyme-Linked Immunosorbent Assay* |
| ***ELR*** | *Glutamic acid-leucine-arginine* |
| ***FACS*** | *Fluorescence Activated Cell Scatter Analysis* |

| | |
|---|---|
| ***FDPs*** | *Fibrinogen Degradation Products* |
| ***FMAIT*** | *Feto-maternal Alloimmune Thrombocytopenia* |
| ***GADAb*** | *Anti-glutamic acid decarboxylase autoantibody* |
| ***GDP*** | *Guanosine diphosphate* |
| ***GM-CSF*** | *Granulocyte- Macrophage Colony Stimulating Factor* |
| ***GM-CSF*** | *Granulocyte- Macrophage Colony Stimulating Factor* |
| ***GO*** | *Graves' ophthalmopathy* |
| ***GP*** | *Glycoproteins* |
| ***GTP*** | *Guanosinetriphosphate* |
| ***HCV*** | *Hepatitis C Virus* |
| ***Hge*** | *Hemorrhage* |
| ***HIT*** | *Heparin-induced Thrombocytopenia* |
| ***HIV*** | *Human Immunodeficiency Virus* |
| ***HLA*** | *Human Leukocyte Antigen* |
| ***HPA*** | *Human platelet antigen* |
| ***IDDM,*** | *Insulin-Dependent (type 1) Diabetes Mellitus* |
| ***IFN*** | *Interferon* |
| ***IgG*** | *Immunoglobulin G* |
| ***IP-10*** | *IFN-γ-induced Protein 10;* |
| ***ITP*** | *Immune Thrombocytopenic Purpura* |
| ***IVIgG*** | *Intravenous immunoglobulin G* |
| ***LADA*** | *Latent autoimmune Diabetes in Adults* |
| ***LAMP*** | *Lysosomal associated membrane protein* |
| ***MCS-F*** | *Macrophage Colony Stimulating Factor* |
| ***MPS*** | *MyeloprolifrativeSyndromes* |
| ***MPV*** | *Mean Platelet Volume* |
| ***MQ*** | *Macrophages* |
| ***MS*** | *Multiple Sclerosis* |
| ***NC-IUPHAR*** | *Nomenclature Committee of the International Union of Pharmacology* |
| ***OA*** | *Osteoarthritis* |

| | |
|---|---|
| ***PAF*** | *Platelet Activating Factor* |
| ***PBL*** | *Peripheral Blood Lymphocytes* |
| ***PBMNC*** | *Peripheral Blood Mononuclear Cells* |
| ***PCR*** | *Polymerase Chain Reaction* |
| ***PF4*** | *Platelet Factor 4* |
| ***PG*** | *Prostaglandins* |
| ***PS*** | *Phosphatidylserine* |
| ***PTP*** | *Post Transfusion Purpura* |
| ***RA*** | *Rheumatoid Arthritis* |
| ***RBC*** | *Red Blood Cell* |
| ***RES*** | *Reticuloendothelial System* |
| ***ROC*** | *Receiver operating characteristic curve* |
| ***SC*** | *Subcutaneous* |
| ***SCCS*** | *Surface-Connected Canalicular System* |
| ***SCF*** | *Stem Cell Factor* |
| ***SCF*** | *Stem Cell Factor* |
| ***TA2*** | *Thromboxane A2* |
| ***TB*** | *Tuberculosis* |
| ***Th1*** | *Type 1 Th* |
| ***Th2*** | *Type 2 Th* |
| ***TTP*** | *Thrombotic Thrombocytopenic Purpura* |
| ***UL- vWF*** | *Ultra large von Willebrand factor* |
| ***VEGF*** | *Vascular Endothelial Growth Factor* |
| ***vWF*** | *von Willebrand factor* |

# INTRODUCTION

Immune thrombocytopenic purpura (ITP) is an organ specific autoimmune disorder characterized by increased platelet clearance through the production of autoantibodies against antigens on the platelets membranes, resulting in enhanced Fc–mediated destruction of the platelets by macrophages in the reticuloendothelial system ***(Cines and Blanchette, 2002).***

The etiology of ITP remains unclear, but it is widely accepted that multi dysfunction of immune system contributes to the development of that disease such as complement mediated lysis, ineffective thrombopoiesis or direct T cell cytotoxicity ***(Olsson et al., 2003).*** Dysfunctional cellular immunity is considered important in ITP pathophysiology. The balance between Th 1and Th 2 subsets is dependent on the level of the cytokines at the site of immune activation. Several studies have reported evidence supporting a type – 1 cytokine polarization and high Th1/Th2 ratio of the immune response in ITP ***(Panitsas et al., 2004).*** The autoimmune process is believed to be sited in the spleen; memory platelet-specific T cells are released into the peripheral circulation ***(Kaushansky, 2002).***

The chemokines are small proteins that induce chemotactic activities in various types of leukocytes and

regulate their trafficking through the appropriate chemokine receptors expressed on these cells ***(Zlotnik et al., 2000).*** In addition to their role in cell recruitment, chemokines have a specific role in immunoregulatory activities such as Th1/Th2 cell induction ***(Nakajjma et al., 2004).***

CXCL 10, also called interferon gamma inducible protein 10, is the prototype of the CXC family; has chemotactic activity mainly for activated Th1 cells and is involved in the pathogenesis of various Th1 dominant autoimmune diseases. CXCL 10 elicits its effects by binding to the cell surface CXCR3 ***(Lee et al., 2009).***

Baseline pretreatment plasma levels of CXCL 10 are elevated in patients with many autoimmune diseases e.g.; Graves' disease, SLE, type I DM ***(Lit et al., 2006).*** CXCL10 has therefore gained much attention in autoimmune ITP which is associated with a Th1 type immune response. However, its preclinical characteristics and clinical applicability in such disease are still demanding further elucidation.

# AIM OF THE WORK

The aim of this study is to evaluate the plasma level of CXCL10/IP10 in patients with immune thrombocytopenia, and investigate its role in the diagnosis of such cases as well as discuss its preliminary role in the pathogenesis of this disease.

# PLATELET FORMATION AND PHYSIOLOGY

Platelets play a fundamental role in haemostasis and are a natural source of growth factors. They circulate in the blood and are involved in hemostasis, leading to the formation of blood clots. Dysfunction or low levels of platelets predisposes to bleeding, while high levels, although usually asymptomatic, may increase the risk of thrombosis ***(Sunitha and Munirathnam, 2008).***

## I- Platelet Morphology:

Platelets are extremely small and discoid anucleated cells 2 to 3μm in diameter. They circulate at a concentration of 150.000-400.000 cells/μl blood with a mean volume of 7-11 fL (Fig 1&.2 a, b). The glycoprotein of the surface coat are particularly important for the platelet interactions of adhesion and aggregation which is the initial events leading to initial plug formation during haemostasis ***(Hoffbrand et al., 2006).***

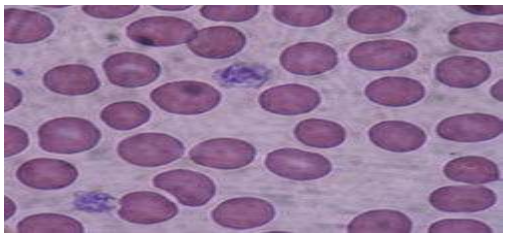

**Figure (1):** Light microscopy of Wright-stained smear reveals platelets as small, anucleate fragments with occasional reddish granule ***(Gawaz, 2001).***

**Figure (2a):** Electron microscope showing resting platelets as discoid form ***(Gawaz, 2001).***

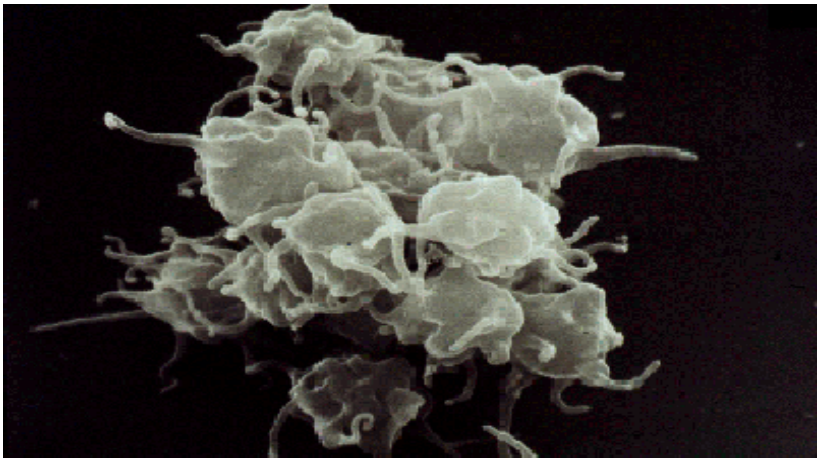

**Figure (2b):** Activated platelets showing pseudopodia emission (x5, 000) ***(Gawaz, 2001).***