Serum Levels of Galectin-3 in Children with Juvenile Rheumatoid Arthritis

Thesis

Submitted in the partial fulfillment of requirements of Master degree in Pediatrics

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2010

مستوى جالاكتين-3 فى مصل الأطفال المصابين بمرض الروماتويد المفصلى الحدثى

رسالة توطئة للحصول على درجة الماجستير فى طب الأطفال

مقدمة مــن

الطبيب /أيمن عثمان يوسف عثمان
بكالوريوس الطب والجراحة العامة ـ جامعة عين شمس, 2003

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Acknowledgment

الحمد لله رب العالمين

At first and foremost thanks to "Allah" who gave me the power to finish this work.

I find no words by which I can express my deepest thanks and gratitude to my honored professor, ***Dr. Mohamed Hesham Mohamed Ezzat Abd El-Hameed, Professor of Pediatrics, and Professor of Pediatric Allergy and Immunology, Faculty of Medicine, Ain Shams University,*** *for the continuous kind encouragement, support and guidance, he gave me throughout the entire work. It has been an honor and privilege to work under his generous supervision.*

The sincere help and enormous effort of ***Dr. Kareem Yehia Aly Shaheen, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University****, is truly acknowledged. He gave me a lot of his valuable time to achieve the laboratory part of this work.*

I would like to express my endless gratitude and appreciation ***to Dr. Tarek Mohey Abdelmegeed EL-Gammasy, Assistant Professor of Pediatrics, Faculty of Medicine, Ain Shams University,*** *for giving me the opportunity to work under his meticulous supervision. His honest assistance and patience make me truly indebted to him.*

To my parents, colleagues and to every one who participated in a way or another in this work, I owe my thanks and appreciation.

A great deal of my gratitude goes to my patients and their parents for their kind cooperation and patience wishing them a very rapid and complete permanent recovery.

Aiman Osman Yousef Osman

CONTENTS

LIST OF ABBREVIATIONS

ACCP:	**Anti-cyclic citrullinated peptide**
ACR:	**American College of Rheumatology**
ANA:	**Antinuclear antibodies**
APCs:	**Antigen presenting cells**
APRIL:	**A proliferation-inducing ligand**
ASSURE:	**Abatacept Study of Safety in Use with other Rheumatoid arthritis thErapies**
BAFF:	**B-cell activating factor**
BLyS:	**B lymphocyte stimulator**
COMP:	**Cartilage oligomeric matrix protein**
CRD:	**Carbohydrate Recognition Domains**
DAS28:	**Disease Activity Score 28-joint count**
DCs:	**Dendritic cells**
DEXA:	**Dual energy X-ray absorptiometry**
DMARDs:	**Disease modifying anti-rheumatic drugs**
EBV:	**Epstein Barr Virus**
ESR:	**Erythrocyte sedimentation rate**
EULAR:	**European League Against Rheumatism**
FGFs:	**Fibroblast growth factors**
FINRACO:	**Finnish RA combination therapy trial**
gp-39	**glycoprotein-39**
HAQ:	**Health Assessment Questionnaire**
HIF-1:	**Hypoxia-inducible factor-1**
HGF:	**Hepatocyte growth factor**
Hp:	**Haptoglobin**
HSPs:	**Heat shock proteins**
IFN-γ:	**Interferon-γ**
Ig:	**Immunoglobulins**
IL:	**Interleukin**

JRA:	**Juvenile rheumatoid arthritis**
Kd:	**Kilo Dalton**
LLN:	**Lower limit of normal**
MAb:	**Monoclonal antibodies**
MCSF:	**Macrophage colony-stimulating factor**
MHC:	**Major-histocompatibility-complex**
MMPs:	**Matrix Metalloproteinases**
MPs:	**Microparticles**
MTX:	**Methotrexate**
NF-κB:	**Nuclear factor-κB**
NK:	**Natural killer cells**
NSAIDs:	**Non-steroidal anti-inflammatory drugs**
PDGF:	**Platelet-derived growth factor**
PMPs:	**Platelet-derived MPs**
QKRAA:	**Q: glutamine / K: lysine / R: arginine / A: alanine**
RA:	**Rheumatoid arthritis**
RANK:	**Receptor activator of NF-kappa B**
RANKL:	**Receptor activator of NFκB ligand**
RANTES:	**Regulated Upon Activation, Normal T Cell Expressed And Secreted**
RF:	**Rheumatoid factor**
SAARDs:	**Slowly acting antirheumatic drugs**
SD:	**Standard deviation**
SF:	**Synovial fluid**
SFL:	**Synovial fluid lymphocytes**
SLE:	**Systemic lupus erythematosus**
SM:	**Synovial membrane**
sRANKL	**soluble Receptor Activator of Nuclear Factor kappa-B Ligand**
TcR:	**T-cell receptor**

TGF:	**Transforming growth factor**
TICORA:	**Tight control for rheumatoid arthritis study**
TIMPs:	**Tissue inhibitors of matrix metalloproteinases**
TNF:	**Tumor necrosis factor**
TRANCE:	**TNF related activation induced cytokine**
TRAF:	**Tumour necrosis factor receptor associated factor**
VEGF:	**Vascular endothelial growth factor**

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, debilitating, systemic inflammatory autoimmune disease that affects approximately 1.3 million individuals in the United States. Untreated, RA can lead to dysfunction, deformation, and destruction of affected joints; over time this can result in significant morbidity, including early mortality ***(Müller-Ladner and Pap, 2005).***

Research during past years has shown that in addition to inflammatory cells and their mediators, resident fibroblasts of the synovial membrane play an important role in the pathogenesis of the disease. These cells exhibit features of stable cellular activation that is maintained in the absence of continuous inflammatory stimuli. In contrast to normal synovial fibroblasts, RA synovial fibroblasts show an up regulation of proto-oncogenes and transcription factors, which in a self-perpetuating manner mediate the expression of adhesion molecules and matrix degrading enzymes, and result in alterations in apoptosis. As a consequence, these activated fibroblasts attach to cartilage and bone and progressively destroy articular structures. A better understanding of the molecular mechanisms that lead to the stable activation of synovial fibroblasts in RA is, therefore, of utmost importance for elucidating the pathogenesis of RA as well as for the development of novel therapeutic strategies aimed at inhibiting joint destruction ***(Müller-Ladner and Pap, 2005).***

Galectins are a family of carbohydrate-binding proteins with specificity for N-acetyl-lactosamine (LacNAc)-containing

glycoproteins ***(Elola et al., 2007).*** At least 14 mammalian galectins share structural similarities in their carbohydrate recognition domains (CRD). Most fall within two groups called prototype (one CRD) or tandem-repeat (two CRDs). Galectin-3, also known as Mac-2 and previously as L29, CBP35, or eBP, is the only known Galectin with one CRD and a unique N-terminus. It is often termed a chimeric Galectin ***(Dumic et al., 2006).*** Galectins lack classical signal peptides but are present and active both within and outside of the cell. They are involved in cell adhesion, migration, survival, and apoptosis and are often up- or down-regulated in cancer ***(Elola et al., 2007).***

Human and mouse Galectin-3 share approximately 86% amino acid sequence identity. The 250 amino acid, 29 - 36 kDa human Galectin-3 is widely expressed in various cells, including macrophages, activated T cells, osteoclasts, epithelial cells, tumor cells, and fibroblasts ***(Dabelic et al., 2006, and Ohshima et al., 2003).*** Galectin-3 expression is unregulated in macrophages as compared to monocytes or dendritic cells ***(Papaspyridonos et al. 2008).***

Increased serum Galectin-3 has been noted in rheumatoid arthritis (also in synovial fluid) ***(Ohshima et al., 2003),*** Behçet's disease ***(Lee et al., 2007),*** and a variety of cancers, especially when they are metastatic ***(Iurisci et al., 2000).*** Cleavage of Galectin-3 in tumors is highly indicative of matrix metalloproteinase activity ***(Mazurek et al., 2007).***

Galectin-3 is highly pleotrophic in function and has numerous intracellular and extracellular binding partners ***(Ochieng et al., 2004).*** The Galectin-3 CRD recognizes terminal, unsialated LacNAc structures that are present on approximately 20% of all serum proteins and many extracellular matrix proteins, while its eight tandem repeats

within the unique N-terminal domain participate in protein-protein interactions ***(Krześlak and Lipińska, 2004).***

Nuclear Galectin-3 can alter gene expression, while in the cytosol; it can inhibit apoptosis and participate in exocytosis, caveolin-mediated endocytosis, and macrophage clearance of apoptotic cells ***(Dumic et al., 2006).*** Extracellular Galectin-3 is involved in innate immunity, binding carbohydrates on specific pathogens such as Candida albicans and Streptococcus pneumoniae, and acting as an opsonin. It is chemotactic for macrophages and induces innate immune responses in neutrophils ***(Elola et al., 2007).*** It cross-links CD98 to activate macrophages by the alternate pathway. Although many of its extracellular functions are pro-inflammatory and pro-apoptotic, it can be anti-inflammatory by down-regulating macrophage responses to bacterial lipopolysaccharides ***(Farnworth et al., 2008).***

Galectin-3 binding to endothelial cells stimulates angiogenesis, while fibroblast binding can promote fibrosis ***(Dumic et al., 2006).*** Some of these activities involve its adhesion to or regulation of certain integrins. Galectin-3 is monomeric, but it can form pentamers when the CRD is engaged by a carbohydrate, creating lattice structures on the cell surface. These lattices are involved in regulating focal adhesion dynamics and CD45-mediated control of T cell receptor signaling. It participates in lipid rafts and influences receptor dimerization or clustering, potentially regulating signaling, adhesion, and receptor endocytosis ***(Goetz et al., 2008 and Hsu et al., 2009).***

Aim of the Work

We sought to evaluate the expression of Galectin-3 in the serum and synovial fluid of children with JRA during disease activity and quiescence. Also, we investigated its relation to the clinical, laboratory, and radiological variables of disease activity andseverity.

JUVENILE RHEUMATOID ARTHRITIS

Epidemiology

Rheumatoid arthritis (RA) is the most common chronic inflammatory and destructive arthropathy that cannot be cured and that has substantial personal, social, and economic costs. The long-term prognosis is poor: 80 percent of affected patients are disabled after 20 years, and life expectancy is reduced by an average of 3 to 18 years ***(Heiberg et al., 2005).***

RA affects about 1 percent of the general population worldwide. Juvenile RA (JRA) is the most common rheumatic disease in children. It is one of the most frequent chronic illnesses of childhood ***(Choy and Panayi, 2001).*** The incidence of JRA is approximately 13.9/100,000 children (16 years or less) per year with a prevalence of approximately 113/100,000 children ***(Schaller, 1997).*** In Egypt, ***El-Gamal, (1998),*** reported a frequency prevalence of 9.8 JRA patients per year per 100,000 children attending the outpatients' clinic, Children's Hospital, Ain Shams University.

Although it is properly considered a disease of the joints, it is important to recognize that it can exhibit a variety of extra-articular manifestations. These manifestations clearly show that the disease has a systemic feature that is capable of involving a variety of major organ systems. Current slow-acting antirheumatic drugs (SAARDs) have limited efficacy and many side effects. Moreover, they do not improve the long-term prognosis of the disease ***(Loetscher and Moser, 2002).***

By definition, JRA begins before the age of 16 years. Even though onset before 6 months of age is unusual, the age of onset is characteristically young (1 to 3 years) but with a substantial number of cases beginning throughout childhood. Systemic onset is an exception with no increased frequency at any particular age. JRA is one of many chronic inflammatory diseases that predominate in females. The ratio of female to male patients ranges from 2:1 to 4:1, except, for systemic onset type in which the sex ratio is equal. A 30 to 50 percent concordance rate has been reported in monozygotic twins when one twin is affected compared with 1 percent for the general population. The risk for a fraternal twin of a patient with RA is also high (2-5 percent), but this is not more than the rate for other first-degree relatives ***(Cassidy, 2001).***

Etiology and Pathogenesis of JRA

Although the etiology of JRA remains a mystery, a variety of studies suggested that a blend of environmental and genetic factors is responsible; a contribution of either one is necessary but not sufficient for full expression of the disease. A guess, based on available data, is that several environment stimuli, possibly viruses especially retroviruses, infect an individual with the appropriate genetic background and through some mechanisms the inflammatory response is focused in the joints ***(Loetscher and Moser, 2002).***

The synovial membrane in patients with JRA is characterized by hyperplasia, increased vascularity, and an infiltrate of inflammatory cells, primarily $CD4^{+}$Th cells, which are the main orchestrator of cell-mediated immune responses. In genetic studies, JRA is strongly linked to the major-histocompatibility-complex (MHC) class II antigens HLA-DRB1*0404 and DRB1*0401 ***(van Bilsen et al., 2004).***

Ezzat et al., (2005), reported the association of DRB1 *04, and *14 alleles with JRA susceptibility, and DRB1*08 with protection. A double allelic dose of SE particularly *04 and *01 alleles contributed to the risk of developing severe forms of JRA, and were strong determinant of disease progression and aggressiveness.

The main function of HLA class II molecules is to present antigenic peptides to $CD4^{+}$Th cells, which strongly suggests that rheumatoid arthritis is caused by an unidentified arthritogenic antigen. The antigen could be either an exogenous antigen, such as a viral protein, or an endogenous protein ***(van Bilsen et al., 2004).*** A number of possible endogenous antigens, including citrullinated protein, human cartilage glycoprotein-39 (gp39), and heavy chain-binding protein, have been identified ***(Suzuki et al., 2003).*** The possible infectious organisms that may be incriminated in the pathogenesis of JRA are summarized in table (1).

Table (1): Possible Infectious Causes of JRA

Infectious Agent	Potential Pathogenic Mechanisms
Mycoplasma	Direct synovial infection; superantigens
Parvovirus B19	Direct synovial infection
Retrovirus	Direct synovial infection
Enteric bacteria	Molecular mimicry (QKRAA)
Mycobacteria	Molecular mimicry (proteoglycan, QKRAA)
Epstein-Barr virus	Molecular mimicry (QKRAA)
Bacterial cell walls	Macrophage activation

QKRAA: Q: glutamine / K: lysine / R: arginine / A: alanine

(Cassidy, 2001)