Urinary excretion of deoxypyridinoline in Perthes’ disease

A PROSPECTIVE, CONTROLLED COMPARATIVE STUDY IN 83 CHILDREN

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Our aim was to investigate the relationship between urinary excretion of deoxypyridinoline (DPD) as a marker of bone resorption, and Perthes’ disease. There were 39 children with Perthes’ disease in the florid stage who collected first-morning urine samples at regular intervals of at least three months. The level of urinary DPD was analysed by chemiluminescence immunoassay and was correlated with the radiological stage of the disease as classified by Waldenström, and the severity of epiphysial involvement according to the classification systems of Catterall and Herring. The urinary DPD levels of a group of 44 healthy children were used as a control.

The median urinary DPD/creatinine (CREA) ratio was significantly reduced (p < 0.0001) in the condensation stage and increased to slightly elevated values at the final stage (p = 0.05) when compared with that of the control group. Herring-C patients showed significantly lower median DPD/CREA ratios than Herring-B patients (p = 0.03). The significantly decreased median DPD/CREA ratio in early Perthes’ disease indicated a reduced bone turnover and supports the theory of a systemic aetiology. Urinary levels of DPD may therefore be used to monitor the course of Perthes’ disease.

Perthes’ disease is a self-limiting avascular necrosis of the femoral head occurring in children from four to ten years of age. The incidence in boys is 1:3000 and is four times more common than in girls. It is generally accepted that an avascular event occurs in the capital epiphysis of the femur but the aetiology is still obscure. Local factors (vascular disorders, increased intra-articular or intra-osseous pressure) as well as systemic disorders (genetic, endocrine, haemostasiological factors) have been implicated. Most likely, it is of multifactorial origin.

Until now the diagnosis of Perthes’ disease has required confirmation either by radiography or by MRI. Laboratory tests are made to exclude inflammatory or malignant disease. There are no known markers which are modulated regularly by Perthes’ disease. The course of the disease has been described radiologically by Waldenström and the stages classified as initial, condensation, fragmentation, re-ossification and residual. The duration is usually between two and four years. The extent of epiphysial necrosis has been classified by Catterall and Herring et al.

Because of the ischaemic process, the growth of the ossific nucleus stops and the bone becomes dense. The dense bone is subsequently resorbed and replaced by new bone. During this process the mechanical properties of the femoral head are altered in such a way that it tends to flatten and enlarge. Healing occurs with remodelling of the femoral head.

During bone resorption the level of breakdown products of collagen is higher in the blood and urine. These breakdown products are composed of collagen cross-links. Extracellular collagen fibrils are cross-linked to stabilise the collagen chains. There are two types of cross-link; pyridinoline which is found in almost all types of tissue containing collagen types I, II, III and IX (cartilage, bone, tendons, dentine and others) and deoxypyridinoline (DPD) which is specific for collagen type I.

Because both of these cross-links do not occur in the skin and the turnover of collagen in cartilage and tendons compared with bone is quite low, they can be used as markers of bone resorption. Of the two, DPD is more specific. Histomorphometric and radio-isotopic measurements of bone resorption have shown that the urinary excretion of these cross-links is highly correlated with bone turnover in adults. In children there are no similar studies. Analysis of pyridinium cross-links can be made from either morning or 24-hour urine samples. The excretion rate is influenced...
by a number of factors including bed rest, pregnancy and age (in children the cross-link excretion is approximately five times higher than that in adults). Dietary factors are of no relevance. An increased urinary pyridinium cross-link excretion is documented for several bone-resorbing processes such as osteoporosis, tumour-associated hypercalcaemia, primary hyperparathyroidism and others.

The relationship between Perthes’ disease and the urinary excretion of DPD has not yet been evaluated. The aim of our study, therefore, was to investigate urinary levels of DPD at different stages of Perthes’ disease.

**Patients and Methods**

We included in the study 39 consecutive children with Perthes’ disease who presented in our outpatient clinic. Inclusion criteria were Perthes’ disease in the initial, condensation or fragmentation (florid) stage according to Waldenström’s criteria were florid bilateral Perthes’ disease and any other condition which may have influenced bone metabolism.

A control group of 44 healthy children, of corresponding age and gender, with no evidence of bone disease was recruited (Table I).

The parents of the children were informed of the study and gave their consent. The study complied with the Statement of the Central Ethics Committee of the German Medical Association (Bundesärztekammer) for the Usage of Human Body Material for Medical Research.

**Urine samples and radiographs.** Children in the Perthes’ disease group were asked to collect samples of their first morning urine at regular intervals of at least three months. Anteroposterior and lateral radiographs were obtained every three to four months during the florid phase of the disease and every three to six months in the re-ossification and final stages. They were all evaluated by one of the authors (BW), an experienced paediatric orthopaedic surgeon. The stage of the disease was classified according to Waldenström and the degree of epiphyseal involvement according to the classification of Catterall and Herring et al (Tables II and III).

For comparison, the morning urine samples of the 44 healthy control children were collected. Four samples on a weekly basis were analysed.

The urine samples were stored at -25°C. In total, 256 samples from the Perthes’ disease patients were analysed, a mean of 6.56 samples per patient (1 to 11). In the control group 175 samples were collected from 44 children. One child could contribute only three samples.

The samples were analysed for DPD and creatinine (CREA). Urinary DPD concentrations were measured on an automated chemiluminescence immunoassay system (Immulite; Pyrilinks-D, DPC Biermann, Bad Nauheim, Germany). In order to correct for variations in urinary flow, the results were normalised to urinary CREA levels. The latter were determined by an enzymatic colorimetric assay on a Modular P analyser (CREA plus; Roche Diagnostics, Mannheim, Germany). The results were expressed as a DPD/CREA ratio (nmol/mmol).

**Statistical analysis.** In the study group each urine sample was compared with the radiological grading. In case more than one result was obtained for a child in one radiological stage, the mean of the DPD/CREA ratio was calculated and used for further analysis. The mean of four values obtained for each of the 44 control subjects was also used for further analysis.

Statistical analysis was by a one-way analysis of variance (ANOVA). Because the DPD/CREA values found for the different radiological stages were not independent of each other, the Friedman test was performed to compare the median DPD/CREA ratios of the study groups. In case of significance the pairwise Wilcoxon signed-rank test was used to compare the median DPD/CREA values of subsequent stages. To compare the DPD/CREA ratio of the control group with the DPD/CREA ratio in the different radiological stages, the Mann-Whitney test was performed. A p value < 0.05 was considered to be significant. To avoid spuriously significant results because of multiple testing, the significance level was adjusted using the Bonferroni method. Confidence limits for the percentiles were based on order statistics (ranks) as described by Hahn and Meeker. The statistical analysis was performed using SPSS 12.0 (SPSS Inc., Chicago, Illinois) and SAS 9.1.3 software (SAS Institute Inc., Cary, North Carolina).
Results

The test for normality showed that the DPD/CREA ratios of the study and control groups were not all normally distributed. Analysis of the intra-individual variability resulted in a within-subject root mean square error of 5.0 during the condensation stage, of 3.6 during the fragmentation stage, of 5.4 in re-ossification and of 10.5 in the final stage. The within-subject variability of the control children was 11.5. The median of the urinary DPD/CREA ratio in the control group was 29.1 nmol/mmol. In the study group it was 12.4 nmol/mmol during condensation, 19.6 nmol/mmol during fragmentation, 25.6 nmol/mmol during re-ossification and 33.4 nmol/mmol in the final stage (Table IV).

The initial stage was observed in only two children and was therefore not used in the following calculations. Seven children had complete observations for the remaining four stages. Using these children the Friedman test rejected the hypothesis of no change at all (p = 0.0003).

The pairwise Wilcoxon signed-rank test was used to compare the medians of the DPD/CREA ratios of subsequent stages of the disease. Again, only data from children with results for both stages which were considered in the pairwise comparisons were taken into account. To adjust for multiple testing, the results of the three pairwise comparisons were considered to be significant if the p values were smaller than 0.05/3 = 0.016. This was the case for all three comparisons, namely, the condensation and the fragmentation stages (n = 13, p = 0.001), the fragmentation and re-ossification stages (n = 21, p < 0.0001) and the re-ossification and final stages (n = 24, p < 0.0001). The result is clear, since each child showed an increase from one stage to the next, except for one at the change from the condensation to the fragmentation stage.

To compare each DPD/CREA ratio of the various radiological stages with the DPD/CREA ratio of the control group, the Mann-Whitney test was performed. Since four tests were carried out at a time, the level of significance for a difference in the medians of the DPD/CREA ratios was reduced to 0.0125 after Bonferroni correction. The median of the DPD/CREA value of the condensation stage was significantly lower than that of the control group (p < 0.0001). This was also the case for the fragmentation (p < 0.0001) and the re-ossification stages (p = 0.003). Only the median DPD/CREA ratio of the final stage did not differ significantly from the control group (p = 0.05).

Since the sample size was rather small in some stages, the exact versions of the tests were also computed. The results were almost the same, or better.

To analyse the relationship between urinary DPD excretion and the extent of epiphyseal necrosis during the fragmentation stage according to the classification systems of Catterall and Herring et al (Table V).

Discussion

Urinary cross-link levels reflect the turnover of bone. DPD is a degradation product of collagen type 1, which is found

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**Table IV.** Comparison of the urinary DPD/CREA ratio (nmol/mmol) in children with Perthes’ disease in different Waldenström radiological stages with that of a group of control children

<table>
<thead>
<tr>
<th>Waldenström stage†</th>
<th>IN</th>
<th>CO</th>
<th>FR</th>
<th>REO</th>
<th>FIN</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>2</td>
<td>25</td>
<td>26</td>
<td>30</td>
<td>24</td>
<td>44</td>
</tr>
<tr>
<td>Median†</td>
<td>N/A</td>
<td>12.4</td>
<td>19.6</td>
<td>25.6</td>
<td>33.4</td>
<td>29.1</td>
</tr>
<tr>
<td>Minimum</td>
<td>34.9</td>
<td>8.0 to 16.5</td>
<td>16.8 to 22.0</td>
<td>22.9 to 26.9</td>
<td>30.6 to 39.2</td>
<td>27.2 to 31.9</td>
</tr>
<tr>
<td>Maximum</td>
<td>36.9</td>
<td>24.8</td>
<td>32.8</td>
<td>35.2</td>
<td>54.0</td>
<td>64.6</td>
</tr>
<tr>
<td>5th percentile</td>
<td>N/A</td>
<td>5.4</td>
<td>7.3</td>
<td>18.3</td>
<td>19.2</td>
<td>17.7</td>
</tr>
<tr>
<td>95th percentile</td>
<td>N/A</td>
<td>24.7</td>
<td>32.5</td>
<td>35.1</td>
<td>52.6</td>
<td>61.0</td>
</tr>
<tr>
<td>p value</td>
<td>N/A</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.003</td>
<td>0.05</td>
<td>N/A</td>
</tr>
</tbody>
</table>

* IN, initial stage; CO, condensation; FR, fragmentation; REO, re-ossification; FIN, final; N/A, not applicable
† CI 95% confidence interval

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**Table V.** The DPD/CREA ratio (nmol/mmol) in relation to the extent of epiphyseal necrosis during the fragmentation stage according to the classification systems of Catterall and Herring et al. (n = 26)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Catterall 3</th>
<th>Catterall 4</th>
<th>Herring B</th>
<th>Herring C</th>
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<tbody>
<tr>
<td>Number</td>
<td>4</td>
<td>22</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Median</td>
<td>12.6</td>
<td>19.8</td>
<td>21.7</td>
<td>18.1</td>
</tr>
<tr>
<td>CI*</td>
<td>7.3 to 20.4†</td>
<td>18.1 to 23.0</td>
<td>18.2 to 29.5</td>
<td>11.4 to 19.9</td>
</tr>
<tr>
<td>Minimum</td>
<td>7.3</td>
<td>11.2</td>
<td>8.9</td>
<td>7.3</td>
</tr>
<tr>
<td>Maximum</td>
<td>20.4</td>
<td>32.8</td>
<td>32.8</td>
<td>22.0</td>
</tr>
</tbody>
</table>

* CI, confidence interval
† 88% cover
almost exclusively in calcifying tissues such as bone and dentine. It is the most specific marker of bone resorption. Our aim was to compare the urinary DPD level of children with Perthes’ disease with that of normal children. The median DPD/CREA ratio in the first morning urine sample in the control group was 29.1 nmol/mmol and the geometric mean was 30.2 nmol/mmol (17.7 to 64.6) which corresponds well to the results of Shaw et al\textsuperscript{29} who found a geometric mean of 31 nmol/mmol (reference range 7.1 to 135) in a similar age group.

We were able to show a relationship between the urinary DPD level and Perthes’ disease, but the median urinary DPD level did not, as hypothesised, reflect the bone-resorption process by an increase during the florid stage. On the contrary, we found that the lowest median DPD level occurred during the condensation stage in which the necrotic process had finished, and a continuous increase in the median urinary DPD level during the subsequent stages, in which the bone was remodelling. Therefore we concluded that the locally-increased bone degradation in the proximal femoral epiphysis with consecutive degradation of collagen and release of cross-links was minimal when compared with overall bone turnover.

The analysis of within-subject variance showed a relatively high variability for both the control and the study groups during the final stage. A possible explanation may be that the parents of the children in the control group did not comply with the instructions for the collection of the urine samples. In the same way the parents of the children in the study group, after healing of the hip disease, may not have complied with the protocol for urine collection.

There are many diseases in which there is an increased urinary DPD level, and a reduced level is found in children with growth hormone deficiency. During therapy with growth hormone the levels rise.\textsuperscript{20,21,30}

In children with Perthes’ disease, growth and developmental disorders are often observed. Many authors have reported skeletal retardation in Perthes’ disease mainly at the beginning of the disease which is more pronounced in younger children.\textsuperscript{31-35} In the later stages of the disease the skeletal maturation accelerates and at the end of the disease the skeletal age corresponds to the chronological age.\textsuperscript{36-38}

Studies concerning hormonal, especially growth hormone, metabolism in children with Perthes’ disease should give an explanation for the skeletal retardation. A disturbance of thyroid metabolism was excluded by Rayner, Schwalbe and Hall.\textsuperscript{39} The effect of growth hormone which is most important for longitudinal growth is mediated by the so-called somatomedins or insulin-like growth factors I and II (IGF\textsubscript{I} and IGF\textsubscript{II}). Somatomedin C is identical in structure and action to IGF\textsubscript{I}, whereas somatomedin A corresponds essentially to IGF\textsubscript{II} but may also include IGF\textsubscript{II}.

Studies concerning somatomedin metabolism in children with Perthes’ disease have given varying results. Normal somatomedin C/IGF\textsubscript{I} levels were found by Kitsugi et al\textsuperscript{41} and Kealey et al.\textsuperscript{42} Burwell et al\textsuperscript{43} and Harrison and Burwell\textsuperscript{44} found increased levels in boys with Perthes’ disease aged between three and five years, but not in those aged between six and 11 years. They also observed an absence of the physiological age-dependent increase in the serum activity of somatomedin. Decreased levels of somatomedin C were found by Motokawa\textsuperscript{45} and Neidel, Zander and Hackenbroch.\textsuperscript{46} In addition, the latter found no physiological age-related increase in somatomedin C during the early stages of Perthes’ disease.\textsuperscript{46,47} The limitation of all of these studies is that they do not comment on the stage of the disease when the blood sample was taken. Our study is the first to evaluate a metabolic parameter in relation to the radiological stage of the disease.

Our results show a decrease in the median urinary DPD/CREA ratio in the early stages of Perthes’ disease. The increase during the subsequent course of the condition corresponds well with the results of those authors who describe exactly this growing pattern in children with Perthes’ disease.\textsuperscript{36-38} The results of Neidel et al,\textsuperscript{46} with a decreased level of somatomedin C in the early stage, which is one possible explanation for the retardation in growth, corresponds well with the decreased median urinary DPD/CREA levels. The hypothesis of Burwell et al\textsuperscript{45} of a reduced number, or a reduced affinity, of somatomedin receptor sites on chondrocytes in growing skeletal tissues, explains a reduced bone turnover followed by a reduced urinary DPD excretion. Our results support those who propose that Perthes’ disease is not simply a local ischaemic disorder of the epiphysis of the femoral head in an otherwise healthy child, but is a systemic disorder of the growing child which contributes to a local structural reduction of mechanical strength of the femoral head.\textsuperscript{39,44,48,49}

Analysis of the urinary DPD/CREA ratio and the severity of Perthes’ disease resulted in significant differences when correlated with the Herring classification system.\textsuperscript{5} Children with more severe involvement of the femoral head (Herring grade C) showed significantly lower median DPD/CREA levels than grade B patients. This could be interpreted as indicating that a more severe femoral involvement follows a more pronounced reduction in bone turnover and therefore a more severe systemic disturbance. Comparison using the Catterall classification system\textsuperscript{4} was not possible because there were no patients in Catterall groups 1 and 2 and only a few in group 3. Further studies are needed to show whether or not the DPD/CREA level correlates only with the extent of the necrosis and the stage of the disease, but also with the final results and the prognosis. The DPD/CREA ratio could then be established as an additional tool for monitoring the course of the disease which until now has been followed only by clinical and radiological examinations every three to four months.

In conclusion we have demonstrated that the median urinary DPD/CREA ratio is significantly decreased in children with Perthes’ disease compared with control children, with the lowest level occurring during the condensation stage indicating a reduced bone turnover. This is in accor-
dance with the observation of skeletal retardation occurring mainly at the beginning of the disease. Further stage-dependent investigations are necessary to explain these results and to analyse whether or not the amount of decrease of DPD has any influence on the prognosis.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References