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Tryptophan is Linking Metabolism to Inflammation in Juvenile Idiopathic Arthritis

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Abstract

This study aimed to evaluate tryptophan and its metabolite kynurenine as possible diagnostic biomarkers for Juvenile Idiopathic Arthritis (JIA) and their role in therapeutic decision-making.The levels of tryptophan (Trp) and kynurenine (Kyn) in 44 sera and nine samples of synovial fluid (SF) of 25 children with JIA were compared with 18 sera of peers with non-inflammatory diseases. Trp and Kyn concentrations were determined by reverse-phase high-performance liquid chromatography (RP-HPLC). Serum levels in JIA patients did not significantly differ from those of the control group. The comparison of SF with serum levels revealed that Trp in SF (mean 57.2 ± SD 19.5 µmol/L) was lower than in serum (mean 36.3 ± SD 18.4 µmol/L). Therefore, the Kyn/Trp ratio was higher (mean 77.8 ± SD 44,4 vs. 41.6 ± 13.7 µmol/mmol). There exists a significant positive association between neopterin concentrations and tryptophan breakdown as expressed by the kyn/trp ratio (rs = 0,427, p<0.01) indicating an involvement of indoleamine 2,3-dioxygenase (IDO-1) in the breakdown of tryptophan. However, this was localized only in the SF. Conclusion: The analyzed parameters did not show relevant differences in serum levels between patients and the control group. However, the SF from JIA patients did show a significantly decreased Trp concentration and a higher Kyn/Trp ratio. This finding suggests a local upregulation of tryptophan breakdown due to increased IDO-activity in inflamed joints.

Keywords: Indoleamine 2,3-dioxygenase; Juvenile idiopathic arthritis; Tryptophan

Introduction

JIA is the most common chronic rheumatic disease in children [1]. Incidence rates vary between 1.6 to 23 and prevalence between 3.8 to 400/100,000. Both proportions were almost twice as high in girls than in boys [2]. Oligoarthritis is the most common subtype (pooled incidence rate 3.7 [3.5 - 3.9] with a prevalence of 16.8 [15.9 – 17.7]/100,000) [2], and the most frequent subset in JIA patients, characterized by early-onset asymmetric arthritis predominantly affecting knee joints or ankles. Rheumatoid factor-positive polyarthritis is considered the childhood equivalent of the RF-positive rheumatoid arthritis (RA) seen in adults [1]. This subset represents only 3% of the JIA cases, concerning particularly female adolescents [3]. The etiology of JIA is still poorly understood, but appears to involve a combination of multiple genes, as well as environmental factors [4,5] Patients with systemic JIA (sJIA) present a distinct inflammatory profile . Mounting evidence indicates that a dysregulated innate immune system induces increased production of autoinflammatory cytokines such as IL-6 and IL-18 [6,7]. The sJIA unique pathogenesis was, therefore, an exclusion ground from this study.

The chronic inflammatory processes of joints in JIA are comparable to those observed in adult RA, accompanied by villous hypertrophy and hyperplasia of the synovial lining layer. The subsynovial layers are hyperaemic, oedematous, and massively infiltrated by inflammatory cells such as mononuclear cells, T cells, B cells, macrophages, dendritic cells, and plasma cells [8,9]. The chemokine receptors CCR5, CXCR3, and CD45RO, are primarily expressed by activated T cells type 1 (Th1), which produce the cytokine profiles interferon-g (IFNγ), tumor necrosis factor (TNF) -α, and IL-2 [10]. The diagnosis of JIA is based on clinical examination, patient's history, and exclusion of other possible causes. Currently, laboratory examination can only support the clinical diagnosis, as there are no reliable laboratory tests or combinations of studies [1]. L-tryptophan (Trp) is the rarest essential amino acid found in food. It is an intermediate for protein synthesis and a building block of several biologically essential metabolites such as 5-hydroxytryptamine (T5H),

kynurenines, and nicotinamide adenine dinucleotide (NAD+). The first step of the kynurenine pathway can be catalyz ed either by tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO) [11].

TDO is predominantly expressed in the liver but also in the brain, the epididymis, and the mucous membranes. TDO preserves blood homeostasis of Trp and may also play a role in immunoregulation after liver transplantation [12]. IDO has only been found in mammals and yeasts and is not expressed in prokaryotes [13]. IDO is the rate-limiting enzyme of the kynurenine pathway and transforms L-Trp into N-formylkynurenine, which is rapidly metabolized into L-kynurenine (Kyn) (Figure 1). Kyn can enter the bloodstream or be further converted to downstream kynurenine metabolites like kynurenic acid and quinolinic acid [14]. The highest levels of IDO expression can be detected in professional antigen-presenting cells (APC), like monocyte-derived macrophages, dendritic cells (DC), and fibroblasts. IDO is mainly responsive to the Th1-type cytokine IFN-γ [15,16] but can also be activated by hormones such as human chorionic gonadotropin [17] and estrogen [18]. Besides these soluble molecules, there is also a direct interaction with membrane-anchored co-receptors such as B7 and CTLA4 found on T cells and DCs [19].

In summary, the activation of IDO is generally considered to have immunosuppressive and anti-inflammatory effects [23-25]. Consequently, the pharmacologic inhibition of IDO promotes the activation of autoreactive T cells, which can lead to autoimmunity and loss of peripheral immune tolerance [26].

Although there have been ongoing, extensive investigations into the enzyme's role in rheumatoid arthritis (RA), research on the activity of IDO in JIA patients remains scarce., Patients with RA had a significantly lower level of Trp than healthy blood donors. In addition, a relation between the progressive stages of RA and decreased levels of Trp was identified [27]. However, a correlation between the subjective disease activity within these stages and Trp levels could not be detected [27,28]. A similar study by Y. Ozkam et al. did not corroborate these results. The authors concluded that serum levels of Trp and Kyn did not differ between RA patients and controls [29]. L. Zhu et al. discovered that DCs isolated from synovial fluid of RA patients expressed higher levels of functionally active IDO than DCs derived from healthy donors. In contrast, there was no detectable IDO in DCs isolated from peripheral blood, neither in patients with RA nor in healthy blood donors [30].

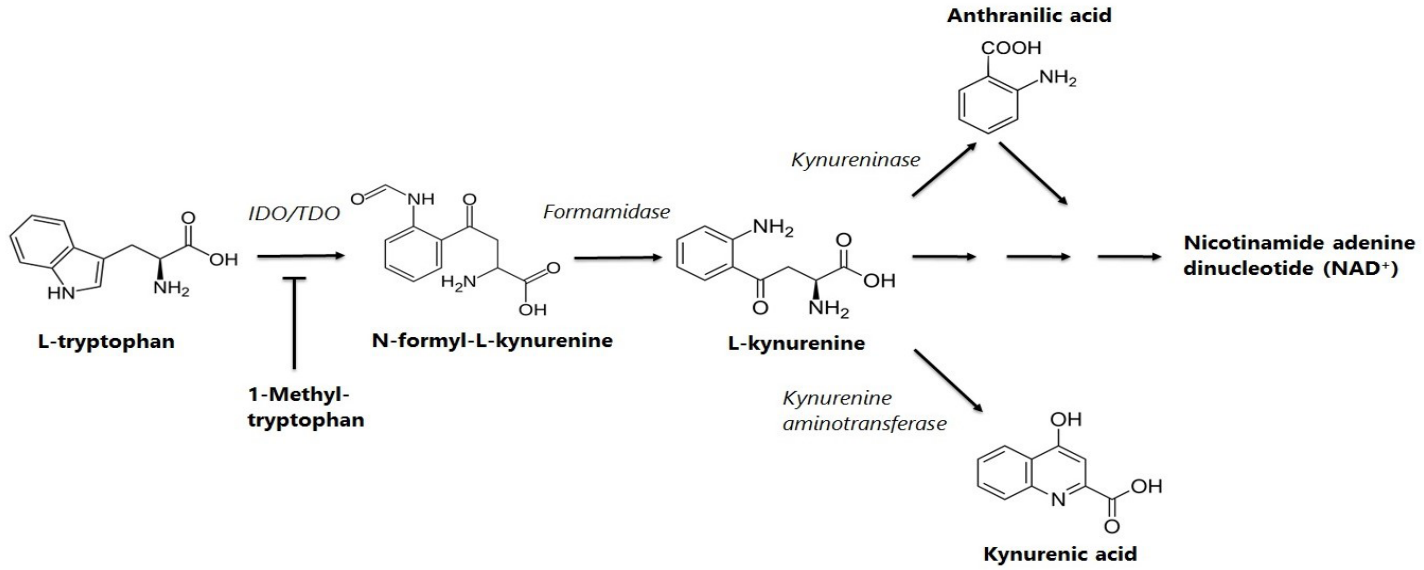

Figure 1: Kynurenine Pathway

The withdrawal of Trp from the micro-environment through stimulation of IDO inhibits the growth of microbes and has a strong antiproliferative effect on malignant cells [20]. IDO plays a pivotal role in peripheral tolerance [21,22]. Kynurenine is a potent start signal for T-cell apoptosis. Furthermore, withdrawal of Trp causes a stop of T-cell proliferation during mitosis and a loss of activity in the T cell.

However, IDO is not able to sufficiently suppress autoreactive T cells in RA patients, which is likely caused by highly upregulated levels of tryptophanyl-tRNA-synthetase (TTS) in T cells (also induced by IFN-γ). This enzyme enables T cells to preserve Trp, which results in lower susceptibility to IDO-mediated tryptophan deprivation [30]. This may cause the pathogenic persistence of autoreactive T cells in

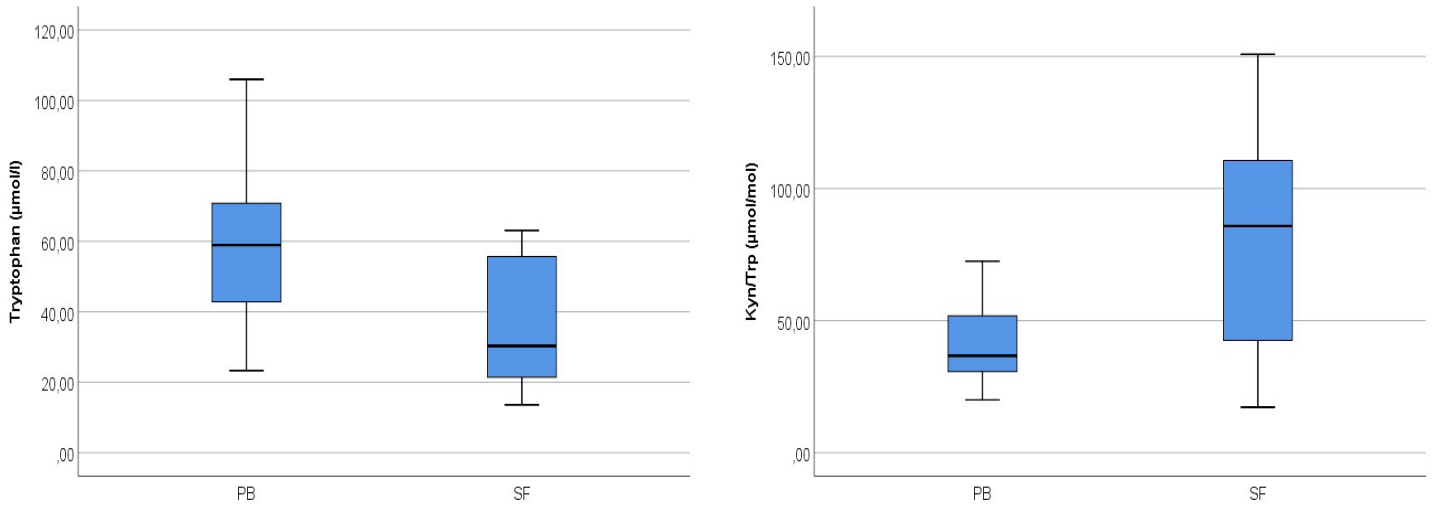

Figure 2: Differences between peripheral blood and synovial fluid in JIA patients.

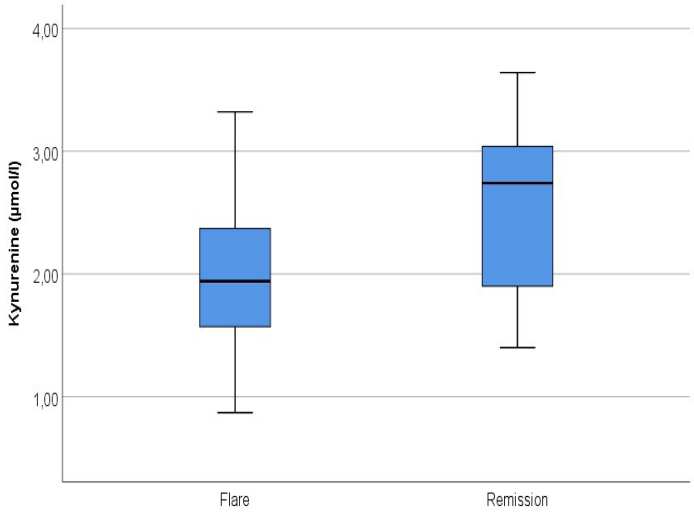

Figure 3: Differences in PB during flare and remission.

patients with autoimmune diseases. A laboratory parameter to ascertain JIA and promote early diagnosis has not yet been established. Therefore, the objective of this study was to evaluate the clinical relevance of Trp and its downstream metabolites as biochemical markers for diagnosis or follow-ups during treatment. These parameters are known to be influenced by chronic inflammatory diseases and could facilitate the diagnostic process. Furthermore, an indicator of disease activity might help the practitioner to intervene promptly in case of exacerbations.

Material and Methods

Forty four sera and nine samples of synovial fluid taken from 25 patients with JIA recruited by the Department of Pediatrics of the Medical University of Innsbruck were analyzed. All patients had been diagnosed using the International League of Associations for Rheumatology (ILAR) Classification Criteria for JIA. Eighteen sera from 18 children with non-inflammatory diseases were used as controls. Due to its particular inflammatory etiology and to gather a more homogenous patient population, children with systemic JIA were excluded from this study. The study was conducted in compliance with the Declaration of Helsinki and was authorized by the Ethics Committee of the Medical University of Innsbruck (AN3731-280/4.5). Informed consent was obtained from all study participants.

Trp and Kyn concentrations were determined by reverse-phase HPLC. Measurements were taken as described by Fuchs et al. [31]. Specimen were analyzed by a Varian ProStar HPLC system equipped with a solvent delivery module (model 210, Varian ProStar), an autosampler (model 400, Varian ProStar), a UV-spectrometric detector (SPD-6A, Shimandzu), and a fluorescence detector (model 360, Varian ProStar). For each specimen, 100 µl serum or synovial fluid were added to 25 µl of 2 mol/l trichloroacetic acid and 100 µl of internal calibrator (25 µmol/l 3-nitro-L-tyrosine). These components were vortexed and centrifuged to precipitate proteins. The generated supernatants were measured. An external calibrator, composed of an albumin-based mixture of 100 µmol/l Trp and 10 µmol/l Kyn, was processed in the same way as the specimens. Separation of sample components was performed by reverse-phase HPLC using a LiChrosorb C18 column (5 µm particle size, Merck, Darmstadt, Germany) and an eluent, consisting of 15 mmol/l acetic acid-sodium acetate solution (pH 4.0). A fluorescence detector identified the natural fluorescence of Trp at an excitation wavelength of 286 nm and an emission wavelength of 366 nm. An ultraviolet light detector measured kyn and internal calibrator 3-nitro-L-tyrosine at a wavelength of 360 nm. The ratio of Kyn/Trp was calculated and set at µmol/

Table 1: Distribution of samples and JIA subtypes. PB: Peripheral Blood; SF: Synovial fluid

Subtypes	Number of patients	Number of specimen	
		PB	SF
Polyarthritis RF-positive	3	8	2
Polyarthritis RF-negative	3	4	0
Oligoarthritis persistent	12	16	5
Oligoarthritis extended	4	11	1
Enthesitis-related arthritis	1	2	0
Psoriatic JIA	2	3	1
	25	44	9

mol [31-33]. Statistical analyses were performed using the software Statistical Package for the Social Sciences (SPSS, Chicago, Illinois). Statistical significance was determined by the independent samples t-test and the Mann-Whitney U test when non-parametric tests were required. The level of significance was set at 0.05.

Results

This study involved 25 participants (17 female, 8 male) aged between one and twenty years old, with any of the JIA subtypes. (Table 1) shows sample distribution and JIA subtypes. Patients' gender did not implicate any statistical influence on the results. The Juvenile Arthritis Disease Activity Score (JADAS) was used to score disease activity on a 0-10 scale based on four parameters: the physicians' global assessment, parents' and patients' evaluation, active joint count, and level of erythrocyte sedimentation rate (ESR) [34]. Trp and Kyn levels in JIA patients' peripheral blood (PB) were (mean ± SD) 57.2 ± 19.5 µmol/l, and 2.2 ± 0.7 µmol/l, with no statistically significant differences compared to those of the control group (57.6 ± 14.8 µmol and 2.1 ± 0.8 µmol/l). Trp concentrations were significantly lower in patients' SF (36.3 ± 18,4) than in their analyzed blood serum ($p<0.05$). The lower Trp level also affected the Trp/Kyn ratio (µmol/mol) at a significant level of $p<0.05$, 41.6 ± 13.7 in PB, and 77.8 ± 44.4 in SF (Figure 2).Sera of patients during active JIA showed significant lower Kyn levels (mean ± SD) 2.0 ± 0.6 compared to patients during remission 2.5 ± 0.7. No discrepancies were noted in other hematological parameters regarding disease activity (Figure 3).

There existed a significant positive association between neopterin concentrations and tryptophan breakdown as expressed by the kyn/trp ratio ($r_s = 0,427$, $p<0.01$) indicating an involvement of indoleamine 2,3-dioxygenase (IDO-1) in the breakdown of tryptophan. However, this was depicted only in the SF and thus a very local metabolic activity.

Discussion

JIA is a systemic autoimmune disease involving an inflammatory process. Even though it is the most common chronic rheumatic disease in children, the diagnosis is still based on the exclusion of other possible diseases. To avoid long-term disability and to minimize the burden of disease, early diagnosis is essential. Evaluation of disease activity hinges on clinical presentation due to a lack of reliable laboratory parameters. This approach can either result in a delayed intervention in case of a flare, or an overtreatment with potentially toxic drugs during remission. Several biochemical markers are already integrated into laboratory diagnostics. HLA-B27, ANAs, rheumatoid factor, and anti-CCP antibodies can support the diagnosis of JIA and suggest the correct classification into JIA subtypes.

CRP and ESR are general markers of inflammation. Unfortunately, CRP is elevated in both infections and sterile inflammatory processes such as JIA. ESR increases belatedly during inflammation. Both markers can indicate the current disease activity but cannot be relied upon to choose the proper medical treatment [35-39]. J. Gerss et al. evaluated the neutrophil activation marker S100A12, the phagocyte activation markers myeloid-related proteins 8 and 14 heterocomplexes (MPR8/14) as well as high-sensitivity CRP as possible biomarkers to stratify a patient's risk of relapse. In absence of clinical or standard laboratory parameters, the levels of S100A12 and MRP8/14 were significantly increased in children with an unstable remission and a higher risk of flare. Therefore, these biomarkers could be instrumental in the risk-adapted treatment and maintain remission in JIA [40]. Thus far, counting joints is still the foundation for classifying JIA subtypes, decision-making, and adopting suitable treatment regimens [41].

This system has inherent limitations, and the necessity of validated and reproducible diagnostic parameters such as biochemical markers, remains a concern. Although the pathogenesis of JIA remains unclear, the process involves immunological alterations, such as a highly activated cell-mediated immune system. In particular, previous studies have described higher populations of Th1 cells in the SF [10]. These cells are known to release high amounts of IFN-γ, as well as TNF-α and IL-2, triggering the activation of macrophages and subsequent synthesis of IDO. Insight into these mechanisms in the pathogenesis of JIA connotated the IDO pathway as a possible biomarker.

In our study, mean values of Trp and Kyn or Kyn/Trp ratio in sera did not show statistically significant deviations in patients compared to controls. Previous studies on IDO

metabolism in RA patients showed inconsistency in terms of tryptophan levels. K. Schroeksnadel et al. found decreased Trp concentrations in RA patients [27], while the study population of Y. Ozkan showed no significant differences in Trp [29]. A statistically significant decreased Trp level and a higher Kyn/Trp ratio could be observed in SF compared to serum levels in our study. In theory, this could be caused by a higher Trp turnover due to IDO activation, although no higher kynurenine level was measurable. An upregulation of IDO in DCs derived from SF in RA patients could also be shown in a previous study by L. Zhu et al. [30].

As we could not perform arthrocentesis on healthy joints of children, the SF could only be compared with JIA patients' peripheral blood and not with SF from controls. In two previous studies on adults with RA, Trp metabolism in SF could be compared to SF derived from patients with osteoarthritis. T. Igari et al. described activation of IDO in the SF and higher Trp, Kyn, and anthranilic acid levels in RA patients compared to those with osteoarthritis (OA), while other downstream metabolites like kynurenine acid and NAD+ had decreased [42-46]. In contrast, K. Kang et al. detected a lower metabolism rate in RA patients than in OA patients [47]. Age-related changes in IDO metabolism may hinder the comparison of these results [48]. Results did not reveal differences between the markers of the kynurenine pathway in patients' and controls' sera.

However, once JIA patients were grouped according to different stages of disease activity, samples taken during active phase/flare surprisingly showed a statistically significant lower Kyn level than the inactive phase/remission. Consequently, this might indicate that inflammation does not stimulate IDO activity, in line with the theory that IDO activation could have anti-inflammatory properties [23-25]. Several studies have confirmed that IDO plays a role in the complex pathogenesis of autoimmune diseases, including JIA. However, as IDO is currently under extensive examination, there are no definitive conclusions regarding its activity and induction. Due to the inconsistency in the results obtained so far, larger multicenter studies are needed to evaluate metabolites of the IDO pathway as biomarkers for JIA.

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