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Plasma Glycosaminoglycans (GAGS) Levels as a Biomarker in Renal Cell Carcinoma Patients

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ABSTRACT

Objective: To determine the diagnostic accuracy of plasma Glycosaminoglycans (GAGs) in Renal Cell Carcinoma (RCC), taking histological findings as the reference standard.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, in collaboration with Armed Forces Institute of Urology, Rawalpindi Pakistan, from Sep 2020 to Jun 2021.

Methodology: The study comprised sixty-two (62) diagnosed cases of RCC. All the patients had nephrectomy, and histopathological findings confirmed the diagnosis. Plasma samples for GAG levels were collected in EDTA tubes and assayed by manual ELISA. The Receiver Operating Characteristic (ROC) curve was constructed, and the Area Under the Curve (AUC) was calculated.

Results: The mean age of the study population was 52.7+10.5 years. At a cut-off of 34 ng/ml, the sensitivity and specificity of plasma GAG levels were 83.9% and 94.2%, respectively, in diagnosing RCC taking biopsy as a gold standard. Positive predictive value (PPV) and negative predictive value (NPV) at this cut-off were 93.7% and 84.9% respectively. The area under the curve (AUC) for plasma GAG levels was 0.97, which further supported the use of this test in diagnosing RCC.

Conclusion: Plasma GAG levels can be used as a promising diagnostic test in patients with RCC. It can prove relatively more convenient and cost-effective for diagnosing such cases.

Keywords: Biomarker; Diagnostic accuracy; Glycosaminoglycans; Renal cell carcinoma.

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INTRODUCTION

Kidney cancer is the world's thirteen (13th) most prevalent cancer and the third most common cause of genitourinary malignancies.[1] Renal cell carcinoma (RCC) is the most common kind of renal cancer and accounts for around 90% of all cases. It is also the leading cause of mortality in the United States.[2,3] Clear cell carcinoma, commonly known as conventional RCC, is the most frequent histological form, accounting for 75–80 per cent of RCC cases. The remaining are papillary (10–15%), chromophobe (5%), and other unusual types such as collecting duct carcinoma (<1%).[4] At presentation, one-fourth to one-third of patients have metastatic disease, while bilateral tumours are seen in approximately 2% of cases.[5]

Due to its accuracy and minimally invasive nature, GAG is gaining traction as a new biomarker for RCC diagnosis and prognosis prediction compared to histological biopsy.[6,7] Because of GAG's possible relevance as an RCC diagnostic marker, this study aimed to see how accurate GAG was in diagnosing cancer when compared to histology results.[8] It can be a more convenient and practical way of diagnosing RCC. This study also reviewed diagnostic procedures and identified challenges for managing patients with RCC.

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METHODOLOGY

In collaboration with the Armed Forces Institute of Urology, cross-sectional study was conducted at Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology Rawalpindi Pakistan, from September 2020 to June 2021. The Institutional Review Board (IRB) approved this study (IRB no. MP-CHP19-6/READ-IRB/20/645). The sample size was estimated by the WHO Calculator using the global prevalence of renal cell carcinoma as 2%.[9]

Inclusion Criteria: All patients suspected of RCC and referred for histological biopsy for definitive diagnosis were included regardless of age and gender.

Exclusion Criteria: Patients with pre-existing endocrine disorders, bone and soft tissue disorders like osteoarthritis, osteosarcoma, bladder carcinoma and small cell carcinoma were excluded from this study.

A standardized, and pre-tested questionnaire was utilized in the study. After receiving informed written consent, samples were taken from selected participants

of the study population. Whole blood (3ml) was collected from the antecubital vein in ethylenediaminetetraacetic acid (EDTA) tubes for GAG level. Samples were transported to the laboratory and centrifuged for 20 minutes at 2000-3000 RPM within two hours after collection. Plasma GAG was assayed by non-competitive enzyme-linked immunosorbent assay (ELISA) based on antigen-antibody reaction by sandwich technique. All the patients underwent a biopsy for histopathological diagnosis as well as a CT scan to see the extent of the disease. The final diagnosis was based on histopathology, and staging was done based on the TNM system. For biochemical diagnosis of RCC, the cut-off GAG levels were taken as ≥34mg/L.

Statistical Package for Social Sciences (SPSS) version 25.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Using histopathological results as the gold standard, diagnostic accuracy was assessed utilizing specificity, sensitivity, and positive and negative predicted values. The ROC curve was constructed by sensitivity and specificity to compare different cut-off levels.

RESULTS:

Sixty-two (62) patients were enrolled in the study, including 28(45.2%) men and 34(54.8%) women. Renal cell carcinoma staging was based on the TNM staging and classified accordingly. Most cases had low-grade tumours [37(61.7%) T2N0M0], while no distant metastasis cases were observed. Mean GAG levels were significantly raised in patients of RCC compared to the disease-free population, as shown in Table-I.

Table-I: Plasma Glycosaminoglycans levels among Patients of RCC compared to the Disease-Free Population

Parameters	GAG levels in Male (mg/L) Mean±SD	*p*-value
RCC patients (n=62)	48.90±4.72	<0.001
Disease free (n=120)	24.12±6.45	

Patients of RCC had significantly higher levels pre-operatively compared to the postoperative period (p<0.001) (Table-II).

Table-II: Plasma Glycosaminoglycans levels in Pre-Operative and Post-Operative Patients (n=62)

Parameter	Plasma GAGs (mg/L) Mean±SD	*p*-value
Pre-Operative (n=62)	48.90±4.71	<0.001
Post-Operative (n=62)	37.13±4.21	

ROC was applied to compare different cut-off levels and identify the best cut-off for RCC diagnosis, as shown in Figure. The area under the curve (AUC) for plasma GAG levels was 0.97, which reflected the test's significance in diagnosing RCC. The diagnostic performance of GAG was evaluated at different cut-offs. At a plasma GAG cut-off of 34, the sensitivity and specificity for the RCC diagnosis were 83.9% and 94.2%, respectively. At this cut-off, the negative predictive value (NPV) and positive predictive value (PPV) were 84.9% and 93.7%, respectively, as summarized in Table-III.

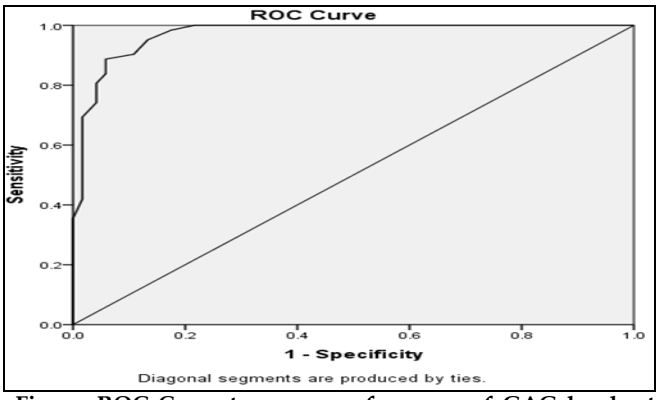

Figure: ROC Curve to assess performance of GAG levels at different cut-offs (n=62)

Table-III: Diagnostic parameters of plasma gag levels (n=62)

	GAG level (>34 mg/L)	Histopathology
Sensitivity	83.9%	100%
Specificity	94.2%	100%
PPV	93.7%	-
NPV	84.9%	-
PLR	14.46	-
NLR	0.17	-
Accuracy	88.9%	99.9%

DISCUSSION

Our study found plasma GAG levels to be an effective marker for diagnosing renal cell carcinoma. The cut-off value was 34ng/ml, and the sensitivity and specificity were 84.9% and 93.7%/ml. This high sensitivity and specificity can help us to deal with the invasive procedure of biopsy, which is inconvenient, cumbersome, costly, & associated with complications.

Corresponding to our study, one previous study reported a GAG score in their study. The GAG score had 93.5 per cent sensitivity and 94.7 per cent specificity for identifying RCC from healthy samples. A total of 108 RCC patients were included, which was independently validated. A novel GAG score was generated in this study that was independent of

histology, tumour stage, size, or grade and was unaffected by age or sex.[10] Whereas in our study, the results were dependent on the age and gender of the patient as well as the grade of the carcinoma according to TNM staging.

A prospective randomized clinical trial (RCT) compared radical with partial nephrectomy in solitary T1-2 N0M0 renal tumours <5 cm with normal contralateral kidney function. The cancer-specific survival (CSS) was 98.5% after radical nephrectomy versus 97% after nephron-sparing surgery (NSS).[11] In our study, the overall GAG levels were calculated as 24.12±7.9 mg/l in blood samples against the reference range of 11.48-36.76 mg/l. Another study observed significantly coordinated control of glycosaminoglycan (GAG) manufacture at the transcript and protein levels using genome-scale metabolic modelling to investigate metabolic reprogramming in 481 ccRCC cases. They compared 18 GAG features in 34 mccRCC samples to 16 healthy plasma and/or urine samples. In mccRCC, the GAG profiles were significantly altered. They came up with three GAG ratings that correctly identified mccRCC patients 93.1% of the time. They validated the score accuracies in a separate cohort (up to 18 mcc RCC versus nine healthy) and confirmed that the scores normalized in eight patients with no signs of disease.[12]

Finally, we discovered differences between the diseased and healthy populations in this investigation. Another limitation of these investigations is the need for repeatability of the marker detection test. Immunohistochemistry, in reality, is semiquantitative and highly reliant on several variables, including antibody selection, antibody concentrations, fixation methods, interpretation and classification criteria variability, sample handling and technical process inconsistency.[13,14] Noninvasive assays can be used to measure molecules in serum, according to certain writers. Using immunoassays, such as mass spectrometry (MS) enzyme-linked immunosorbent assay (ELISA) or immunonephelometry, the investigators detected changed serum protein expression in RCC patients, which can give helpful diagnostic and prognostic knowledge.[15,16]

One study conculded that 94 stage I, 58 stage II–III, and 22 stage IV patients were in the RCC group. The novel GAG score discriminated RCC from healthy samples with an area under the receiver operating characteristic curve (AUC) of 0.999 in the initial discovery set (n=67). The GAG score has an AUC of 0.991 in the validation set (n=108), with 93.5% sensitivity. GAG levels were higher in RCC samples than healthy samples, regardless of stage, grade, histology, age, or gender.[17] In our study, the results were dependent on the age, gender, and tumour grading of the patient according to TNM classification.

Varied ethnicities and lifestyles may result in various plasma GAG baseline values in different groups. Therefore, a broad cohort study is further suggested. For metastatic ccRCC, there is presently no diagnostic biomarker that has established standard procedure. As a result, if alterations in the GAG profile could be used as a predictor of disease development, it would surely be a significant clinical advance. This fast-growing and lucrative field of research seeks to create novel, effective medicines for cancer diagnosis and prediction, drug administration, and molecularly targeted treatment.[18]

LIMITATIONS OF STUDY

The current study was limited to GAG levels in plasma but not in urine to investigate its role in diagnosing RCC, which has proven effective in this study.

CONCLUSION

We concluded that plasma GAG levels could be a promising biomarker to differentiate RCC patients from healthy individuals. They can aid in the definitive diagnosis of RCC patients with quite acceptable sensitivity and specificity. It will benefit the patient and the treating surgeon as it is a minimally invasive and less costly test than histopathological biopsy.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

HM & MA: Conception, data analysis, drafting the manuscript, approval of the final version to be published.

UBK & FBK: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AB & ZHH: Data acquisition, drafting the manuscript, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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