URINARY GLYCOSAMINOGLYCANS IN SYSTEMIC LUPUS ERYTHEMATOSUS: MARKER OF RENAL AFFECTION AND RELATION TO DISEASE ACTIVITY

HEBA ABDUL-WAHAB SELIEM, OMAYMA ZAKAREYA SHEHATA, AHMAD ABDUL-MONIEM EMEIRA AND YOUSRY ABUL-MAGD*

Rheumatology & Rehabilitation and Biochemistry* Departments Zagazig University Faculty of Medicine

KEY WORDS: URINARY GLYCOSAMINOGLYCANS, LUPUS NEPHRITIS, SLE DISEASE ACTIVITY.

ABSTRACT

Objective: To evaluate glycosaminoglycans (GAG), heparan sulphate (HS) and chondroitin sulphate (CS) levels in the urine of systemic lupus erythematous (SLE) patients. Also, to determine its possible use as a marker for lupus nephritis and its correlation with disease activity.

Methodology: This study was conducted on 30 patients suffering from SLE. They were subdivided according to disease activity and renal affection. Ten apparently healthy subjects were taken as a control group. GAGs were isolated from urine with ion exchange chromatography on DEAE sephacel. Determination of HS and CS levels were done with ELISA.

Results: There was a significant increase of GAGs and HS levels in SLE patients than in controls (p<0.05). There was a highly significant difference (p<0.01) between active and inactive SLE patients as regards CS/HS ratio. GAGs and CS/HS ratio were significantly higher in active patients with lupus nephritis (p< 0.001).

Conclusion: Urinary GAGs may represent an additional, non-invasive diagnostic approach for lupus nephritis. It could be used as a parameter for disease activity and lupus nephritis.
INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by loss of immunologic self-tolerance and subsequent development of autoantibodies (Gehi, et al., 2003). This autoimmune process plays a crucial role in the pathogenesis of SLE (Kozak, et al., 2000). Autoantigens in SLE are highly diverse in terms of structure and location in control cells (White & Rosen, 2003). Lupus nephritis (LN) remains a leading cause of morbidity and mortality in SLE. Progression to LN in SLE is dependent on the host breaking immune tolerance and forming autoantibodies that deposits in the kidney (Oates and Gitkeson, 2002).

Glycosaminoglycans (GAGs) are highly negatively charged molecules, with extended conformation that imparts high viscosity to the solution. GAGs are located primarily on the surface of cells or in the extracellular matrix. Along with the high viscosity of GAGs comes low compressibility, which makes these molecules ideal for a lubricating fluid in the joints. At the same time, their rigidity provides structural integrity to cells and provides passageways between cells, allowing for cell migration (Michael & Marchesini, 2003).

Proteoglycans (PG) play a role in the control of the anatomic and functional integrity of the kidney. PG, and in particular heparan sulphate proteoglycans (HS-PG), play an important role in the control of charge. Selectivity in the glomerular capillary wall is an important component of the glomerular basement membrane (De Muro et al., 2001).

In normal subjects GAG can be detected in considerable amounts in the urine, but only in very low concentrations in the serum (Mitsuhashi et al., 1993).

Most investigations indicate that urinary GAG originates form the plasma by glomerular filtration, although part of the HS fraction may originate form renal tissue (De Muro et al., 2001).

An increase in urinary GAG, heparan sulfate and chondroitin sulfate are considered to be markers of early renal involvement (Ilhan et al., 2003)

Aim Of The Work:

To evaluate glycosaminoglycans (GAG), heparan sulphate (HS) and chondroitin sulfate (CS) levels in the urine of systemic lupus erythematosus patients. Also, to determine its possible use as a marker for lupus nephritis and its correlation with disease activity.
SUBJECTS AND METHODS

This study was carried out on 40 subjects: 30 had systemic lupus erythematosus (SLE) and ten healthy volunteers who were matched in age and sex with patients and served as a control group. All subjects were selected from the patients attending the Outpatient and Inpatient Clinic of the Rheumatology & Rehabilitation Department, Zagazig University Hospitals.

The SLE patients were 29 females and one male, their age ranged from 16 to 45 years with a mean value of (26.2 ± 8.2) years, and disease duration ranged from 6 months to 11 years with a mean value of (26.2 ± 8.2) years. All patients fulfilled the American College of Rheumatology (ACR) revised criteria for classification of SLE (Tan et al., 1982).

The controls were 2 males and 8 females, their ages ranged from 25-45 years with a mean of 3.3 ± 2.7 years.

SLE patients were subdivided according to disease activity index (Morrow et al., 1982) into:

Group I: Inactive: 17 patients.
Group II: Active: 13 patients.

We re-divided them according to renal involvement into 4 subgroups as follow:

Group I (Inactive): I a- with renal involvement 7 (23.3%).
I b- without renal involvement 10 (33.3%).

Group II (Active): II a- with renal involvement: 6 (20%).
II b- without renal involvement 7 (23.3%).

All patients did not suffer from any diseases that might affect urinary GAG as diabetes mellitus or liver diseases. None of them was taking drugs that can interfere with GAG synthesis.

All patients and controls were subjected to complete history taking and thorough clinical examination.

They underwent laboratory investigations as:

- Complete blood picture.
- Complete urine analysis (24 hours proteinuria).
- Kidney function tests (urea and creatinine levels)
- ANA and anti DNA antibody.

Thirteen patients had lupus nephritis, diagnosed on the basis of proteinuria, hematuria, increased anti DNA titer and impaired renal functions. GAGs were isolated from urine with ion exchange
chromatography on DEAD-sephacel (Pharmacia) according to (Staprans et al., 1981). The GAG composition was determined, after solubilization with water, with electrophoresis on acetate cellulose strips in a discommons buffer. Bitf composition was expressed in terms of relative percentages based on densitometric scanning of alcien Blue stained strips using a scan analysis program. GAG identification was performed by treating aliquots of the sample (containing about 100 μg of hexuronate) at 37ºC for 18 hs before electrophoresis with specific elements.

Determination of heparan sulfate (HS) and chondroitin sulfate (CS) levels were done with Enzyme-linked immunosorbent assay.

All data were coded; entered and analyzed using EPIINFO (version 6.1) software computer package (Dean et al., 1999).

**RESULTS**

Table (1): Clinical findings of the SLE patients.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthralgia and/or myalgia</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>Raynaud's phenomenon</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>Vasculitic skin rash</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>Neuropsychiatric manifestations</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>Serositis</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Bleeding tendency</td>
<td>2</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Table (1) shows the clinical findings of SLE patients while table (2) shows significant statistical difference (p < 0.05) between SLE patients and control group as regard total urinary GAG level and HS level, while no statistical difference was found between the two groups regarding CS level. But there was a highly significant difference (p< 0.001) between SLE patients and the control group as regards the CS/HS ratio.

In table (3) the levels of GAG, Cs and Hs were highly significantly elevated (p< 0.001) in active SLE patients than in the inactive group. Also, the Cs / Hs ratio had a significant statistical difference (p< 0.05) between the active and inactive patients.
Table (2): Shows comparison between SLE patients and control group as regard total urinary GAG level, Hs, Cs levels and Cs / Hs ratio.

<table>
<thead>
<tr>
<th></th>
<th>SLE patients</th>
<th>Control</th>
<th>T</th>
<th>p</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary GAG level</td>
<td>Range 5.88 – 19.33</td>
<td>4.39 -12.01</td>
<td>2.2</td>
<td>&lt; 0.05</td>
<td>Sig.</td>
</tr>
<tr>
<td></td>
<td>X ± SD 10.9 ± 4.79</td>
<td>7.4 ± 2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hs level</td>
<td>Range 2.28 – 7.11</td>
<td>1.59 – 3.50</td>
<td>3.3</td>
<td>&lt; 0.05</td>
<td>Sig.</td>
</tr>
<tr>
<td></td>
<td>X ± SD 4.15 ± 17</td>
<td>2.35 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cs level</td>
<td>Range 2.01 – 5.33</td>
<td>2.13 – 4.67</td>
<td>0.65</td>
<td>&gt; 0.05</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td>X ± SD 3.4 ± 1.2</td>
<td>3.1 ± 0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cs / Hs ratio</td>
<td>Range 0.73 – 1.33</td>
<td>1.31- 1.39</td>
<td>6.86</td>
<td>&lt; 0.001</td>
<td>H.S.</td>
</tr>
<tr>
<td></td>
<td>X ± SD 0.85 ± 0.20</td>
<td>1.33 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Shows comparison between urinary GAG level, Cs, Hs levels and Cs/ Hs ratio in active and inactive SLE patients.

<table>
<thead>
<tr>
<th></th>
<th>GAG level</th>
<th>Cs level</th>
<th>Hs level</th>
<th>Cs / Hs ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± SD</td>
<td>X ± SD</td>
<td>X ± SD</td>
<td>X ± SD</td>
</tr>
<tr>
<td>Inactive</td>
<td>8.7 ± 3.2</td>
<td>2.7 ± 0.8</td>
<td>3.6 ± 1.1</td>
<td>0.75 ± 0.008</td>
</tr>
<tr>
<td>Active</td>
<td>18.6 ± 0.6</td>
<td>5.2 ± 0.15</td>
<td>6.9 ± 0.2</td>
<td>0.99 ± 0.3</td>
</tr>
<tr>
<td>T.</td>
<td>10.19</td>
<td>10.81</td>
<td>11.58</td>
<td>3.4</td>
</tr>
<tr>
<td>P.</td>
<td>&lt; 0.001 (Hs)</td>
<td>&lt; 0.001 (Hs)</td>
<td>&lt; 0.001 (Hs)</td>
<td>&lt; 0.05 (sig.)</td>
</tr>
</tbody>
</table>

Table (4): Comparison between SLE Subgroups and control group as regard urinary GAG, CS, HS level and CS / HS ratio in relation to renal involvement.

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Urinary GAG level</th>
<th>CS level</th>
<th>Hs level</th>
<th>CS / HS total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X ± SD</td>
<td>X ± SD</td>
<td>X ± SD</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>7.42 ± 2.6</td>
<td>2.74 ± 0.8</td>
<td>2.35 ± 0.6</td>
<td>1.336 ± 0.02</td>
</tr>
<tr>
<td>Subgroup I: in active</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. With renal involvement</td>
<td>7</td>
<td>9.15 ± 2.1</td>
<td>3.4 ± 0.7</td>
<td>4.56 ± 1.0</td>
<td>0.75 ± 0.008</td>
</tr>
<tr>
<td>b. Without renal involvement</td>
<td>70</td>
<td>6.6 ± 0.6</td>
<td>2.1 ± 0.15</td>
<td>2.86 ± 0.2</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>Subgroup II: active</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. With renal involvement</td>
<td>6</td>
<td>17.9 ± 2.4</td>
<td>4.9 ± 0.6</td>
<td>6.6 ± 0.8</td>
<td>1.27 ± 0.75</td>
</tr>
<tr>
<td>B. Without renal involvement</td>
<td>7</td>
<td>11 ± 3.02</td>
<td>3.71 ± 0.7</td>
<td>2.9 ± 0.4</td>
<td>0.75 ± 0.004</td>
</tr>
<tr>
<td>F</td>
<td>10.85</td>
<td>79.85-</td>
<td>53.44</td>
<td>209.38</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>
Our results in table (4) revealed that urinary GAG, CS, HS levels and CS / HS ratio were higher in SLE patients with active and renal involvement than other subgroups, with a higher significant difference between SLE subgroups and control group (p< 0.001).

**DISCUSSION**

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that can involve multiple organ systems. The kidney is the most common visceral organ affected by SLE (Trouw et al., 2004). Lupus nephritis remains a major cause of morbidity and mortality in SLE patients (Cortes et al., 2003).

Glycosaminoglycans (GAGs) are heteropolysaccharides present as integral components of the extra cellular matrix (ECM) and basement membranes. GAGs play an important role in the immune and inflammatory response because of their ability to interact with cytokines and chemokines, promoting the localization of these molecules onto ECM or cell membrane at specific anatomical sites (Fernandez et al., 2002).

Since GAGs originate from different kinds of connective tissues, their measurement in urine may be useful to evaluate the metabolic state of various organs (Ilhan, et al., 2003). The urine contains several forms of GAGs but the vast majority of them are either chondroitin sulphate or heparan sulphate (Tencer et al., 1997).

Our work was performed in an attempt to evaluate glycosaminoglycans (GAG) level in the urine of systemic lupus erythematosus (SLE) patients with and without renal involvement and to determine its role as a marker for lupus nephritis and its correlation with disease activity.

In this study, it was found that there was a significant difference between SLE patients and control groups as regard the total urinary GAGs levels (p< 0.05).

This result coincided with the results of the studies of (Parildar et al., 2003, Ilhan et al., 2003 and De Muro et al., 2001) who found that urinary GAGs levels were higher in lupus patients than in control groups.

In our study, there was a significant difference between SLE patients and controls as regard HS levels (p<0.05) while there was no statistical difference between the two groups regarding CS levels. These results coincided with the results of the studies (Parildar et al., 2003 and De Muro et al., 2001) who observed a reduction of CS levels in urine of SLE patients while HS levels were higher than in controls.
It may be hypothesized that the reduced CS urinary excretion is due to decreased glomerular synthesis of GAGs or too increased incorporation in different pathological glomerular structures as observed by Tancer et al. (1997).

Also, in this work, we found that urinary GAGs levels were higher in active SLE patients with renal affection as compared to subgroups of SLE patients and control groups. These results are in agreement with (Parildar et al., 2003) who found that total urinary GAGs levels were higher in the lupus nephritis patients and they also found that GAGs values in cases 3 nephritis were higher than both class 2 and 4 nephritis. These results also coincided with the results of the study done by (Ilhan et al., 2004).

In contrast (DeMuro et al., 2001) found that urinary GAGs levels were higher in SLE patients with extra renal affection without renal affection as compared to the control group and they hypothesized that this could have been due to activation of T-lymphocytes.

Also, we found in our study a highly significant difference between SLE subgroups and the control group (p< 0.001) as CS and HS levels in relation to especially active patients with renal involvement. These results agreed with the results of the study of (Ilhan et al., 2003).

Finally, we found that the ratio between CS/HS was higher in active SLE patients than inactive patients with the highest value in active patients with renal affection.

These results agreed with the results of the study done by DeMuro, et al. (2001) who found this ratio to be significantly reduced in SLE patients in remission but, he also, observed that the CS/HS ratio was independent of renal involvement.

**Conclusion:**

The analysis of urinary GAGs may represent an additional, non-invasive diagnostic approach in SLE patients. It might indicate the presence of an early abnormal permeability of the renal filter in patients without other appreciable signs of kidney alteration, and there is strong correlation with disease activity.

**Recommendation:**

We recommend evaluating the urinary GAG excretion in a group of untreated SLE patients and studying a larger number of patients with close observation and evaluation.
REFERENCE


مركب الجليكوژ أمينوجليكازن في بول مرضي الذنبة الحمراء كمؤشر لتاثر الكلى وعلاقته بمدى نشاط المرض

أبو المجد

فما الذي يحدث في هذا المرض؟

 byćğı, oğul, 

مرضاً ذنبية حمراء هو مرض مناعي مزمن يؤثر على العديد من أجزاء الجسم ويتميز بإنتاج أقسام مضادة ذاتية والتي تدورها مع وسائط الانكماش تدمر الأنسجة. وتعتبر إصابات الكلى في مرض الذنبة الحمراء من أجزاء المصابات التي تسبب اعصار الجسم المختلفة في هذا المرض، كما تعتبر أيضاً أحد الآثار الرئيسية تدمر هذا الالكلي، وتتأثر الكلى إلى حد ما في معظم المرضى وترجع هذا التأثر ما بين أصابع طفيفة إلى نسيج كلي واضح. ومركب الجليكوژ أمينوجليكازن يعتبر جزء رئيسي من القالب الخارجي للخلايا ويتعرض من الجسم عن طريق البول.

والهدف من إجراء هذا البحث: هو قياس نسبة مركبات الجليكوژ أمينوجليكازن في البول وعلاقته بمدى نشاط مرضي الذنبة الحمراء وتقييم علاقته أيضاً بتاثر الكلى بالمرض وقد أجريت الدراسة على 30 مريضاً يعانون من مرض الذنبة الحمراء وعشرة أشخاص أصحاء، اخذوا كمجموعة ضابطة.

وقد أظهرت الدراسة وجود علاقة ذات دلالات إحصائية لمستوي مركب الجليكوژ أمينوجليكازن في البول لمريضي الذنبة الحمراء وعناء في الأصحاء كما توجد علاقة ذات دلالات إحصائية واضحة في المرضى أثناء نشاط المرض خاصة عند تأثر الكلى.

وأظهرت أيضاً النتائج أن مستوي الهي بالر ين سلبى و الكتيربيوتين سلبب في البول والنيجة بينه ترتفع في مرضي الذنبة الحمراء أيضاً أثناء نشاط المرض وعند تأثر الكلى.

ومن هذه النتائج: تستطيع أن نستنتج أن قياس مستوى كل من الجليكوژ أمينوجليكازن والهيبارين سلفينت و الكنديروتين سلفينت والنسبة بينهم في البول في مرضي الذنبة الحمراء يمكن استخدامه كدليل على نشاط المرض وتاثر الكلى.