Original Article

Plasma Glycosaminoglycans: A new Promising tool for Assessment of Non-Metastatic Renal Cell Carcinoma Patients Following Nephrectomy

Awais Ali[1*], Abdelkarem Omneya[2], Kashif Adil[3]

[1]Department of Biochemistry, Abdul Wali Khan University Mardan, Mardan, Pakistan
[2]Department of Chemical Pathology, Medical Research Institute, Alexandria University, Alexandria, Egypt.
[3]Department of Urology, Hayatabad Medical Complex Hospital, Pakistan

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Abstract

Background: Non-invasive detection of renal cell carcinoma (RCC) recurrence is a major challenge that could radically affect patient survival. To date, there are no approved biomarkers for inclusion in the monitoring and follow-up of RCC; therefore, assessment of treatment response is lacking.

Materials and Methods: A Cross-sectional study was carried out on biopsy-proven renal cell carcinoma patients scheduled for nephrectomy at Hayatabad Medical Complex (HMC) Hospital, Peshawar, Pakistan, between September 2022 and January 2024.

Results: A total of 160 samples were included in the study. Eighty samples were obtained from biopsy-proven non-metastatic renal cell carcinoma patients, of which 40 were collected pre-nephrectomy, 40 were collected post-nephrectomy from the same patients, and 80 samples were collected from age and gender-matched healthy individuals. Total plasma glycosaminoglycans (GAGs) levels were analyzed through a manual enzyme-linked immunosorbent assay using a non-competitive sandwich technique. Quantitative variables were summarized as means and standard deviation, while qualitative variables were summarized as frequency and percentage. A paired t-test was performed to check whether the difference between the mean plasma GAG levels in pre-nephrectomy and post-nephrectomy groups was significant.

Conclusion: The results revealed that post-nephrectomy GAG levels have significantly decreased compared to pre-nephrectomy levels ($P<0.001$). Plasma glycosaminoglycan levels could be promising markers for monitoring renal cell carcinoma patients post-nephrectomy.

Keywords: Glycosaminoglycans, Nephrectomy, Renal cell carcinoma

***Corresponding Author:** Awais Ali, Department of Biochemistry, Abdul Wali Khan University Mardan, Mardan, Pakistan. Email: awaisali@awkum.edu.pk. ORCID: https://orcid.org/0000-0002-4514-9509

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Introduction

Renal cell carcinoma (RCC) stands as the most common form of kidney malignancy, contributing to approximately 179,000 deaths each year globally. According to the data of GLOBOCAN in 2020, kidney cancer is the 14th most common malignancy worldwide, with an estimated about 431 thousand patients newly

diagnosed in 2020[1]. Kidney Cancer (KC) ranks as the sixth most prevalent cancer in males and the tenth in females on a global scale[2]. Pakistan ranks eighth among the countries with a high rate of kidney diseases, with 17 million people suffering from kidney problems[3]. However, there are no updated statistics about the incidence of kidney cancer cases.

Several factors contribute to increased risk for RCC, including male sex, old age, central adiposity, tobacco smoking, alcohol intake, persistent hemodialysis, high blood pressure, and polycystic kidney disease[4]. Additionally, advances in the genetic landscape have recognized that both germline and somatic mutations could contribute to the development of KC. According to the Cancer Genome Atlas analysis, it's estimated that approximately 6–9% of submitted KC cases revealed a germline alteration in genes linked to cancer predisposition. However, due to the lack of extensive population-wide studies, researchers have overlooked much data regarding potentially influential autosomal recessive factors and gene variants that impact KC[4–9].

Around 75% of individuals diagnosed with renal cell carcinoma (RCC) have clear cell subtype (ccRCC), which commonly begins in the tubular epithelium of the kidney and has the potential to spread to various organs such as the liver, bones, lungs, and brain[10].

Over 40% of RCC patients have no overt symptoms[11–13]. RCC is mainly asymptomatic, with reports indicating that 20–40% of cases are diagnosed at the metastatic stage, which is universally deemed incurable[14,15]

The primary treatment approach for patients with non-metastatic RCC is surgical resection, either through partial or radical nephrectomy (M0 RCC). Nevertheless, around one-third of all M0 RCC patients encounter recurrence within five years following surgery[11]. Despite the implementation of contemporary targeted therapies, patients with metastatic RCC still experience notably worse median survival rates[16]. Existing follow-up protocols focus on early detection of recurrence because symptomatic recurrences typically carry a worse prognosis than those detected through routine follow-up[17,18]. Current follow-up protocols primarily depend on radiological imaging, typically involving chest and abdomen computed tomography every 6–12 months for 3–5 years, depending on the recurrence risk post-surgery[19]. Non-invasive detection of recurrence is a major unmet clinical need that could improve follow-up. To date, the absence of approved biomarkers in clinical management leads to delayed diagnosis or insufficient evaluation of treatment response. Although recent technologies involving methylated cfDNA and metabolomics show promise as markers, these assays are complex, costly, and lack standardized kits[20,21]. Therefore, there is a need for an early non-invasive biomarker for diagnosis, disease surveillance, and RCC monitoring after therapy.

The extracellular matrix (ECM) constitutes a significant portion of the acellular compartment within the tumor microenvironment (TME), comprising major components such as proteoglycans (PGs) and hyaluronan (HA). A substantial portion of PGs is composed of repetitive chains known as glycosaminoglycans[22].

Interestingly, plasma GAGs are emerging as a promising marker for diagnosing and monitoring surgically treated RCC patients. Glycosaminoglycans are structurally diverse heteropolysaccharides with viscous, lubricating characteristics similar to mucous secretions; therefore, they are also known as mucopolysaccharides[19]. These molecules can be located within the extracellular matrix or on the surfaces of animal cells. They have been discovered to bind and regulate various proteins, such as cytokines, chemokines, enzymes, growth factors (GF), morphogens, and adhesion molecules[20]. The complex array of GAG sulfation and epimerization patterns, referred to as the GAGome, is increasingly recognized as a non-genetic code governing numerous biological functions, many of which play a role in cancer. Additionally, GAGs can bind to various ligands and are essential mediators of homeostasis and other pathological processes like tumorigenesis, wound healing defects, and blood clotting disorders[21,22]. In Renal cell carcinoma patients, GAG excretion in the urine is considered a reliable diagnostic marker for determining tumor size and unilocularity or multilocularity[23].

Based on the importance of GAG levels in diagnosing RCC, our study aimed to evaluate plasma GAG levels as non-invasive biomarkers for monitoring post-nephrectomy RCC patients.

Methods

Study design: A cross-sectional study was conducted at Hayatabad Medical Complex (HMC) Hospital, Peshawar, Pakistan, between September 2022 and January 2024, to assess the role of glycosaminoglycans in monitoring patients with RCC post-nephrectomy (Ethical code: HMC/PMC-EB/MS/00343). The study was carried out according to the Helsinki Declaration 2013 and was approved by the HMC ethical committee. Patients diagnosed with biopsy-proven non-metastatic renal cell carcinoma scheduled for nephrectomy were enrolled in the study. A total of 160 samples were included in the study. Eighty samples were obtained from biopsy-proven renal cell carcinoma patients, of which 40 samples were collected pre-nephrectomy and 40 samples were collected post-nephrectomy from the same patients. Another 80 samples were collected from age and gender-matched healthy individuals as control samples.

Meanwhile, patients with urological cancer, bladder cell carcinoma, or any concurrent cancer were excluded. Written informed consents were obtained from all patients before participation in the study. All of the enrolled patients agreed to have their samples for research purposes.

GAG measurements: Three mL blood samples were collected from the study subjects in the antecubital vein and placed in an ethylenediaminetetraacetic acid (EDTA) vacutainer tube. The sample was then processed by centrifugation at 2000-3000 RPM for approximately 20 *minutes* within two hours of sample collection, and the supernatant was collected and stored at -80 C until the analysis. Human total plasma GAG levels were analyzed through manual enzyme-linked immunosorbent assay (ELISA). The principle was based on a non-competitive sandwich ELISA technique, antigen-antibody interaction[24]. The human GAG antibody was pre-coated on the plate. A GAG antigen in the sample was added, bound to the antibody, and coated on the well. The results of the samples were analyzed using an ELISA plate reader

Statistical analysis: Data were entered in a statistical software package (SPSS *version* 21). The normality of the data was tested using the Kolmogorov-Smirnov test. Mean and standard deviation were used to describe normally distributed quantitative variables. Qualitative variables were described as frequency and percentage. The paired t-test was used to compare normally distributed variables within dependent groups. A p-value of ≤ 0.05 was considered statistically significant.

Results

Patient characteristics: A total of 160 samples were included in the study. Eighty samples were obtained from biopsy-proven renal cell carcinoma patients, of which 40 samples were collected pre-nephrectomy and 40 samples were collected post-nephrectomy from the same patients. Another 80 samples were collected from age and gender-matched healthy individuals as control

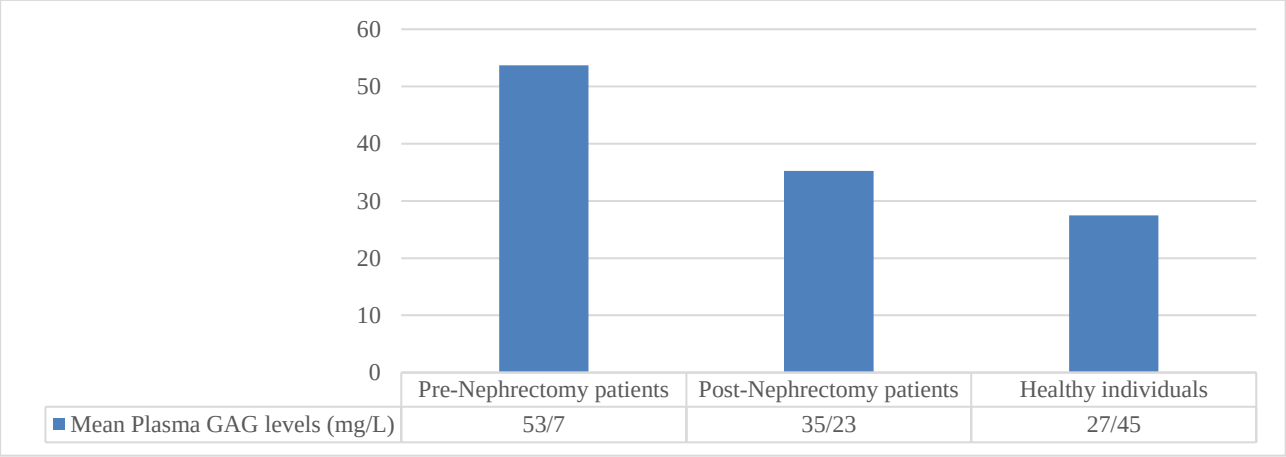

Figure 1. Mean plasma glycosaminoglycans levels in the studied groups.

samples.

The mean age of pre-operative patients is 59.50 ± 5.5 years compared to 58.60 ± 5.7 years for controls. The mean age of males was 59.24 ±6.5 years, and the mean age of females was 60.24 ± 6.7 years. 26 (65 %) of the pre-operative patients were males, while 14 (35 %) were female. According to TNM staging, 39.6% of our RCC patients were T2NM0 stage while 24.3% were in the T3NM0 stage, and 36.1% were in the T4NM0 stage.

Plasma GAG levels in renal carcinoma patients pre and post-nephrectomy: The pre-nephrectomy mean plasma GAG levels was 53.70±6.72 (mg/L) which was significantly higher than the post-nephrectomy mean plasma GAG levels 35.23 ± 5.28 (mg/L), (t=19.31, P<0.001). The mean plasma GAG levels of the healthy individual were 27.45±4.82 (mg/L). Plasma GAG levels were significantly higher in pre-operative and postoperative samples than controls (p<0.001 and p=0.03). There was no significant difference in plasma GAG levels between males and females, whether pre-operative or postoperative (p>0.05).

Discussion

The discovery of promising biomarkers for monitoring cancer prognosis is one of the most significant challenges in oncologic research. Recently, numerous biomarkers have been demonstrated to reliably predict poor cancer prognosis through alterations in the concentration of proteins or small molecules[25–27].

Our study aimed to assess the role of plasma GAGs as a non-invasive biomarker for monitoring RCC patients' response post-nephrectomy. Our study included 160 samples, 80 from histologically proven renal cell carcinoma patients, of which 40 samples were obtained pre-nephrectomy and 40 were obtained post-nephrectomy from the same patients. Another 80 samples were collected from age and gender-matched healthy individuals as control samples. According to TNM staging, 39.6% of our RCC patients were T2NM0 stage, while 24.3% were in the T3NM0 stage, and 36.1% were in the T4NM0 stage.

Our results showed that the mean of pre-operative plasma GAG levels was 53.70±6.72 (mg/L), which was significantly higher than the postoperative plasma GAG levels 35.23±5.28 (mg/L), (t=19.31, P<0.001). The mean plasma GAG levels were significantly higher in pre-nephrectomy patients compared to healthy individuals (controls), confirming the role of plasma GAG levels as diagnostic marker for RCC. Additionally, the significant reduction in plasma GAG levels in post-nephrectomy patients could be explained as an early reaction towards the tumor. This underscores the significance of plasma GAG levels as a non-invasive marker for monitoring RCC patients post-nephrectomy.Our findings were consistent with Gatto et al.(2018) who reported that GAG scores were elevated in surgically treated RCC patients compared to healthy individuals, irrespective of the stage, grade, histology, age, or gender[28]. Additionally, they concluded that GAGs are susceptible diagnostic and promising markers indicators in surgically managed RCC, regardless of stage, grade, or histological characteristics.

Interestingly, another study conducted by Gatto et al. (2023), showed a correlation between the GAG scores and progression-free survival (PFS) and overall survival (OS) in a prospective cohort of clear cell renal cell carcinoma (ccRCC) patients previously enrolled in another study[29,30].

Remarkably, Gatto et al. (2022) observed that plasma and urine-free GAGomes are correlated with postsurgical recurrence in M0 RCC, as reported in a single-center prospective cohort study[29].

Additionally, changes in GAG profile were observed in metastatic clear cell (mcc)RCC patients. Moreover, they developed three GAG scores that facilitated precise identification of mccRCC patients. They successfully confirmed the score's reliability using an independent group of 18 patients with mccRCC and nine healthy individuals[31].

Our results showed that plasma GAG levels were significantly higher in a post-nephrectomy group than controls (p =0.03); the timing of the post-nephrectomy sampling could explain this within 24 hours after the surgical procedure. However, Gatto et al., reported that glycosaminoglycans scores normalized in eight disease-free patients; however, these cases were assessed many years after the surgery. Therefore, frequent measurement of post-nephrectomy plasma GAGs is recommended to detect the accurate time of

normalization of plasma GAGs[31].

Importantly, It was found that GAGs are completely changed in RCC tissue compared to normal adjacent tissues, confirming the tumor's effect in modifying GAG profile and supporting the fact that serum GAG levels play a crucial role in RCC diagnosis[26].

Currently, there is no approved biomarker for metastatic clear cell RCC in routine practice. Changes in the GAG profile are considered promising biomarkers for monitoring RCC patients and early detection of recurrence. Likewise, our findings suggest that plasma GAG levels could have clinical relevance in the monitoring and follow-up of non-metastatic RCC.

These biomarkers offer numerous potential benefits. Firstly, plasma GAGs offer diagnostic advantages for patients with non-metastatic RCC, as non-invasive biomarkers allow for dynamic disease monitoring. Secondly, the biological significance of GAGs supports their use as early biomarkers of cancer recurrence being involved in extracellular matrix interactions with chemokines activation. Both processes are crucial for tumor metastasis [23].

Despite the strong potential of plasma GAG levels as promising biomarkers for monitoring RCC patients, our study has some limitations. Firstly, we did not investigate the urine GAG levels post-nephrectomy in the enrolled patients. Secondly, our study is a Cross-sectional study with a relatively small sample size, therefore conducting further studies in prospective cohort design with larger sample size is highly encouraged. Finally, further prognostic studies, such as survival analysis, are needed to confirm the role of plasma GAG levels as a prognostic biomarker to guide patient risk stratification and clinical decision-making.

Some challenges in using GAGs as biomarkers need to be addressed in future studies. Firstly, there is a need for commercially standardized assays. Secondly, the a need for investigating the effect of ethnic variation and lifestyle factors on plasma GAG baseline levels in different populations.

Conclusion

Total Plasma GAGs are promising biomarkers for monitoring non-metastatic RCC patients. Post-nephrectomy. The non-invasive nature of plasma GAGs and simple method of detection will facilitate frequent monitoring of such patients and early detection of cancer recurrence with subsequent improvement in the patient-centered care. Future studies to validate our results in multi-centric studies in different ethnic groups are highly recommended.

Authors' contributions

Awais Ali contributed to the research idea data collection, performed the laboratory work, interpreted the results, reviewed the literature, and wrote the manuscript.

Abdelkarem Omneya contributed to the research design, reviewed the laboratory work and results interpretation, reviewed the literature, and wrote the manuscript.

Kashif Adil contributed to the study participants' research design, recruitment, and clinical assessment and wrote the manuscript.

All authors have read, edited, and approved the final version of the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

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