MELANOMA INHIBITORY ACTIVITY (MIA) 
& TRANSFORMING GROWTH FACTOR-β 
(TGF-β) LEVELS IN THE SERA OF 
JUVENILE ARTHRITIS

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ABSTRACT

Aim of Study: We measured serum levels of MIA & TGF-β by means of ELISA and investigated whether they provide clinically relevant parameters in juvenile arthritis patients. We also evaluated their correlations with clinical and laboratory parameters

Methodology: Serum was obtained from 10 poly-articular juvenile arthritis (pa-JRA) patients, 10 childhood systemic lupus erythematosus (ch-SLE) patients, 10 childhood systemic sclerosis (ch-SSc) patients and 10 apparently healthy children as a control group.

Results: Both MIA and TGF-β serum concentrations were found to increase in both destructive (JRA) and non-destructive (ch-SLE and ch-SSc) juvenile arthritis. We found no correlation with disease activity, clinical or laboratory parameters except RF.

Conclusion: MIA and TGF-β are not serum markers of a specific disease. In addition, the presence of elevated MIA in the sera of juvenile arthritic patients very likely reflects passive release from necrotic or apoptotic chondrocytes and is partly due to chondrocyte activation. This is supported by the fact that MIA level was only associated with RF positivity and not with markers of inflammation.
INTRODUCTION

Pediatric rheumatic diseases are rare and are different from those occurring in most adult forms of arthritis. To date there is no single test, blood, or otherwise, which allows making a firm diagnosis of juvenile arthritis. This is probably due to the fact that the exact causes of these diseases are not yet known. Another factor is the overlap between the different types of arthritis and many other diseases. As a result, the diagnosis can only be made on the basis of signs and symptoms and in particular by ruling out other diseases, which might cause similar problems.

In addition, the differential diagnosis of juvenile rheumatoid arthritis (JRA) vs. other non-joint destructive rheumatic diseases, such as childhood systemic lupus erythematosus (ch-SLE) and childhood systemic sclerosis (ch-SSC) frequently appears difficult.

Similar to JRA, invasive growth of tumors is associated with the destruction of tissue matrix. Recently, a novel protein, named melanoma inhibitory activity (MIA) has been characterised. It is originally identified as an 11 KDa protein secreted from malignant melanoma cells. It acts as a potent inhibitor for these malignant cells. Whereas in malignancies, elevated serum levels are predominantly found in metastatic melanoma, and to a lesser extent in ovarian, pancreatic and breast cancer (Bosserhoff et al., 2001). MIA in non-malignant tissue expression is predominantly seen in developing and mature cartilage (Muller-Ladner et al., 1999). Beside MIA, melanoma cells produce many growth factors as TGF-β (Colombo et al., 1992).

TGF-β is a multifunctional cytokine modulating the onset and course of autoimmune diseases. In high concentrations it has an immunosuppressive effect and in small amounts it can stimulate T-cell down-regulatory activity (Kanzou et al., 1998 and Ohtsuka et al., 1999). A potential role of TGF-β in RA is inhibition of cartilage or bone destruction and the down-regulation of the immune response (Choe & Kim, 1998). In SLE, T-cells are activated in the presence of this cytokine leading to down-regulation of autoantibodies (Gray et al., 1999).

Aim of Work:

To determine whether MIA might also be a marker for cartilage and bone degradation in juvenile rheumatic diseases, supported by the fact that it is produced by chondrocytes. MIA & TGF-β serum levels were quantified by means of a non-radioactive ELISA and investigated whether they provide clinically relevant parameters in juvenile destructive diseases patients, and evaluated their correlations for clinical and laboratory parameters.
MATERIALS AND METHODS

Patients:

Serum was obtained from 10 juvenile polyarticular arthritis (po-JRA) patients, 10 childhood systemic lupus erythematosus (ch-SLE) patients and 10 childhood systemic sclerosis patients (ch-SSC). The 3 diseases were diagnosed according to the American College of Rheumatology Criteria presented by Brewer et al. (1977) and Tan et al. (1982) and its Diagnostic and Therapeutic Criteria Committee (1980) respectively. All patients and controls used to attend the Outpatient Clinic of the Rheumatology and Rehabilitation Department of Ain Shams University Hospitals. Controls consisted of 10 apparently healthy individuals. None of the studied subjects suffered from malignant melanoma.

Assessment of disease activity in JRA was performed according to Pinals et al. (1981). A patient was considered in activity if he had at least three of the following five criteria, otherwise he could be considered in remission:

1) Tenderness of more than six joints.
2) Swelling of more than three joints
3) Morning stiffness for more than 45 minutes.
4) Articular index of more than 20 (according to Ritchie et al., 1968).
5) ESR more than 28 mm (1st h)

Disease activity in SLE patients was assessed according to SLE DAI indicated by Bombardier et al. (1992). The patient was considered to be active if he had SLE DAI more than nine.

Serum samples were aliquoted and stored immediately at -20°C. In addition, the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), of antinuclear antibodies (ANA) titer, anti double stranded nuclear antibody (ds-DNA) and red and white blood cell counts were evaluated on the day of the visit.

Enzyme-Linked Immunosorbent Assay (ELISA) For Detection Of MIA:

Serum concentration of MIA was measured using a recently developed quantitative ELISA, which is now commercially available (Bosserhoff et al., 1997). In brief, two monoclonal and COOH-terminal peptides were raised and conjugated to horseradish peroxidase and biotin, respectively. Ten microliters of serum or standard were incubated with 200μl of the reagent containing biotinylated 2F7 antibody and horseradish peroxidase-TA12 antibody in a streptavidin-coated 96-well plate for 45 minutes with continuous shaking. After washing three times in phosphate-
buffered saline, 200 μl of 2.2-azino-di-ethylbenz-thiazoline sulphonate were added to the wells and measured colorimetrically at 45 nm. Standardization was performed using solutions of purified MIA derived from stably MIA-transfected Chinese hamster ovary cells in a range from 0.1 to 50 ng/ml. The standard curve was calculated in a linear fashion. All serum samples and standards were measured in duplicate and results never varied more than 5%. MIA serum concentrations of the different patients groups were compared with the serum concentrations of healthy individuals.

**Enzyme-linked immunosorbent assay (ELISA) for the detection of TGF-β:**

This is again commercially available provided by Biosource Europe S.A Zoning Industrial B. 1400 Nivelles Belgium. The optimal density of each well was measured at more length 450 nm.

**Statistical Analysis:**

For comparison of MIA and TGF-β serum concentrations to the controls, the Mann-Whitney test for non-paired parameters was applied. For evaluation of the association between MIA and TGF-β in sera and RF, ESR, CRP, ANA, and DNA one way ANOVA test was used. p values of < 0.05 were regarded as significant.

**RESULTS**

Serum samples for a total of 36 subjects (10 po-JRA, 10 childhood SLE, 10 childhood systemic sclerosis, and 10 controls) were assayed for MIA and TGF-β. Table (1) shows the characteristics of the patients and controls.

**Table (1): Characteristics of patients and controls.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of patients</th>
<th>Mean age in years</th>
<th>MIA ng/ml</th>
<th>TGF-β ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M SD SE M SD SE</td>
<td></td>
</tr>
<tr>
<td>JRA</td>
<td>10</td>
<td>11.2</td>
<td>12.5 5.2 1.6</td>
<td>92.3 9.0 2.8</td>
</tr>
<tr>
<td>SLE</td>
<td>10</td>
<td>14.8</td>
<td>11.1 3.5 1.1</td>
<td>90.9 19.0 6.0</td>
</tr>
<tr>
<td>SSc</td>
<td>6</td>
<td>10</td>
<td>10.7 1.8 0.7</td>
<td>84.0 4.98 2.0</td>
</tr>
<tr>
<td>control</td>
<td>10</td>
<td>10.3</td>
<td>4.7 1.2 0.4</td>
<td>40.8 1.9 0.6</td>
</tr>
</tbody>
</table>

M=mean/SD=Standard Deviation/SE=Standard Error

Using the ELISA test system, healthy individuals showed MIA serum concentrations ranging from 3.2 to 6.8 ng/ml. MIA values of healthy individuals followed a Gaussian distribution, and were age independent.
Applying 3 SD. to base line of healthy individuals, MIA values of at least 6.5 ng/ml (ELISA cut-off) were regarded as elevated.

All JRA patients showed enhanced MIA serum concentrations ranging from 8.0 to 23.0 ng/ml, with a mean of 12.5 ± 1.6 ng/ml. The Serum concentrations showed a statistically significant difference from the controls p= 0.000.

Interestingly, all patients with non-joint-destructive rheumatic diseases, such as childhood SLE and SSc, showed increased MIA serum concentrations (except one childhood SLE patient who had a MIA serum concentration of 6.2 ng/ml). Serum concentrations in childhood SLE ranged from 6.2 to 15.5 ng/ml with a mean of 11.1 ± ng / ml. Serum of childhood SSc ranged from 9.5 to 13.0 ng/ml with a mean of 4.7 ± 0.4. Concentrations of MIA in both childhood SLE and SSc showed statistically significant difference from controls where p= 0.000. All data are summarised in table (1) and figure (1).

The inter-correlation studies of serum MIA revealed no statistical significance between JRA patients and neither the childhood SLE patients nor the childhood SSc patients where p was > 0.05.

All 3 groups of patients showed enhanced TGF-β serum concentrations, ranging from 72.8 to 104.0 ng/ml with a mean of 92.3 ± 2.8 ng/ml in JRA, from 57.2 to 109.2 mg/ml with a mean of 90.9± 6.0 in childhood SLE and from 78.0 to 89.0 ng/ml with a mean of 84.0 ±ng/ml in childhood SSc.
The concentration of TGF-β in all 3 studied groups of patients was statistically different from controls where \( p = 0.000 \). The intercorrelation studies of the serum TGF-β revealed no statistical significance between any of the 3 studied groups. All data are summarised in table (1) and figure (2).

The one way ANOVA test was used for the comparison between both MIA and TGF-β in sera and RF, ESR, CRP, ANA, and DNA in the 3 groups of patients. No significant differences were recorded between the serum levels of MIA and the previous mentioned parameters where \( p > 0.05 \), except for RF (JRA \( p = 0.01 \), SLE \( p = 0.014 \), SSc \( p = 0.02 \)). In contrast TGF-β levels showed no significant differences between it and RF in all groups except in systemic sclerosis where \( p = 0.007 \). TGF-β levels showed no significant difference between it and the rest of the parameters studied in all of the 3 groups of patients, except for 2 significant differences which were recorded between the levels of TGF-β and both anti ds-DNA and ANA in systemic sclerosis patients (\( p \) in both=0.000).

Results showed that 6 JRA patients and 7 SLE patients were in activity. We found a non-significant correlation between disease activity (in JRA & SLE) and MIA & TGF-β. Again, there was no significant correlation to any of the clinical or laboratory parameters except for RF.
DISCUSSION

Little is now known about the regulation of MIA synthesis and its biological properties in cartilage metabolism; but there are indications that in healthy individuals MIA expression is limited to cartilaginous tissue. However, in both developing as well as in mature cartilage MIA mRNA and protein were detected (Dietz & Sandell, 1996). This is supported by the fact that in the study performed by Muller-Ladner et al. (1999), cultured RA and OA synovial fibroblasts, which are known to be involved in joint destruction, in particular at the sites of invasion of the proliferating synovial tissue into adjacent cartilage, did not produce enhanced amounts of MIA (Gay et al., 1993). This is in contrast with cartilage oligomeric matrix protein (COMP), which has been considered as a marker for cartilage destruction, but is produced by both chondrocytes and synovial fibroblasts (Hummel et al., 1998).

A most recent study conducted by Bosserhoff & Buettner (2002) showed that MJA is a highly specific and sensitive marker for clinical follow-up & therapy monitoring of malignant melanoma patients. In addition, data suggested a further potential application as a surrogate marker for measuring cartilage destruction in rheumatoid arthritis that will certainly attract further interest and investigations.

In addition, mechanisms of matrix degradation are similar in metastasis of melanoma and diseases leading to joint destruction. Matrix metalloproteinases and cysteine proteases are found at the sites of articular degradation and in patients suffering of melanoma (Boike et al., 1992 and Muller Ladner et al., 1999).

In this study we examined the presence of MIA and TGF-β in the serum of patients suffering of different juvenile rheumatic diseases (JRA, childhood SLE and SSc) and its association with laboratory parameters. To our knowledge, there are no previous studies on juvenile arthritis as regards MIA & TGF-β, while there are many reports on adult arthritis. Muller Ladder et al. (1999) found a high level of MIA in RA with a significant difference from controls. This result is in agreement with our study. Moreover, they did not find a significant correlation to disease activity. This is confirmed by our results and another Egyptian study reported by El-Kady et al. (2001). Contrary to these results, Neidholt et al. (2000) found a positive correlation between MIA and inflammatory parameters (ESR, CRP).

Our SLE and SSc patients showed elevated serum MIA with a significant difference than controls. In contrast to these findings, Muller-Ladner et al. (1999) found a non-significant difference in their SLE and SSc
patients. Fahmy et al. (2000) showed the same result in SLE patients only. Therefore, it can be speculated that juvenile arthritis is different from the adult form of arthritis not only in the clinical picture but also in its pathogenesis.

In this study, MIA was increased in both destructive joint disease as JRA and non-destructive childhood SLE and SSc. We speculated that MIA release in juvenile arthritis is mainly due to passive release from necrotic or apoptotic chondrocytes, and is in part due to an active release from chondrocytes during the process of joint destruction.

Interestingly, elevated MIA levels were only associated with RF positivity in all 3 groups of patients, but no association was found with any of the inflammatory parameters studied. This hypothesis that MIA reflects chondrocyte activation, but not disease activity per se, is not only supported by our data, but also by the findings of Muller-Ladner et al. (1999) and El Kady et al. (2001). Earlier findings showed progressive radiological joint destruction without inflammation in numerous RA patients (Mulherin et al., 1996).

In an interesting study performed by Neidhart et al. (2000) who measured the serum level MIA and COMP in marathon runners. They found that MIA was elevated to a level comparable to that reported in RA. They concluded that elevated levels of COMP and MIA might reflect increased joint matrix turnover and/or damage due to prior extreme physical training. Thus, we speculate that MIA can be a marker of chondrocyte metabolism and/or damage in both disease and health during sports.

Numerous similarities of melanoma cells and chondrocytes exist in the metabolic pattern and the interaction with matrix. Both of them are known to produce numerous growth regulating factors such as transforming growth factors (TGFs), basic fibroblast growth factor (bFGF) and insulin-like growth factor (IGF) (Verschure et al., 1996). Growth factors enhance both catabolic as well as anabolic pathways in articular chondrocytes. The main cartilage anabolic factor is the insulin growth factor-1. It shows great safety, but has little contribution in diseased cartilage because of its non-responsiveness to arthritic chondrocytes.

TGF-β can overrule interleukin-1 (IL-1) catabolic effects and can enhance cartilage repair in arthritic tissue. This is unlike bone morphogenetic protein-2 that only is capable of enhancing chondrocyte proteoglycan synthesis in the absence of IL-1. TGF-β and bone morphogenetic protein–2 induces chondrocyte forming at the margins of the joint (Van den Berg et al., 2001). On the other hand, TGF-β acts as a tumor
suppressor in the early stages of carcinogenesis. However, it has also been suggested to produce tumor progression at later stages (Weeks et al., 2001).

In our study, all 3 groups of juvenile arthritis patients showed enhanced TGF-β as compared with healthy individuals. In a study done by Feldmann et al. (1996) on RA patients, TGF-β, was higher in patients than controls, which is in accordance with our findings as well as other reports by Choe & Kim (1998) and Demiralp et al. (1999). On the other hand, Kanzuo et al. (1998) found that TGF-β was lower in RA patients. All of them found no correlation to disease activity that is in consistence with our study. Dharmapotni et al. (2001) found that the serum level of TGF-β was within normal limits in systemic sclerosis patients. Badre et al. (2001) detected a lower level in their SLE patients.

It is clear now that cartilage destruction in arthritis is linked to aberrant cytokine and growth factor expression in the affected tissue. Hence, it becomes that the balance between the different factors is more important in the net destruction than the absolute levels of each mediator. This may explain the destructive nature of the adult form when compared to the early onset of JRA.

Conclusion:

MIA & TGF-β serum concentrations increase in both destructive (JRA) and non-destructive (childhood SLE and SSc) juvenile arthritis. Therefore, they are not serum markers of a specific disease. In addition, the presence of elevated MIA in the sera of juvenile arthritic patients very likely reflects passive release from necrotic or apoptotic chondrocytes and is partly due to chondrocyte activation; supported by the fact that MIA levels is only associated with RF.

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