Serum TNF-Related Apoptosis-Inducing Ligand (TRAIL) Levels in Children with Juvenile Rheumatoid Arthritis and Systemic Lupus Erythematosus

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مستوى (TRAIL) فى مصل الأطفال المصابين بمرض الروماتويد المفصلى الحدثى و الذئبة الحمراء

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LIST OF ABBREVIATIONS

AIF:	**Apoptosis Inducing Factor**
Anti-dsDNA:	**Anti-double stranded-DNA**
Apaf-1:	**Apoptotic protease activating factor**
BAD:	**BCL2 antagonist of cell death**
BAG:	**BCL2 associated athanogene**
Bcl2:	**B cell leukemia/lymphoma 2 locus**
BID:	**BH3 interacting domain death agonist**
BIK:	**BCL2 interacting killer**
BLK:	**Bik-like killer protein**
CAD:	**Caspase-Activated DNAse**
CIA:	**Collagen-induced arthritis**
CTLs:	**Cytotoxic T lymphocytes**
DR:	**Death receptor**
ESR:	**Erythrocyte sedimentation rate**
FADD:	**Fas-associated death domain**
FasL:	**Fatty acid synthetase ligand**
FLSs:	**Fibroblast-like synoviocytes**
IAP:	**Inhibitor of Apoptosis Proteins**
ICAD:	**Inhibitor of Caspase Activated DNAse**
IFN:	**Interferon**
JIA:	**Juvenile idiopathic arthritis**
JSLE:	**Juvenile-onset systemic lupus erythematosus**

MPT:	**Mitochondrial permeability transition**
NuMA:	**Nuclear mitotic apparatus protein**
PBL:	**Peripheral blood lymphocytes**
PBMC:	**Peripheral blood mononuclear cells**
PGA:	**Physician global assessment**
RA:	**Rheumatoid arthritis**
RIP:	**Receptor-interacting protein**
SLE:	**Systemic lupus erythematosus**
SLEDAI:	**Systemic lupus erythematosus disease activity index**
TEM:	**Transmission electron microscopy**
Th2:	**Type 2 helper T cells**
TNF:	**Tumor necrosis factor**
TRADD:	**TNF receptor-associated death domain**
TRAIL:	**TNF-Related Apoptosis-Inducing Ligand**
TUNEL:	**Terminal dUTP Nick End-Labeling**
TWEAK:	**TNF-like weak inducer of apoptosis**

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INTRODUCTION

Apoptosis is induced by binding of death receptor ligands, members of the tumor necrosis factor (TNF) superfamily, to their cognate receptors. The Fas-Fas ligand pathway has been studied extensively in relation to systemic lupus erythematosus (SLE) ***(Castellino et al., 2007)***, and rheumatoid arthritis (RA) ***(Xie et al., 2007).*** However, other death pathways are also considered important. TNF-related apoptosis-inducing ligand (TRAIL), another ligand of the TNF superfamily, induces apoptosis in sensitive cells. There is, however, increasing evidence that the presence and accumulation of apoptotic cells play a role in autoimmunity. Increased apoptosis may induce autoimmune conditions. ***(Lub-de Hooge et al., 2005).***

TRAIL, also called apoptosis 2 ligand (Apo2L) for its similarity in sequence, structure, and function to Fas Ligand/Apo1L, is a TNF superfamily (TNFSF) member designated TNFSF10. TRAIL was cloned from human heart atrium, peripheral blood lymphocyte, and placenta cDNA libraries based on its similarity to regions highly conserved in the TNFSF. TRAIL is a 281 amino acid (aa), approximately 32 kDa, type II transmembrane protein expressed on the cell surface. It lacks a signal sequence, has a highly conserved and singly glycosylated C-terminal extracellular domain, a transmembrane domain, and a short N-terminal cytoplasmic domain. Like other members of the TNFSF, TRAIL also exists in a soluble form. SDS-PAGE analysis indicates that soluble TRAIL has an apparent molecular weight of approximately 24 - 28 kDa

while gel filtration analysis suggests that it exists as a trimer of approximately 66 - 80 kDa ***(Pan et al., 1997).***

Crystallography studies confirm that TRAIL is a homotrimeric jelly roll protein, but unlike other TNFSF members, TRAIL contains a zinc ion within the trimer interface. Three inward-facing Cys230 residues coordinate the zinc ion. These residues and the zinc ion are crucial for TRAIL trimer stability, receptor binding, and function. Within the TNFSF, TRAIL is most closely related to Fas Ligand with 28% aa identity. Across species, TRAIL is 65% identical at the aa level to its mouse homolog ***(Pan et al., 1997).***

TRAIL has an extremely broad expression pattern based on Northern blot analysis. It is expressed in fetal kidney, liver, and lung, as well as in adult colon, heart, kidney, lung, ovary, peripheral blood lymphocytes, placenta, prostate, skeletal muscle, small intestine, spleen, and thymus. TRAIL is variably expressed in tumor cell lines ***(Wiley et al., 1995).***

Both cell-surface and soluble TRAIL induce apoptosis in a variety of lymphoid and non-lymphoid tumor cell lines. These effects are mediated through binding TRAIL receptors 1 and 2 (TRAIL R1 [DR4] and TRAIL R2 [DR5]), both of which are expressed in many tissues as well. TRAIL also binds TRAIL R3 (DcR1), TRAIL R4 (DcR2), and osteoprotegrin (OPG), but is not able to affect apoptosis through these receptors. TRAIL R3, TRAIL R4, and OPG have become known as decoy receptors and are thought to be critical to the regulation of TRAIL signaling by competing for TRAIL binding ***(Ashkenazi and Dixit, 1998).***

Differential sensitivities to TRAIL-induced apoptosis may be due to the balance of TRAIL receptor and TRAIL decoy receptor expression patterns on particular cells as well as the relative expression of intracellular signaling regulatory proteins. Since TRAIL is capable of preferentially inducing apoptosis in tumor cells over normal cells, it has become an exciting prospect as a cancer chemotherapeutic ***(Ashkenazi and Dixit, 1998).***

Although the pathogenesis of juvenile idiopathic arthritis (JIA) disease is unclear, it is well known that T cells play a major role in both development and perpetuation of JIA through activating macrophages and B cells. Evidences for alterations in TRAIL/TRAIL receptor expression on peripheral T lymphocytes in the molecular mechanism of JIA development had been widely explored. ***Bisgin et al., (2010)*** reported up regulation of TRAIL and its receptors (both death and decoy) on both CD4+ and CD8+ T cells in rheumatoid patients compared to control individuals. TRAIL, TRAIL R1 and TRAIL R4 were expressed by many of the cells expressing CD68 (macrophages) ***(Dharmapatni et al., 2009).***

Moreover, the infiltration and accumulation of T cells in rheumatoid arthritis synovial fluid are hallmarks of disease. Accumulating evidences suggest the functional relevance of APO2L/TRAIL in the persistence of T cells in the rheumatoid synovial fluid. In addition, the presence of an aggressive population of activated synovial fibroblasts and pseudo-tumoral expansion of fibroblast-like synoviocytes (FLSs) that are

prominently involved in the destruction of articular cartilage and bone are constant hallmarks of JIA disease ***(Bisgin et al., 2010)***.

AIM OF THE WORK

It has recently been reported that TRAIL plays various roles in many autoimmune diseases as diabetes, multiple sclerosis, ankylosing spondylitis, RA, and adult-onset SLE. However, it has still remained unclear whether there is a close relationship between TRAIL and juvenile-onset SLE and JIA. The aim of this study was to determine the expression of TRAIL in the serum of JSLE and JIA children. The results were compared with those obtained from a group of healthy age-and sex-matched children serving as controls, to clarify the clinical usefulness of such biomarker and its relevance to clinical and laboratory variables in terms of disease severity, activity and response to therapy.

Apoptosis

Introduction

The term apoptosis (a-po-toe-sis) was first used in a now-classic paper by Kerr, Wyllie, and Currie in 1972 to describe a morphologically distinct form of cell death, although certain components of the apoptosis concept had been explicitly described many years previously ***(Kerr et al., 1972; Paweletz, 2001; Kerr, 2002).*** The understanding of the mechanisms involved in the process of apoptosis in mammalian cells transpired from the investigation of programmed cell death that occurs during the development of the nematode *Caenorhabditis elegans* ***(Horvitz, 1999)***. In this organism 1090 somatic cells are generated in the formation of the adult worm, of which 131 of these cells undergo apoptosis or "programmed cell death." These 131 cells die at particular points during the development process, which is essentially invariant between worms, demonstrating the remarkable accuracy and control in this system. Apoptosis has since been recognized and accepted as a distinctive and important mode of "programmed" cell death, which involves the genetically determined elimination of cells. However, it is important to note that other forms of programmed cell death have been described and other forms of programmed cell death may yet be discovered ***(Debnath et al., 2005)***.

Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents ***(Norbury and Hickson, 2001)***. Although there are a wide variety of stimuli and conditions, both physiological and pathological, that can trigger apoptosis, not all cells

will necessarily die in response to the same stimulus. Irradiation or drugs used for cancer chemotherapy results in DNA damage in some cells, which can lead to apoptotic death through a *p53*-dependent pathway. Some hormones, such as corticosteroids, may lead to apoptotic death in some cells (e.g., thymocytes) although other cells are unaffected or even stimulated ***(Elmore, 2007).***

Some cells express Fas or tumor necrosis factor (TNF) receptors that can lead to apoptosis via ligand binding and protein cross-linking. Other cells have a default death pathway that must be blocked by a survival factor such as a hormone or growth factor. There is also the issue of distinguishing apoptosis from necrosis, two processes that can occur independently, sequentially, as well as simultaneously ***(Zeiss, 2003)***. In some cases it's the type of stimuli and/or the degree of stimuli that determines if cells die by apoptosis or necrosis. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia and cytotoxic anticancer drugs can induce apoptosis but these same stimuli can result in necrosis at higher doses. Finally, apoptosis is a coordinated and often energy-dependent process that involves the activation of a group of cysteine proteases called "caspases" and a complex cascade of events that link the initiating stimuli to the final demise of the cell ***(Elmore, 2007).***

Morphology of Apoptosis

Light and electron microscopy have identified the various morphological changes that occur during apoptosis ***(Hacker, 2000)***. During the early process of apoptosis, cell shrinkage and pyknosis are visible by light microscopy ***(Kerr et al., 1972)***. With cell shrinkage, the