Serum prolidase activity in benign hypermobility syndrome

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**Serum prolidase activity in benign hypermobility syndrome**

**Objective:** Moderate joint laxity is widespread in many joints of the body, and this condition is considered to be caused by an abnormality in the collagen structure. The purpose of this study was to determine the serum prolidase activity in female patients with benign hypermobility syndrome (BJHS), evaluate the clinical features of these patients and investigate the correlation between clinical features and prolidase.

**Methods:** A total of 45 patients with benign hypermobility syndrome and 40 healthy controls were included in the study. All of the patients with BJHS met the Beighton diagnostic criteria. All the patients and the control group underwent a comprehensive examination of the locomotor system and took the New York Posture Rating Test. The examination and test results were recorded. Serum prolidase activity was measured in both of the groups.

**Results:** Prolidase activity was significantly lower in patients with BJHS compared to the healthy controls (479.52 ± 126.50, 555.97 ± 128.77, respectively; \( p=0.007 \)). We found no correlation between serum prolidase activity and Beighton scores or New York rating test scores. On the other hand, mean prolidase activity was significantly lower in patients with pes planus or hyperlordosis compared to those without ( \( p=0.05 \), \( p=0.03 \), respectively). We did not find such a correlation with the other clinical features.

**Conclusions:** Significantly lower prolidase activity in patients with benign hypermobility syndrome suggests that prolidase may affect the collagen metabolism and cause hyperlaxity.

**Key words:** hypermobility, ligament, New York rating test, prolidase

**Introduction**

Benign joint hypermobility syndrome is a hereditary disease characterised by musculoskeletal symptoms in patients with widespread joint laxity, independent of a systemic rheumatoid disease [1].

The frequency of BJHS varies with age, sex and ethnicity [2]. The frequency of BJHS varies between 5-57% in young females and 2-35% in males [3]. Besides, the incidence of BJHS is higher in females compared to males and decreases with age [4].

The prevalence of BJHS is 10% in the European community and 25% in the other ethnic groups [1].
Hypermobility may not cause any problems, but on the other hand, it may predispose some individuals to a wide variety of soft tissue injuries as well as internal joint derangements, arthritis, arthralgia and myalgia, which may require medical attention [5].

Range of motion in a joint depends on the collagen structure, surface of the joint and the neuromuscular tone [5].

Type I collagen is the most abundant collagen of the human body. Typically, type I collagen is highly found in such connective tissues as tendons, ligaments, joint capsules, skin, non-mineralised bones and nerve receptors with a high tensile strength [6]. It is suggested that moderate joint laxity is caused by an abnormal collagen structure [5].

BJHS may develop with some musculoskeletal symptoms which are affiliated with bones, tendons, muscles, ligaments, joints and spine. Impaired stability in the joint causes pathologies in the joint [7]. Insufficient support of the collagen connective tissue in the non-joint tissues may lead to certain clinical conditions such as instability, trauma-related damages and early degenerative changes, which are frequently seen in patients with BJHS [8].

Reduced thickness of collagen fibrils in patients with BJHS, as suggested by the electron microscopic examination of the skin biopsy, supports this view [9].

Collagen is an abundant protein of the body. It is a major component of skin, tendons, ligaments, joint capsules and blood vessels [10]. Ligaments are dense bands of articular tissues that connect bones together. Such ligaments as those found in the knee are hypocellular ligaments and are composed of type I and III collagens, proteoglycans, elastin and water [11]. Maximum possible range of motion is determined by the tension of ligaments, hence by their motion restricting features. Therefore, the primary underlying cause of the hypermobility is the ligament laxity [12].

Prolidase is an important enzyme that takes part in the collagen formation and degradation. It is particularly influential in the last stage where the imminopeptidases which contain C terminal proline and hydroxyproline split [13,14].

This enzyme promotes recycling of the residues of proline, acquired from collagen degradation. This recycling is particularly important to the collagen synthesis and the cellular growth [15].
Prolidase deficiency affects the recycling of residues of proline in the collagen re-synthesis [16,17], consistent with the findings by some earlier studies that have reported increased prolidase activity in the fibrotic process of the liver [18].

This evidence supports the view that prolidase, which supplies proline for collagen biosynthesis, can regulate the collagen turnover and act as a restricting factor in the regulation of collagen formation [19].

Patients with BJHS have impaired collagen structures [20]. Plasma prolidase might be an index of liver fibrosis in rats [21]. We considered that defects in the collagen structure might be responsible for ligament laxity in patients with BJHS, and decided to conduct this study, assuming that prolidase deficiency might lead to a reduction in the proline content of the collagen and change the collagen structure. We evaluated the existing clinical features in patients with BJHS, compared the patients to the healthy controls in regard to prolidase activity, and investigated the correlation between prolidase and clinical features.

**Materials and Methods**

**Study Population and Assessments**

A total of 45 female patients of reproductive age who were diagnosed as having joint hypermobility, and 40 healthy controls were included in the study. Ethical board’s approval was obtained, patients and healthy controls were fully informed about the study, and their written consent was taken before the study was performed. Exclusion criteria were as follows: pregnancy, breastfeeding, use of oral contraceptives, menstrual irregularity and existence of any neurologic, rheumatoid, skeletal, metabolic or collagen disease.

Medical histories of the participants were taken. Furthermore, the participants underwent physical examinations and were checked for routine hematologic and biochemical parameters. Physical examinations and assessments were conducted by the same team of physiatrist early in the morning, at the room temperature, with patients wearing only underwear and no shoes. Beighton scores were used in an effort to diagnose patients with BJHS (Table 1) [22]. The patients and healthy controls were assessed for the existence of joint pain, widespread pain throughout the body, carpal tunnel syndrome, joint subluxation, hyperkyphosis, hyperlordosis and pes planus. New York Posture Rating Test was used to assess the posture [23]. The
patients and healthy controls were examined from both sides i.e. from the front and the back. Posture changes which might occur in 13 different regions of the body were evaluated and scored. The participant was scored 5 if he/she had a straight posture, 3 if he/she had a moderately impaired posture and 1 if he/she had a severely impaired posture.

**Blood Sample**

Following the examination of locomotor system, the Beighton manoeuvres and the New York posture analyses, 3-4 ml of blood was taken from the antecubital veins of the patients to determine the prolidase activity. Serum was isolated from the blood after it was kept at room temperature for 30 minutes and centrifuged at 3000 rpm for 15 minutes. Serum samples were coded and kept at -40°C for further spectrophotometric analyses.

**Determination of Prolidase**

Levels of plasma prolidase (U/L) were determined by a spectrophotometric method that measured the proline levels produced by prolidase. The supernatant was diluted up to two folds by normal saline. Twenty-five microliters of mixture was preincubated with 75 µL preincubation solution (50 mmol/L Tris HCl buffer pH 7.0 containing 1 mmol/L glutathione and 50 mmol/L MnCl₂) at 37°C for 30 minutes. The reaction mixture containing 144 mmol/L gly–pro, pH 7.8 (100 µL), was incubated with 100 µL preincubated sample at 37°C for 5 minutes. 1 mL glacial acetic acid was added to the mixture in order to cease the incubation reaction. After adding 300 µL Tris HCl buffer, pH 7.8, and 1 mL ninhydrin solution (3 g/dL ninhydrin was melted in 0.5 mol/L orthophosphoric acid), the mixture was incubated at 90°C for 20 minutes and then cooled with ice. Later on, absorbance was measured at a 515 nm wavelength to determine the proline by using the method recommended by Myara et al.[18]. This method is a modified version of Chinard’s method [19]. The intra-assay and interassay coefficients of variation (CVs) were both lower than 7%.

**Statistical Analysis**

Measurement variables were expressed in mean ± standard deviation, while categorical variables were presented in numbers and percentages (%). Kolmogorow-Smirnov test was used to analyse the compliance of datasets with the normal distribution. Student-t test was
used to compare the mean values of the group that displayed a normal distribution, and Mann-Whitney U test was used for the group that did not. Furthermore, Spearman’s correlation test was used to assess the correlation between serum prolidase levels and joint pain, myalgia, carpal tunnel syndrome, shoulder impingement, joint subluxation, hyperkyphosis, hyperlordosis and pes planus. Varying frequencies among the categorical groups were evaluated by Chi-square test. Fisher’s exact test was used when the expected values were lower than 5. A $p$ value below 0.05 was regarded to be statistically significant. All the analyses were performed by Statistical Package for Social Sciences software version 15.0 for Windows.

**Results**

We found no statistically significant difference between the two groups in terms of age, weight, height and menstrual status ($p>0.05$). Mean serum prolidase level was significantly lower in the patients (479.52 ± 126.50 pg/mL) compared to the control group (555.97 ± 128.77 pg/mL) ($p=0.03$). New York Posture Rating Test scores were significantly lower in patients with BJHS (49.0 ± 16.2) compared to the control group (58.8 ± 3.5) ($p=0.001$) (Table 2).

Clinical features of the patients and the controls are shown in Table 3. The frequency of joint pain ($p=0.000$), myalgia ($p=0.01$), shoulder impingement ($p=0.05$), pes planus ($p=0.01$) and hyperkyphosis was significantly higher in patients with BJHS compared to the control group. No significant difference was found between the groups with regard to the frequency of other clinical parameters ($p>0.05$) (Table 3).

Serum prolidase levels were significantly higher in patients with BJHS who had pes planus compared to those patients who did not ($p=0.05$). Furthermore, serum prolidase concentration was significantly higher in patients with BJHS who had hyperlordosis compared to those patients who did not ($p=0.03$).

No significant correlation was found between the serum prolidase levels and age, BMI, Beighton scores and New York posture test scores.
Discussion

Collagen is the main component of ligament structure. Therefore, we chose to work on prolidase as an enzyme which can influence the collagen structure in patients with BJHS. In this study, we evaluated the serum prolidase activity and the locomotor system in an effort to investigate the collagen metabolism in female patients with BJHS. There is a study in the literature which evaluates the prolidase activity in children with hypermobility [24], but this is the first study to investigate the prolidase activity in adult females. What makes this study different from the others is its adult target group and its efforts to find out the correlation between prolidase and clinical features.

In this study, we found that mean serum prolidase activity was significantly lower in patients with BJHS compared to the healthy controls. Besides, the frequency of joint pain, myalgia, shoulder impingement, hyperkyphosis and pes planus was significantly higher in patients with BJHS. Serum prolidase activity was significantly low in patients with pes planus and hyperlordosis. On other hand, we found no significant correlation between prolidase and Beighton scores or New York posture test scores. Similarly, there was no significant correlation between prolidase and age, height or weight. Benign hypermobility is a clinical syndrome characterised by greater than normal active or passive range of motion in joints, independent of a systemic rheumatoid disease. The frequency of such conditions as arthralgia, myalgia, carpal tunnel syndrome, impingement syndrome, subluxation and ligament injuries is higher in patients with BJHS compared to normal individuals. These conditions are usually overlooked and underestimated in this population [25]. However, the quality of life may significantly be reduced and various symptoms such as anxiety and depression may occur in those patients suffering from chronic pain [26].

At the same time, increased laxity causes limitations in patients' functions, resulting in ligament injuries. Therefore, etiological studies and clinical assessments are significant in hypermobility. Race, ethnic origin, and environmental and genetic factors are considered to be responsible for the etiopathogenesis of BJHS [2]. Although various enzyme and hormone studies have been conducted in this area [27,28], no full light has been shed on the etiopathogenesis of BJHS.

Ligaments are hypocellular and consist of collagen, proteoglycans, elastin and water [11]. Such structural features as stiffness and flexibility of the ligament play an important role in
the creation of normal joint motions. Therefore, changes in the collagen structure and the resulting ligament laxity are considered to be highly responsible for hypermobility.

Prolidase enzyme plays an important role in the regulation of collagen metabolism. Glisin-prolin, a substrate of prolidase, exits in the collagen structure (29). The primary biological functions of this substrate are re-synthesis of collagen degradation products and re-cycling of proline from X-Pro dipeptides [30,31].

Polidase regulates the collagen turnover by supplying prolin for the collagen biosynthesis, and has a restrictive role in the formation of collagens [19].

In a study conducted on children with joint hypermobility, who met the Beighton diagnostic criteria, Yazgan et al. found lower prolidase activity in the patients compared to the controls, which was not statistically significant [24] In another study, they found lower prolidase activity in osteogenesis imperfecta skin fibroblasts [19]. Both of the studies concluded that decreased levels of prolidase activity as such might affect the cellular growth and the collagen metabolism. Similarly, we found significantly lower prolidase activity in patients with BJHS in our study, suggesting that prolidase might affect the collagen metabolism, partially resulting in changes in the ligament structure and paving the way for hypermobility.

Clinical signs and symptoms such as pes planus, hyperkyphosis, subluxation and impingement have an increased frequency in patients with BJHS compared to the normal population [3]. All the clinical signs and symptoms evaluated under this study were significantly higher in patients with BJHS. Besides, such clinical features as joint pain, myalgia, shoulder impingement and hyperkyphosis had a significant correlation with the joint laxity in these patients.

The number of studies that have evaluated the joint and non-joint symptoms in patients with BJHS is rather limited. In their study, Mishra et al. found that 31% of the patients with BJHS had joint pain, while 10% had ligament injury, another 10% had tendinopathy and 4% had subluxation [32]. Furthermore, Shari et al. found that the frequency of joint pain was %35.4 among patients with BJHS [33], whereas Yazgan et al. concluded that 30% of the patients with BJHS had joint pain, 10% had pes planus and 4% had myalgia [24]. Reviewing the literature, we have not seen any study which evaluates the frequency of carpal tunnel syndrome, hyperkyphosis and hyperlordosis in patients with BJHS. Our findings support the literature data about joint pain and tendinopathy. On the other hand, we found lower values
than those in the literature particularly about myalgia, pes planus and subluxation, which can be attributed to the genetic variations and environmental factors.

A person’s posture is determined by internal and external factors. Intrinsic factors of BJHS may allow for extreme joint motions, resulting in abnormal positions which may be acquired at subconscious level and turn into being a habit in time [34]. A study which investigated the postures of patients with BJHS found significant differences between the patients with BJHS and the control group with regard to posture scores and pain [34]. Because BJHS is painless at the outset, abnormal positions which may lead to chronic pain are overlooked in many cases. We used a qualitative method, namely New York posture rating test, in evaluating the postures of patients. We also found significantly lower posture scores in patients with BJHS compared to the control group. We attributed significant joint pain and widespread pain throughout the body to the impaired postures of the patients. Poor posture is significant in the management of BJHS, and its long term effects must be taken into account when planning a treatment for such patients.

Being a cross sectional study is one of the restrictive factors in our study. Another restrictive factor is that our study did not involve any male patients. Given the fact that hypermobility is more prevalent among females, we could not find enough number of male patients and excluded this group from the study.

Conclusions

Prolidase is an important enzyme that takes part in the collagen formation and degradation. It is particularly influential in the last stage where the imminopeptidases which contain C terminal proline and hydroxyproline split. We performed this study, assuming a possible correlation between collagen defects and serum prolidase activity in patients with BJHS. We found that serum prolidase activity was significantly lower in the patients compared to the healthy controls. Therefore, we suggest that lower serum prolidase activity may be an important etiologic factor in the etiopathogenesis of BJHS. However, in order to attain further knowledge about the role of prolidase in joint and the process of the disease, more comprehensive studies on a wider range of population are required.
References


<table>
<thead>
<tr>
<th>Task</th>
<th>Right</th>
<th>Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Passively dorsiflex the fifth metacarpophalangeal joint to ≥90°</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2. Oppose the thumb to the volar aspect of the ipsilateral forearm</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3. Hyperextend the elbow to ≥10°</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4. Hyperextend the knee to ≥10°</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5. Place hands flat on the floor without bending the knee</td>
<td>1</td>
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</tr>
<tr>
<td>Total possible score</td>
<td>9</td>
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</table>
Table 2 Prolidase levels and demographic characteristics of the study groups (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Hypermobile (n=45)</th>
<th>Normal (n=40)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.26 ± 6.69</td>
<td>25.45 ± 7.14</td>
<td>0.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>55.63 ± 8.91</td>
<td>55.73 ± 8.13</td>
<td>0.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.6 ± 5.01</td>
<td>161.32 ± 4.15</td>
<td>0.9</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>21.32 ± 3.55</td>
<td>21.41 ± 3.06</td>
<td>0.5</td>
</tr>
<tr>
<td>Prolidase (pg/mL)</td>
<td>479.52 ± 126.50</td>
<td>555.97 ± 128.77</td>
<td>0.007</td>
</tr>
<tr>
<td>New York posture</td>
<td>49.04 ± 16.22</td>
<td>58.82 ± 3.56</td>
<td>0.001</td>
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<tr>
<td>Rating test scor</td>
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</tbody>
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BMI, Body mass index. Significance was defined as \( p < 0.05 \).
Table 3 The clinical features of study groups

<table>
<thead>
<tr>
<th></th>
<th>Hypermobility n=45 (%)</th>
<th>Normal n=40 (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthralgia</td>
<td>15 (33.3)</td>
<td>10 (25)</td>
<td>0.00</td>
</tr>
<tr>
<td>Myalgia</td>
<td>25 (55.6)</td>
<td>11 (27.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Carpal tunnel syndrome</td>
<td>4 (8.9)</td>
<td>2 (5)</td>
<td>0.11</td>
</tr>
<tr>
<td>Impingement syndrome</td>
<td>5 (11.1)</td>
<td>3 (7.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Subluxation</td>
<td>3 (6.7)</td>
<td>0 (0)</td>
<td>0.24</td>
</tr>
<tr>
<td>Hyperlordosis</td>
<td>30 (66.7)</td>
<td>28 (70)</td>
<td>0.818</td>
</tr>
<tr>
<td>Pes planus</td>
<td>26 (57.8)</td>
<td>12 (30)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hyperkyphosis</td>
<td>28 (62.2)</td>
<td>9 (22.5)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Significance was defined as p < 0.05.
Tablo 4 The levels of prolidase associated with clinical symptoms and findings

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Prolidase, (mean ± SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthralgia</td>
<td>452.34±121.78</td>
<td>513.51±127.04</td>
</tr>
<tr>
<td>Myalgia</td>
<td>452.87±124.96</td>
<td>509.98±124.18</td>
</tr>
<tr>
<td>Carpal tunnel syndrome</td>
<td>483.36±136.82</td>
<td>479.15±127.27</td>
</tr>
<tr>
<td>Impingement syndrome</td>
<td>468.72±116.56</td>
<td>480.87±129.01</td>
</tr>
<tr>
<td>Subluxation</td>
<td>455.70±45.10</td>
<td>481.23±130.50</td>
</tr>
<tr>
<td>Hyperlordosis</td>
<td>422.66±121.60</td>
<td>507.96±120.76</td>
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<tr>
<td>Pes planus</td>
<td>437.23±116.09</td>
<td>510.43±126.92</td>
</tr>
<tr>
<td>Hyperkyphosis</td>
<td>477.34±132.40</td>
<td>483.11±120.02</td>
</tr>
</tbody>
</table>

Significance was defined as p < 0.05.