Evaluation of Plasma Glycosaminoglycan in monitoring the patients of Renal cell carcinoma after Nephrectomy

Hamayun Mumtaz[1], Momna Arooj Malik*[2]

[1]Department of Pathology Poonch Medical College Rawalakot AJK.

[2]National university of Medical science (NUMS).

Corresponding Author email: humayuntitroad@gmail.com

Abstract

Renal cell carcinoma is the thirteenth most widespread malignancy worldwide and is the leading cause of urogenital tract cancers. Worldwide, renal cell carcinoma is sixth most prevalent cancer in males and tenth most in females. Its diagnosis is based on histological findings and CT scans that were performed for isolated symptoms. Treatment options include chemotherapy, radiofrequency ablation, nephron-sparing surgery, and radical nephrectomy. Therefore, there is a need for an early non-invasive bio marker for diagnosis, disease surveillance, as well as for monitoring of RCC after therapy. **Objective:** To determine the role of plasma glycosaminoglycans levels for monitoring and prognosis of renal cell carcinoma patients after nephrectomy. **Methods:** A Cross sectional study was taken place at Armed forces institute of Pathology in collaboration with Armed forces institute of urology from July 2021 to Dec 2022. A total of 124 samples were included in study, out of which 62 samples were collected from pre-operated patients and another 62 from same RCC patients after operation. Plasma Glycosaminoglycans levels were analyzed through manual enzyme linked immunosorbent assay by non-competitive sandwich technique. Quantitative variables were calculated by means and standard deviation while qualitative variables were assessed as frequency and percentage. Paired t test was applied to check statistical significance of the difference between the means of pre-operative and post-operative cases. **Results**: Mean age of total population was 39.24 ± 15.90 years. Taking in account age, gender and staging of pre- and post-operative patients, the results revealed significant post-Surgical decrease in plasma glycosaminoglycans levels (p value <0.05). In renal cell carcinoma patients 74(61.7%) were in T2NM0 stage while 31(25.8%) in T3NM0 stage and 15(12.5%) in T4NM0 stage. Majority of patients lied in T2NM0 stage while T3NM0 stage was common in males and T4NM0 stage in females. **Conclusion:** Plasma glycosaminoglycans levels is a potential biomarker in post-surgical phase of RCC patients. It can be utilised as a promising marker to monitor RCC

patients after nephrectomy. The non-invasive nature of the test will help monitoring of such patients which will prove convenient for both patient and treating surgeon.

Introduction

Renal cell carcinoma (RCC) is the thirteenth most widespread malignancy worldwide and ranks third amongst the leading reasons of urogenital tract cancers [1]. Renal cell carcinoma is responsible for almost 90% of all renal malignancies. Worldwide, RCC is sixteenth most prevalent cancer in males and tenth most in females. It primarily affects patients over the age of 55(median age 64 years) [2]. Although there is lack of reliable information about the incidence of renal cell carcinoma in Pakistan, a study reported 1.8 percent prevalence of RCC among all tumors in males in a sample of 2393 cases performed at Agha Khan University in Karachi [3]. During the last decade, there has been a spike in RCC all over the world. One-third to one-fourth of patients have metastatic cancer at the time of diagnosis, while bilateral tumors are seen in just 2% of cases [4].

Factors leading to increased risk for renal cell carcinoma are male sex, adiposity, old age, alcoholism, persistent hemodialysis, high blood pressure, polycystic kidney disease, and various genetic disorders. Initial clinical appearances of RCC are different and may bring about an assortment of vague and often misattributed side effects. Just 10% of RCC patients come with typical flank mass, discomfort, and hematuria and these individuals frequently have progressed illness. Over 40% of RCC patients have no such symptoms [4].

The best way to detect renal masses is with a triple-phase contrast CT scan. These masses are frequently identified during an abdominal or chest CT scan that was initially performed for isolated symptoms. Hematuria is a warning indication that

necessitates more testing, such as imaging, to arrive at a diagnosis and treatment plan [5]. Active surveillance, chemotherapy, radiofrequency ablation, nephron-sparing surgery (NSS) and radical nephrectomy are all options for treatment. NSS or partial nephrectomy is the preferred therapeutic option for minor isolated renal tumors as compared to radical nephrectomy. NSS provides the benefit of retaining renal function while also lowering cardiovascular mortality [6]. Authoritative diagnosis is based on histological findings, but it is an intrusive procedure and may cause damage to kidneys. Therefore, there is a need for an early non-invasive bio marker for diagnosis, disease surveillance, as well as for monitoring of RCC after therapy.

Plasma Glycosaminoglycan (GAG) levels is emerging as a promising marker for diagnosis and monitoring of surgically treated RCC patients. Glycosaminoglycan has viscous, lubricating characteristics similar to mucous secretions therefore they are also known as mucopolysaccharides [7]. These are found in the extracellular matrix and on the surfaces of animal cells. They have been found to bind and modulate a variety of proteins including cytokines, chemokines, enzymes, growth factors (GF), morphogens and adhesion molecules [8]. Glycosaminoglycans include far reaching capacities inside the body. They assume a critical part in the phone flagging cycle, including guidelines of cell development, multiplication, advancement of cell grip, anticoagulation, and wound repair [9]. Urinary glycosaminoglycan excretion is enhanced in several disseminated cancers. In Renal cell carcinoma patients, GAG excretion in the urine is a reliable diagnostic marker for determining tumor size and unilocularity or multilocularity [10].

Keeping in view, the importance of plasma GAG levels in the diagnosis and prognosis of RCC, our study aims to highlight the importance of GAG levels in post-surgical phase of RCC. This study will play a pivotal role in establishing plasma GAG levels as a significant marker for assessing the efficiency of nephrectomy in such patients. The study will help to establish a non-invasive test for monitoring of post-surgical patients which will be beneficial for both patients and the treating surgeons.

Methods

A cross sectional study was conducted at Armed Forces Institute of Pathology in collaboration with Armed Forces Institute of Urology, Rawalpindi from July 2021 to Dec 2022, to evaluate the role of glycosaminoglycan in monitoring the RCC patients after nephrectomy at a tertiary care

setting. The AFIP Institution review board approved the study (IRB no. MP-CHP19-4/READ-IRB/21/247). Sample size estimation was done by WHO calculator using the formula ($n = z^2 p(1-p)/\varepsilon^2$). After comprehensive literature review and considering 2% prevalence of RCC [11], a sample size of 62 was estimated with a 90% confidence interval. Healthy individuals (n= 120) were included as controls. The study included all renal cell carcinoma patients and individuals sent for a histological biopsy for a conclusive diagnosis. The study excluded all individuals with endocrine abnormalities and bone diseases such as osteoarthritis, osteosarcoma, small cell carcinoma, and bladder cell carcinoma. After getting permission from each subject, sampling was performed using a non-probability convenient sampling approach. A standardized and pre-tested questionnaire was used to estimate socioeconomic and demographic variables.

For estimation of GAG levels, 3ml blood sample was collected from study subjects from the antecubital vein and placed in an ethylenediaminetetraacetic acid (EDTA) tube. Then sample was sent to laboratory and centrifugation was done at 2000-3000 RPM for approximately 20 minutes within two hours of sample collection. Plasma

GAG levels were analyzed through manual enzyme linked immunosorbent assay by non-competitive sandwich technique [Human GAGs (Glycosaminoglycan) ELISA Kit (G-Biosciences)]. The principle was based on antigen antibody interaction. Human GAG antibody was pre-coated on the plate. GAG antigen existing in the sample was added and binds to antibody coated on the well. Result of the samples were analyzed using ELISA plate reader [specifications 96T (8 X 12) storage -20^0C) Data was entered in the statistical software package (SPSS version 21). Quantitative variables were calculated by means and standard deviation while qualitative variables were computed as frequency and percentage. Kolmogrov-Smirnov test was applied to check normality of data. Paired t-test was applied for inferential statistics to check statistically significant difference in means among the groups. P-value of ≤ 0.05 was taken as significant.

Results

Total sample size was 124 out of which 62 samples were collected from pre-operated patients and another 62 from same RCC patients after operation. Healthy individuals (n= 120) were included as controls. Mean age of the total population (n=244) was 39.24±15.90 years. Mean age of pre-

operative and post-operative patients as shown in table 1.

Table 1: Descriptive statistics of the study population

Variables	n	Age Mean ± SD	Male Mean ± SD	Female Mean ± SD
Total Population	244	39.24± 15.90	34.74± 14.88	46.28± 14.92
Pre-operative	62	53.34 ± 8.60	51.71± 9.88	54.68± 7.26
Post-operative	62	53.34 ± 8.60	51.71± 9.88	54.68± 7.26
Control (Healthy)	120	24.67 ± 4.35	24.53± 4.23	25.15± 4.76

Mean age of males was 34.74±14.88 years and mean age of females were 46.28±14.92 years. Total males were 149 (61.1%) while females were 95(38.9%). as shown in figure 1. In pre-operative and post-operative group males were 28 and female were 34.
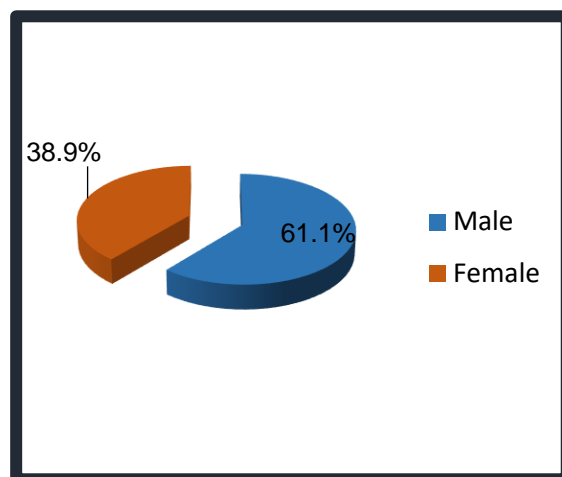

Figure 1. Gender wise frequency distribution of participants.

In Renal cell carcinoma patients 74(61.7%) were in T2NM0 stage while 31(25.8%) in T3NM0 stage and 15(12.5%) in T4NM0 stage. (Shown in figure 2)
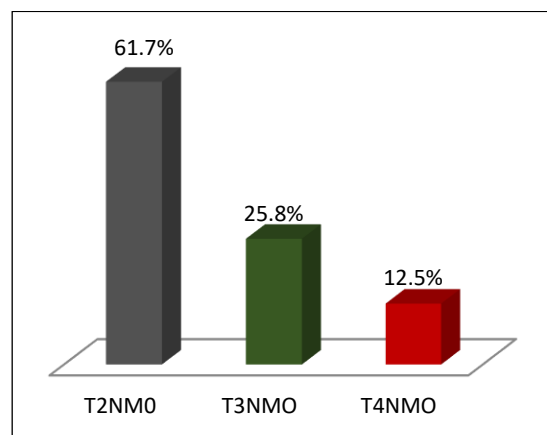

Figure 2: Frequency of Cancer staging in renal cell carcinoma patients

It was observed that most of the patients lied in T2NM0 stage while majority patients were males and females in T3NM0 stage and T4NM0 stage respectively as shown in figure 3.

Evaluation of Plasma Glycosaminoglycan in monitoring the patients of Renal cell carcinoma after Nephrectomy

Table 2. Reference intervals of Glycosaminoglycans levels.

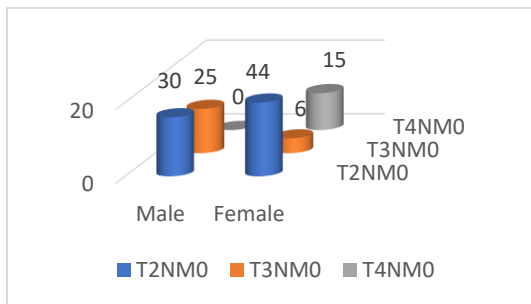| Variables	GAGs levels (Mean ± SD)	Reference Interval (mg/L)
Overall population	24.12±6.45	11.48-36.76
Male	24.67±6.65	11.67-37.7
Female	22.22±5.36	11.71-32.73

Figure 3. Gender wise distribution of renal cell carcinoma staging

Reference Interval

Reference interval computed based on mean and ±2SD because data was

Table 4: Comparison of GAGs levels in

Pre-operative and Post-operative group

parametric and reference interval computed on 120 participants who were disease free.

Reference intervals of Glycosaminoglycans (GAG) levels of local adult population is 11.67-37.7mg/l in males and 11.71-32.73mg/l in females.

Inferential Statistics

Table 3. Glycosaminoglycan levels in Pre-operative and Post-operative group

Variables	Male GAG, s Mean ± SD	Female GAG, s Mean ± SD	P value <0.05
Pre-operative	50.43±13.23	54.68±7.26	0.03
Post-operative	34.43±13.23	37.68±7.26	0.02
Controls	24.53±4.23	25.68±7.4	0.018

The mean value of GAG level in preoperative samples of blood collected from RCC patients was higher than the reference range. Whereas the mean value of GAG level in postoperative samples was limited to the reference range.

Parameter	Plasma GAGs (mg/L)	P value
Pre-Operative: • Male • Female	• 50.43 ± 13.23 • 54.68 ± 7.26	<0.05
Post-Operative: • Male • Female	• 34.43 ± 13.23 • 37.68 ± 7.26	

Paired T test has been applied for the pre-operative and post-operative participants and it gives P value less than <0.05 and this has proved that difference between mean of pre-operative and post-operative is significant.

Discussion

Blood and urine are an ideal wellspring of biomarker, for hypothetical, methodological, and viable reasons. Therefore, the recognizable proof of sub-atomic markers in body liquids which can be utilized for screening, determination, and treatment follow-up in RCC patients, is perhaps the most aggressive difficulties inoncologic research.

Our study aimed to establish a non-invasive test for monitoring of RCC patients during post-nephrectomy phase.

In our study we included 124 samples out of which 62 samples were collected from pre-operated patients and another 62 from post-operated patients of RCC. It was calculated that in renal cell carcinoma patients 61.7% were in T2NM0 stage while 25.8% in T3NM0 stage and 12.5% in T4NM0 stage. Majority of patients (both males and females) lied in T2NM0 stage while T3NM0 stage was common in males and T4NM0 stage in females. A paired t test was used to compare the pre-operative and post-operative groups, and the p value was less than 0.05. This proved that difference between mean of plasma GAG levels of pre-operative and post-operative patients is statistically significant. Thus, emphasising the importance of plasma GAG levels as a diagnostic marker and for surveillance of RCC patients' post-nephrectomy.

Francesco Gatto et al. performed a study to identify highly coordinated control of GAG formation at the mRNA and protein levels in ccRCC patients. GAGs characteristics in mccRCC samples were assessed in comparison to healthy samples. In mccRCC patients the GAG profile changed substantially. Three GAG scores were developed that accurately differentiated mccRCC patients. The score accuracies were validated by comparing 18 mccRCC

with nine healthy subjects and confirmed that the scores were normalized in 8 subjects with no indication of disease. This study concluded that in ccRCC, there is coordinated control of GAG biosynthesis, and non-invasive GAG profiling is useful for mccRCC diagnosis [12].

Lucas Teixeira e Aguiar Batista et al. investigated the expression of heparinase and the profile of glycosaminoglycans in RCC patients. In urine specimens collected before and after nephrectomy, hyaluronic acid $(C_{14}H_{21}NO_{11})_n$ and sulfated GAGs were examined by RT-PCR and agarose gel electrophoresis respectively. In neoplastic tissues, there was a substantial statistical decrease in levels of dermatan sulphate and heparan sulphate and a rise in chondroitin sulphate as compared to non-neoplastic tissues. Between RCC and control samples, the urine GAG profile revealed no substantial difference. The extracellular matrix modifications seen in this study support the idea that heparinase and GAGs can influence the early stages of RCC development [13].

Eleni G. Papaioannou et al. conducted a study to observe changes in levels of serum glycosaminoglycan after renal transplantation. The study comprised forty individuals who had kidney transplantation. GAG levels in the blood were tested before, during, and after surgery. It was observed that after transplantation GAG levels remained considerably increased in individuals with acute graft rejection while overall serum GAG levels decreased in individuals with satisfactory graft function [14].

Pierina De Muro et al. studied patients who suffered issues after receiving a kidney transplant. It was observed that Urine MIG and urinary trypsin inhibitor levels were higher on day 1 after transplant, but chondroitin sulphate relative content was lower and heparan sulfate excretion was absent in the urine. it was stated that evaluating urinary GAG pattern and urine MIG levels can be valuable indicators for diagnosing transplantation difficulties, revealing an early inflammatory state on which immunosuppressive medication could be adjusted properly [15].

Various investigations have indicated that GAGs are completely changed in RCC tissue compared to normal adjacent tissues, confirming the tumor's role in modifying GAG centralization. In this way, it is clear from the research that serum GAG levels play an important role in RCC diagnosis [16]. With respect to GAGs in RCC patients we noticed practically no relationship between the GAG levels used to figure the tumor stage, age, and sexual orientation of

patient. This appears to demonstrate that the adjustments in these plasma GAG levels are incited by the tumor. Basically, tumor trouble converts into bigger changes in GAG plasma levels. Also, these GAG levels seemed, by all accounts, to be amazingly touchy to nephrectomy in this partner, in which RCC patients had a critical decrease in plasma GAG level. These GAG adjustments may start from the tumor and that the liver keeps up plasma GAG homeostasis regardless of changes in the tumor trouble [17]. Nonetheless, this theory matches with the perception that GAG scores appear to diminish postoperatively, which may be adequate to catch variances after surgery.

In past investigations, GAG scores diminished to ordinary levels in eight patients with no disease, however these examples were gotten a very long time after the surgery. The high affectability could be valuable for applications significant in the clinical administration of RCC, for example, observation for repeat after surgery. It has been indicated that plasma GAGs can possibly make up for the absence of insignificantly obtrusive biomarkers in the administration of RCC [18]. In spite of the away from of the GAG level as a biomarker of direct clinical use, this investigation ought to be additionally

assessed to organize phase II and III of clinical trials.

According to some researcher's non-invasive assays can be used to assess particles in serum. They identified altered serum protein production in RCC patients using immunoassays such as ELISA or immunonephelometry, as well as mass spectrometry. That give helpful indicative or potentially prognostic data. It is conceivable that distinctions in identity and way of life can prompt diverse plasma GAG standard levels in various populaces, thus wide associate examination is additionally proposed [19][20].

Right now, there is no symptomatic biomarker that has entered routine practice for metastatic ccRCC. It would without a doubt address a significant clinical headway if changes in the GAG profile could establish a marker of the event of the Infection. This is a quickly developing and promising space of exploration for the plan and advancement of novel incredible specialists for finding and anticipation, drug conveyance and atomic focused on treatment in disease.

The current study is limited to phase-I clinical trial for GAG levels in plasma but not in urine for the purpose of investigating its role in diagnosis of RCC that is proven to be effective by this study. The study has

concise sample size. There is a need to conduct further multi-centric studies before establishing the role of GAG levels in post-nephrectomy patients. More cohort study should be performed proceeding to phase-II and phase-III clinical trials. Further studies should be conducted which include serial monitoring of post-nephrectomy patients with GAG levels. Such studies will help to signify the role of GAG level as a new emerging biomarker in post treatment phase of RCC.

Conclusion

The study concluded that plasma GAG levels differ significantly between preoperative and postoperative RCC patients therefore, it can be utilised as a promising marker to monitor RCC patients after nephrectomy. The non-invasive nature of the test will help monitoring of such patients which will prove convenient for both patient and treating surgeon.

References

1. Capitanio U, Bensalah K, Bex A, Boorjian SA, Bray F, Coleman J, et al. Epidemiology of Renal Cell Carcinoma. Eur Urol. 2019 Jan;75(1):74–84.

2. Dabestani S, Thorstenson A, Lindblad P, Harmenberg U, Ljungberg B, Lundstam S. Renal cell carcinoma recurrences and metastases in primary non-metastatic patients: a population-based study. World J Urol. 2016 Aug;34(8):1081–6.

3. Ahmad Z, Azad NS, Yaqoob N, Husain A, Ahsan A, Khan AN, et al. Frequency of primary solid malignant neoplasms in both sexes, as seen in our practice. J Ayub Med Coll Abbottabad. 2007 Mar;19(1):53–5.

4. Kim CS, Bae EH, Ma SK, Kweon SS, Kim SW. Impact of partial nephrectomy on kidney function in patients with renal cell carcinoma. BMC Nephrol. 2014 Dec;15(1):181.

5. Gray RE, Harris GT. Renal Cell Carcinoma: Diagnosis and Management. AFP. 2019 Feb 1;99(3):179–84.

6. Boorjian SA, Blute ML. A prospective randomized EORTC intergroup Phase 3 study comparing the complications of elective nephron-sparing surgery and radical nephrectomy for low-stage renal cell carcinoma. Urologic Oncology: Seminars and Original Investigations. 2008 Jan;26(1):101–2.

7. Afratis N, Gialeli C, Nikitovic D, Tsegenidis T, Karousou E, Theocharis AD, et al. Glycosaminoglycans: key players in cancer cell biology and treatment:

GAG targeting in cancer cell biology. FEBS Journal. 2012 Apr;279(7):1177–97.

8. Gandhi NS, Mancera RL. The Structure of Glycosaminoglycans and their Interactions with Proteins. Chemical Biology & Drug Design. 2008 Dec;72(6):455–82.

9. Lau W, Blute M, Weaver A, Torres V, Zincke H. Matched comparison of radical nephrectomy vs. nephron-sparing surgery in patients with unilateral renal cell carcinoma and a normal contralateral kidney. Urologic Oncology: Seminars and Original Investigations. 2002 Mar;7(2):86–7.

10. Hennessey PT, Hurst RE, Hemstreet GP, Cutter G. Urinary glycosaminoglycan excretion as a biochemical marker in patients with bladder carcinoma. Cancer Res. 1981 Oct;41(10):3868–73.

11. Padala SA, Barsouk A, Thandra KC, Saginala K, Mohammed A, Vakiti A, et al. Epidemiology of Renal Cell Carcinoma. World J Oncol. 2020 Jun;11(3):79–87.

12. Gatto F, Volpi N, Nilsson H, Nookaew I, Maruzzo M, Roma A, et al. Glycosaminoglycan profiling in patients' plasma and urine predicts the occurrence of metastatic clear cell renal cell carcinoma. Cell Reports. 2021 Mar;34(9):108795.

13. Batista LT e A, Matos LL, Machado LR, Suarez ER, Theodoro TR, Martins JRM, et al. Heparanase expression and glycosaminoglycans profile in renal cell carcinoma: Extracellular matrix and renal cancer. International Journal of Urology. 2012 Nov;19(11):1036–40.

14. Papaioannou EG, Magkou C, Kostakis A, Staikou C, Mitsoula P, Kyriakides S, Sitaras NM. Changes in total serum glycosaminoglycan levels in patients undergoing renal transplantation: preliminary data. Surg Today. 2004;34(8):668-72. doi: 10.1007/s00595-004-2795-4. PMID: 15290396.

15. De Muro P, Faedda R, Masala A, Lepedda AJ, Zinellu E, Ciccarese M, et al. Kidney post-transplant monitoring of urinary glycosaminoglycans/proteoglycans and monokine induced by IFN-γ (MIG). Clin Exp Med. 2013 Feb;13(1):59–65.

16. Ustundağ Y, Huysal K, Guzelsoy M, Genim CE, Yavuz A. Urine and serum glycosaminoglycan levels in the

diagnosis of urological diseases and conditions: A narrative review of the literature. Urologia. 2021 May;88(2):103–9.

17. Wei J, Hu M, Huang K, Lin S, Du H. Roles of Proteoglycans and Glycosaminoglycans in Cancer Development and Progression. IJMS. 2020 Aug 20;21(17):5983.

18. Gatto F, Blum KA, Hosseini SS, Ghanaat M, Kashan M, Maccari F, et al. Plasma Glycosaminoglycans as Diagnostic and Prognostic Biomarkers in Surgically Treated Renal Cell Carcinoma. European Urology Oncology. 2018 Oct;1(5):364–77.

19. Flitcroft JG, Verheyen J, Vemulkar T, Welbourne EN, Rossi SH, Welsh SJ, et al. Early detection of kidney cancer using urinary proteins: a truly non-invasive strategy. BJU International. 2022;129(3):290–303.

20. Zhou Y, Funston G, Lyratzopoulos G, Walter FM. Improving the Timely Detection of Bladder and Kidney Cancer in Primary Care. Adv Ther. 2019 Jul;36(7):1778–85.