Expression of IL-4 (VNTR intron3) and IL-10 (-627) genes Polymorphism in childhood Immune Thrombocytopenic purpura

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Abstract

- **Background:**

Immune thrombocytopenic purpura (ITP) is an acquired autoimmune disorder caused by the production of anti-platelet antibodies. These autoantibodies opsonize platelets for splenic clearance, resulting in low levels of circulating platelets.

Aim:

The current case-control study aimed at detecting the frequency of IL-4 (VNTR intron 3) and IL-10 (-627) genes polymorphism in Egyptian children with ITP as genetic markers for ITP risk, and to clear out their possible role in the pathogenesis of ITP.

- **Materials and Methods:**

IL-4 (VNTR intron 3) and IL-10 (-627) genes polymorphisms were studied in 70 ITP patients and 50 healthy controls by PCR amplification of the target gene followed by allele specific restriction enzyme digestion (RFLP technique). Odds ratios (ORs) along with their 95% confidence intervals (CIs) were computed to compare the distribution of alleles and genotypes between cases and controls.

- **Results:**

More frequent IL-4 RP2 allele and IL-10 A allele among ITP patients than controls. A statistical significant difference between acute and chronic ITP patients as regard IL-10 and IL-4 gene polymorphism distribution with higher A allele and RP2 allele among chronic ITP patients versus acute ITP patients.

Furthermore, combined polymorphism of IL-4 and IL-10 genes was associated with much more increased risk of ITP development.

- **Conclusion:**

Our study suggests the possibility that IL-4 and IL-10 genes polymorphism may contribute to the susceptibility of development of ITP in Egyptian children.

- **Key words:**

ITP, Childhood ITP, Immune thrombocytopenia, IL-4 (VNTR intron 3), IL-10 (-627), Polymorphism.

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List of Abbreviations

aa	:	Amino acid
ALL	:	Acute lymphocytic leukemia
ALPS	:	Autoimmune lymphoproliferative syndrome
ARDS	:	Adult respiratory distress syndrome
APCs	:	Antigen presenting cells
Apls	:	Anti phospholipids antibodies
APS	:	Anti phoshpholipid syndrome
ASH	:	American society of Hematology
BMA	:	Bone marrow aspirate
Bp	:	Base pair
BAFF-R	:	B cell activating factor receptor
Cat	:	Catalog number
CBC	:	Complete blood count
CD	:	Crohn's disease
CRP	:	C reactive protein
C		cytosine
CD	:	Cluster of differentiation
CTL	:	Cytotoxic T lymphocyte
CSIF	:	Cytokine synthesis inhibitor factor
CD40 L	:	Cluster of differentiation 40-ligand
CI	:	Confidence interval
cITP	:	chronic ITP
CVID	:	Common Variable Immune Deficiency
DCs	:	Dendritic cells
DNA	:	Deoxyribonucleic acid
dNTPs	:	Deoxynucleotide triphosphates
EBV	:	Ebstein bar virus
EDTA	:	Ethylene diamine tetra acetic acid
FNIII	:	Fibronectin type III
FcγR	:	Fc gamma receptor
GBS	:	Guillain-Barré syndrome
G-CSF	:	Granulcoytoe colony stimulating factor
GM-CSF	:	Granulocyte monocyte colony stimulating factor
GC	:	Glucocorticoids
GR	:	Glucocorticoids receptor
γC	:	Gamma common
GVHD	:	Graft versus host disease
GpI	:	Glycosylphosphatidylinositol
H.plyori	:	Helicobacter pylori

hIL	:	Human interleukin
Hb	:	hemoglobin
HBV	:	Hepatitis B virus
HCV	:	Hepatitis C virus
HIV	:	Human Immune –deficiency Virus
IBD	:	Irritable bowl disease
ICAM-1	:	Intracellular adhesion molecule -1
IFNγ	:	Interferon gamma
IL	:	interleukin
IL-1 ra	:	Interleukin 1 receptor alpha
IgA	:	Immune globulin A
IgG	:	Immune globulin G
IgM	:	Immune globulin M
Ig E	:	Immune globulin E
ITP	:	Immune Thrombocytopenic Purpura
IVIG	:	Intravenous immunogolbulin
IRS-1	:	Insulin receptor substrate 1
JAK	:	Janus kinase
Kd	:	Kilodalton
Kb	:	Kilo bytes
LFA-1	:	Lymphocyte function-associated antigen-1
MW	:	Molecular weight
MS	:	Multiple sclerosis
MIP1α	:	Macrophage inflammatory protein 1 alpha
M1	:	Macrophage 1
mRNA	:	Messenger ribonucleic acid
mAb	:	Monoclonal antibody
MHC	:	Major histocompatibility
NA	:	Nucleic acid
NK	:	Natural killer cells
NF-KB	:	Nuclear factor kappa beta
OR	:	Odds ratio
P value	:	Probability value
PCR	:	Polymerase chain reaction
PTPN22	:	Protein tyrosine phosphatase non receptor type 22
PRP	:	Platelet rich plasma
PEG-rhMGDF	:	Pegylated recombinant human megakaryocyte growth and development factor
PI3k	:	Phosphoinositide 3 kinase
PFA	:	paraformaldehyde
PBS	:	Phosphate buffered saline
PIFT	:	Platelet immunofluorescense test

RA	:	Rheumatoid arthritis
RBCs	:	Red blood cells
RE	:	Restriction enzyme
RFLP	:	Restriction fragment length polymorphism
rhTPO	:	Recombinant human thromopoietin
SD	:	Standard deviation
SLE	:	Systemic Lupus Erythematosus
SNP	:	Single nucleotide polymorphism
SOCS3	:	Suppressor of cytokine signalling 3
STAT	:	Signal transducer and activator of transcription
T	:	thymine
TAR	:	Thrombocytopenia with absent radii syndrome
TAP1	:	transporter associated with antigen processing 1
TBE	:	Tris-borate EDTA
Tc	:	T cytotoxic
TCR	:	T cell receptor
Th1/Th2	:	T helper
TLC	:	Total leucocytic count
TNFα	:	Tumor necrosis factor alpha
TGFβ	:	Transforming growth factor beta
Tyr	:	tyrosine
TPO	:	Thrombopoietin
TNFRSF	:	Tumor necrosis factor receptor superfamily
UV	:	Ultra violet
VNTR	:	Variable number tandem repeats

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INTRODUCTION AND AIM OF THE WORK

Immune thrombocytopenic purpura (**ITP**), also known as idiopathic thrombocytopenic purpura, is an immune-mediated acquired disease of adults and children characterized by transient or persistent decrease of the platelet count and depending upon the degree of thrombocytopenia, increased risk of bleeding ***(Cooper and Bussel , 2006)***. In most children and some adults, ITP is an acute, self limited disease that resolves or improves spontaneously within months. In a small number of children and in many adults, ITP may be chronic and poorly responsive to treatment ***(Psaila and Bussel, 2007)***.

ITP may be classified as acute or chronic (i.e. thrombocytopenia of less or more than 6 months in duration). This distinction suggests two different clinical courses that may be pathophysiologically distinct, and may warrant different treatment approaches ***(Kuhne, 2006)***.

ITP has been documented to be associated with cytokine response and dysregulation in the cytokine network. Genetic factors have been reported to be associated with ITP. Single nucleotide polymorphisms are the most abundant types of DNA sequence variation in established method for analyzing complex gene-associated diseases. Gene polymorphisms, including those in cytokine genes, have been reported to be associated with adult and childhood ITP ***(Wu et al., 2007)***

The cytokine genes are polymorphic, which accounts for the different levels of cytokine production and are related to regulation of the immune-mediated pathogenetic process. Cytokine gene polymorphisms have recently attracted considerable interest because distinct alleles of cytokine genes have been discovered to be associated with different immunoinflammatory diseases ***(Lin et al., 2003)***.

IL-4 is a highly pleiotropic cytokine that is able to influence T helper cell differentiation. Early secretion of IL-4 leads to polarization of T helper cell differentiation toward T helper 2-like cells. The T helper 2-

cell secretion of IL-4 and IL-10 leads to the suppression of Th1 responses by down-regulating the production of macrophage-derived IL-1 and inhibiting the differentiation of T helper 1-type cells. IL-4 is a key cytokine that induces activation and differentiation of B cells as well as development of T helper subset of lymphocytes. IL-4 gene has a 70-bp variable number of tandem repeats (VNTR) polymorphism in intron3 associated with IL-4 production ***(Wu et al, 2003).***

IL-10 is the most important anti-inflammatory cytokine found within the human immune response. It is a potent inhibitor of Th1 cytokines, including both IL-2 and IFN-γ. This activity accounts for its initial designation as cytokine synthesis inhibition factor; it is mainly produced by macrophages, monocytes, T cells, B cells, dendritic cells, mast cells and eosinophils. It also limits the inflammatory responses and regulates the differentiation and proliferation of several immune cells such as T cells, B cells, natural killer cells, antigen-presenting cells and mast Cells. The gene encoding IL-10 has been identified on chromosome 1q31–32 ***(Nunez et al., 2006).***

Carcao et al, 2003 reported that IL-4 VNTR intron 3, and IL-10 (-627) polymorphisms were associated with the pathogenesis of ITP and contributed to the susceptibility of developing ITP.

Hypothesis

Detection and study of Cytokines gene polymorphism (IL-4 & IL-10) in ITP patients may play a fundamental role in pathogenesis of childhood immune thrombocytopenic purpura and this might be the base for future specific immunomodulatory therapies for ITP as well as providing a tool for early diagnosis of susceptibility to develop ITP.

Aim of the work

The present work aims to study the expression of IL-4 and IL-10 genes polymorphism by PCR-RFLP in childhood immune thrombocytopenic purpura patients.

CHAPTER I

Immune Thrombocytopenic Purpura (ITP)

Introduction:

Immune thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by a low circulating platelet count caused by destruction of antibody-sensitized platelets in the reticuloendothelial system. ITP can be classified based on patient age (childhood versus adult), duration of illness (acute versus chronic), and presence of an underlying disorder (primary versus secondary). Persistence of thrombocytopenia, generally defined as a platelet count of less than 150 x $10^3/cm^3$ for longer than 6 months, defines the chronic form of the disorder ***(Cuker & Cines, 2010)***. Secondary causes of ITP include collagen vascular disorders, such as systemic lupus erythematosus (SLE); immune deficiencies, such as common variable immunodeficiency (CVID); and some chronic infections as HIV and hepatitis C ***(Blanchette & Bolton, 2008).***

Pathophysiology

The pathophysiology of ITP is a complex process with involvement of many players in the human immune orchestera including antibodies, cytokines, antigen-presenting cells, costimulatory molecules, and T and B lymphocytes (including T-helper, T-cytotoxic, and T-regulatory lymphocytes) ***(Blanchette & Bolton, 2008)***.

Immune thrombocytopenic purpura (ITP) is characterized by autoantibody mediated platelet destruction. These autoantibodies opsonize platelets for splenic clearance, resulting in low levels of

circulating platelets ***(Cuker & Cines, 2010).*** Recent studies paint a broader picture of immune dysregulation leading not only to accelerated platelet destruction, but to abnormalities in megakaryocyte growth and development and poorly compensated thrombopoiesis. These findings open new avenues of possibilities in the management of thrombocytopenia ***(Gernsheimer, 2009).***

The underlying defects and the triggering events leading to autoantibody production are unknown, but continued research is providing new insights into the underlying immunopathogenic processes as well as the cellular and molecular mechanisms involved in megakaryocytopoiesis and platelet turnover. Heritability is uncommon, although predisposing polymorphisms in cytokines and Fcγ receptors have been described ***(Kuwana & Ikeda, 2005, Cuker & Cines, 2010).***

Viral infection can induce the autoimmune diseases including ITP. Probably most of acute and / or chronic ITP may be caused by molecular mimicry, and antibodies formed in response to viral infection may cross-react with antigenic targets naturally present on platelets ***(Fujita, 2003)***.

The natural course of ITP in infants is poorly described in the literature. Several features distinguished infants from older children with ITP:

1- Higher male / female ratio which was reported by **Lee et al. (2002).**

2- Less frequent occurrence of infection before ITP than in children 1 to 10 years of age, which may be due to less contact with other children.

3- Less frequent occurrence of chronic ITP ***(Kuhne et al., 2003)***.

Immune thrombocytopenia (ITP) is a haematological disorder in which patients predominantly develop skin and mucosal bleeding**.** Early studies suggested ITP was primarily due to immune-mediated peripheral

platelet destruction ***(Nugent et al., 2009)***, and defective immune complex clearance, perhaps mediated by low-affinity Fcγ receptors. ATh1/Th0 cytokine profile, a reduction in suppressor T-regulatory cells, and an increase in B-cell-activating factor may predispose to emergence of autoantibodies in response to exogenous antigens. Molecular mimicry appears to play a role in the development of self-reactive platelet antibodies after vaccination and certain viral infections ***(Cuker & Cines, 2010)***.

However, increasing evidence indicates that an additional component of this disorder is immune-mediated decreased platelet production that can not keep pace with platelet destruction. Evidence for increased platelet destruction is thrombocytopenia following ITP plasma infusions in normal subjects. The cause of platelet destruction in most ITP patients appears to be autoantibody-mediated. However, cytotoxic T lymphocyte mediated platelet (and possibly megakaryocyte) lysis, may also be important ***(Nugent et al., 2009).***

Studies supporting suppressed platelet production include: reduced platelet turnover in over 80% of ITP patients, morphological evidence of megakaryocyte damage, autoantibody-induced suppression of in vitro megakaryocytopoiesis, and increased platelet counts in most ITP patients following treatment with thrombopoietin receptor agonists ***(Cines & McMillan, 2005).***

Aetiology of ITP

I. A key element in the pathophysiology of ITP is loss of tolerance leading to production of autoantibodies directed against platelet antigens. The antiplatelet factor subsequently was confirmed as an immunoglobulin. Previous studies showed that transfusion of 500 ml of

whole blood or plasma equivalent from ITP patients into healthy recipients showed marked drop of platelet count followed by recovery over the following few days ***(Nugent et al.,2009).***

The study of **Chang and colleagues, (2003)** showed that plasma from patients with ITP containing autoantibodies against gpIb and gpIIb–IIIa significantly suppressed megakaryopoiesis in vitro. They proposed that platelet autoantibodies may affect megakaryocyte maturation or survival, leading to decreased platelet production. The most commonly occurring autoantibodies in about 75% of ITP patients are directed against the platelet surface glycoprotein complexes gpIIb–IIIa and gpIb–IX ***(McMillan, 2000)***. Antibodies against other glycoproteins (Ia–IIa, IV, and V) have been identified, and multiple platelet antigen specificities can be found in most patients should be noted ***(Nugent et al, 2009).***

However these autoantibodies are not detectable in up to 50% of ITP patients, and that remission in ITP can occur despite the presence of platelet autoantibodies ***(McMillan et. al, 2003).***

Although antibodies are primarily of the IgG subtype, IgM and IgA may be found. Platelets are targeted by the attachment of autoantibodies to their gp antigens, bound to Fcγ receptors expressed on tissue macrophages of the reticuloendothelial system and cleared from the circulation ***(Gernsheimer, 2009)***. Macrophages express platelet epitopes on their surface and T-cell secrete cytokines that stimulate initiating CD4+ve T-cell clones and clones with additional specificities ***(Cines & Blanchette, 2002).***

It is increasingly clear that cellular immune mechanisms play a pivotal role in ITP. The production of antiplatelet antibodies by B cells requires antigen-specific, CD4-postive, T-cell help. It also is possible that in some ITP cases, cytotoxic T cells play a role in the destruction of