# The Value of Glycosamynoglycan as a Tumor Marker

## GLİKOZA M İNOG LİKA NIN TÜMÖR MARKER! OLARAK DEĞERİ

Müge A K M A N S U *, Barbaros B A L A B A N L I * *, Ahmet DİRİER*, Nurten TÜRKÖZKAN**, Hüseyin BORA*, İbrahim EGEHAN*

* Depts. of *Radiation Oncology, **Biochemistry, Medical School of Gazi University, Ankara, T U R K E Y

**Summary**

*Some authors have proposed the use of urinary glycosaminoglycan (GAG), as a sensitive and specific tumor marker for bladder cancer and Wilms' tumor. To test the utility of urinary glycosaminoglycan as a tumor marker, we measured the glycosaminoglycan fraction in 24 hours' urine with Whiteman's method. There were 16 patients diagnosed to have malignancy and have not been previously treated in the study group and 20 healty human subjects in the control group. The mean 24-hours' GAG value was found to be 70.2±28.7 mg (27.1-126) in patient group and 16.9±5.3 mg in control group (p<0.01). Since values of patients are statistically higher than controls we are in the opinion that urinary GAG values are neither specific nor sensitive for bladder tumors or the Wilms tumor. However, our study is a preliminary one. In order to obtain net results, the number of malignancy groups and number of cases in each group must be increased.*

Key **Words:** Glycosamynoglycans, Tumor marker

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**Özet**

*Bazı araştırmacılar tarafından idrar glikozaminoglikan miktarlarının ölçümü Wilms' tümörü ve mesane tümörleri için spesifik ve sensitif tümör markerı olarak değerlendirilmektedir. Bu değerlendirmenin doğruluğunu incelemek amacı ile 24 saatlik idrarda Whiteman Metodu ile glikozaminoglikan değerleri ölçüldü. Çalışmaya kontrol amaçlı 20 sağlıklı insan ve malignité tanısı almış halen makroskopik tümörü bulunan daha önce tedaviye başlanmamış 16 hasta alındı. Bu çalışmaya dahil edilen hastaların 24 saatlik idrar glikozaminoglikan değerlerinin ortalaması 70.2±28.7 (27.1-126) ve kontrol grubunun ortalaması ise 16.9±5.3 idi (p<0.01). Malignité grubunun sonuçları istatistiki olarak daha yüksek bulunduğu için bizim düşüncemize göre idrar glikozaminoglilcan değerlerindeki yükseklik Wilms' tümörleri ve mesane tümörleri için ne spesifik ne de sensitif olarak yorumlanamaz. Ancak bizim çalışmamız bir ön çalışmadır ve daha net sonuçlara ulaşmak için grup sayısı ve her gruptaki hasta sayısı artırılmalıdır.*

**Anahtar Kelimeler:** Glikozaminoglikanlar, Tümör marker

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Glycosaminoglycans (GAG) are big complexes made up of small amounts of protein and heteropolysaccharide chains. These complexes constitute a jel like matrix capable of binding water. Hence, the viscous and slippery properties of mucous secretions are due to the presence of glycosaminoglycans. These negatively polarized heteropolysaccharide chains glide and get away from each other just like two magnets with the same polarization. This is what maintains the slippery state of mucous secretions and the synovial fluid. When the glycosaminoglycan solution is compressed, its water content separates and they occupy a smaller volume. When they are decompressed, they readily return to their old volumes by the negative polarizations pushing each other. This property secures the elasticity of the synovial fluid and the aquous humor of the eye (1,2).

The type and quantity of these compounds differ in mature normal tissues from those found during embryonic development (3). Glycosaminoglycans are of the 6 types according to the glycoside bonds and sulphate units they contain. These are briefly; 1) Condroitin-4-sulphate and condroitin-6-

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**Correspondence:** Miige AKMANSU
§airNedim Sok. 21/8
06690 A.Ayranci
Ankara, TURKEY

sulphate; the most abundantly found glycosaminoglycan in the body (cartilage, tendon ligament and the aorta), 2) Keratan sulphate; the most heterogenous of the glycosaminoglycans (the proteoglycan aggregates of the cartilage, the cornea), 3) Hyaluronic acid; maintains slipperiness, reduces the effects of blowes (synovial fluids of joints, humor aquous of the eye, umblical cord blood and loose areolar tissue), 4) Dermatan sulphate (skin, blood vessels and heart valvules), 5) Heparine; especially has anticoaqulant function in the liver, lung and skin, 6) Heparan sulphate (basal membranes and all extracellular surfaces) (2,4).

The polysaccharide chains of the glycosaminoglycan are prolonged through some reactions by spesific transferases. The synthesis of glycosaminoglycan are similar to the synthesis of glycogens, the difference is that glycosaminoglycans are synthesized for extracellular excretion but the synthesis is realized in the cell endoplasmic reticulum and golgy apparatus. The lysis of glycosaminoglycan take place in the lysosomes. In vivo, glycosaminoglycans are not only present in the extracellular matrix but they are present on the cell surface as well (2,4).

In the last 20 years biochemical and histochemical studies regarding glycosaminoglycans have been successfull in determination of the amount of glycosaminoglycans in samples from urine, serum or tissue by using ELISA-like or other methods (1,5).

As can be seen from a study performed in rabbit bladders, especially the chondroitin sulphate and hyaluronic acid types of glycosaminoglycans are densely found in the lamina propria and muscle layers (6). This is in accordance with the human bladder and while hyaluronic acid and dermatan sulphate are the major glycosaminoglycans in the normal human bladder epithelium and the submucosa, dermatan sulphate and heparan sulphate are abundantly found in the normal bladder muscle (7).

In view of this knowledge it has been searched in recent years whether glycosaminoglycans are of value as tumor markers in bladder and kidney tumors (1,7-9).

In this study in order to investigate the tumor marker value of 24-hour urine glycosaminoglycan (GAG) values, GAG levels in various cancer patients have been analysed.

## Materials-Methods

In this study 16 cancer patients who came to Gazi Hospital Radiation Oncology Department and had indication of radiotherapy and 20 healty human subjects as the control group have been included. Of these patients eleven were diagnosed as head and neck tumor, one central nervous system tumor, one non-small cell lung cancer, one recurrent seminoma, one soft tissue sarcoma and one rectum tumor. In all of these patients macroscopic tumor was present prior to radiotherapy. In all of the patients 24-hour urine were collected before beginning therapy and glycosaminoglycan values were detected according to the Whiteman's method (10). For measurement of glycosaminoglycan excretion, a final product in urine, 24-hour urine samples were kept at -20°C without a preservative. The glycosaminoglycan determination method developed by Whiteman was modified. According to this method, the standart glycosaminoglycan solution and centrifuged urine (200 ml) were mixed with 4 ml newly prepared 0.05% w/u alcian b!ue 8Gx and 50 mM Na acetate buffer (pH:5.8). This mixture was kept in room temperature for 2 hours. Following twenty minutes of centrifugation at 2000g the supernatant precipitate was washed two times with ethanol. Then by using 5% Na dodecyl sulphate (4ml), it was read at 620 nm in a 1 cm microcuvette.

## Results

The average urinary GAG values in our control group composed of twenty healthy people were found to be 16.9±5.3 mg/24 hours. These results are in accordance with the value range of the normal population measured in other studies performed by the same method (11,12).

Glycosaminoglycan values of our patients are seen in Table 1. The patients' values in 24-hours urine varied between 27.1 and 126mg (70.2±28.7) and were statistically higher than control group ($p<0.01$)(Table2).

In statistical evaluation of the data, the $X^2$ test in the SPSS computer statistics package program has been used.

**Table 1.** Urinary GAG values of cancer patients

| Number of the patients and their tumor site | GAG (mg/24hour) Mean:70.2±28.7 |
|---|---|
| 1 nasopharynx | 126.0 |
| 2 nasopharynx | 66.0 |
| 3 larynx | 27.1 |
| 4 larynx | 69.7 |
| 5 larynx | 67.5 |
| 6 larynx | 57.4 |
| 7 larynx | 39.3 |
| 8 larynx | 61.8 |
| 9 nasopharnx | 41.7 |
| 10 nasopharnx | 91.4 |
| 11 hypophysis | 54.3 |
| 12 CNS | 90.0 |
| 13 lung | 96.0 |
| 14 testis | 50.0 |
| 15 soft tissue | 126.0 |
| 16 rectum | 58.0 |

**Table 2.** Mean±SD values of urinary GAG in control and patient groups ($p<0.01$)

| | Control group (n:20) | Patient group (n: 16) |
|---|---|---|
| GAG (mg/24 hours) | 16.9±5.3 | 70.2±28.7 |

## Discussion

Until now, many elements including monoclonal antibodies and cell surface antibodies have been employed as a tumor marker in the diagnosis and follow-up of various tumors. A tumor marker must be both sensitive and spesific for a tumor type, must be inexpensive, noninvasive at the same time. It must be possible to use it for screening. As yet the tumor marker which meets this aim is prostat spesific antigen (PSA). All other substances which can be used as a tumor marker are not 100% spesific and sensitive. Sensitivity is the proportion designated positive by the screening test among all individuals who have the disease, whereas spesificity is the proportion designated negative by the test among all those who do not have the disease.

In many recent studies urinary glycosaminoglycan values were described as having up to 92% spesificity and sensitivity as tumor markers in bladder, Wilms' and some renal tumors (1,13,14). However, it has been determined that glycosaminoglycans increase not only in urologic tumors but in some other tumor groups as well. In a study undertaken by Hernandez, glycosaminoglycan detection was made in the bronchoalveolar washing fluid in bronchogenic carcinomas. Those patients included in that study were 81 patients with a malignant and 34 patients with a benign diagnosis of lung disease. As a result of a series of analyses, glycosaminoglycan values were found to be higher if there is an accompanying infection in benign diseases as well. However, the glycosaminoglycan values in the bronchoalveolar washing fluid of especially small cell lung carcinomas has been statistically found to be higher than the value belonging to noninfectious benign lung diseases (15). The result which can be derived from the study (15) is that glycosaminoglycan values also increase in case of infection and that especially hyaluronic acid values may rise not only in Wilms' tumor and bladder tumor but in different tumor groups as well. Additionally, it is known that hyaluronic acid synthesis increases in malignant lung mesotheliomas which is contrary to lung adenocarcinomas (16). Some researchers believe that the amount of urinary glycosaminoglycan is an index reflecting the metabolism in the joint cartilage and in studies it has been observed that urinary glycosaminoglycan discharge is elevated in inflammatory and degenerative rheumatoid diseases such as rheumatoid artritis and osteoarthrosis (17-19).

Urinary glycosaminoglycan values, particulary the hyaluronic acid component is found to be raised in childhood extrahepatic biliary atresia. What is more, hyaluronic acid concentrations reach to the values 200 times the normal value in liver diseases, cirrhosis in particular (19,20). Blood and tissue glycosaminoglycan (hyaluronic acid) levels increase in Werner's Syndrome as well, a rare hereditary disease (19).

In a study performed by Resnick, hyaluronic acid which is a type of glycosaminoglycan, has been described as the basic component in the extracellular matrix of the central nervous system (13). Jaworski, on the other hand, has determined that in surgically obtained tissue samples of malignant glioma there is a tissue-tumor spesific extracellular "barin enriched hyaluronan-binding protein (BEHAB)" which is not encountered in the normal

adult cortex or in non-glioma tumors and he argued that this may be a unique and selective marker (14).

In conclusion, an increase in the glycosaminoglycan values in urine, tissue or serum can be present in infectious states and inflammatory disease as well as in various malignant tumors. In our results the rise in urinary glycosaminoglycan values in all the patients although none of them had a kidney or bladder tumor indicates that these values may increase not only in bladder and renal tumors but in all other tumor types as well. However, our study is a preliminary one and in order to obtain net results, the number of disease groups and number of cases in each group must be increased.

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