Original Research

**Determination of reference ranges of plasma glycosaminoglycans in a tertiary care centre**

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**Objective:** To determine reference ranges of plasma glycosaminoglycans (GAGs) in a population presenting at a tertiary care centre of Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi from January 2020 to December 2020.

**Methodology:** An observational cross-sectional study which involved one hundred and twenty (120) disease-free healthy population was selected by non-probability consecutive sampling at a 90% confidence interval with a 5% margin of error. Plasma glycosaminoglycans were assayed by manual ELISA technique. The study population was stratified according to gender. Normality of data was assessed by Kolmogorov-Smirnov test. Dixon range test was employed to find outliers. After removing the outliers, mean ± SD was calculated for plasma GAG levels (mg/L). Sex-specific reference values were determined.

**Results:** In our study, the total male population was 62 (51.7%), while the female was 58 (48.3%) in the disease-free population (n=120). 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. In males, GAG levels were found as 24.67±6.65 against a reference range of 11.67-37.7 mg/l, while in females, it was 22.22±5.36 against the reference interval of 11.71-32.73 mg/l.

**Conclusion:** The reference range for plasma GAG was found 11.48-36.76 mg/l in our study population. GAG levels differ significantly among males and females with reference ranges of 11.67-37.7 mg/l and 11.71-32.73 mg/l, respectively.

**Keywords:** Glycosaminoglycans; reference range; malignancy; ELISA

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Introduction:

Long-chain polysaccharides made up of repeated disaccharide units are called glycosaminoglycans (GAGs) or mucopolysaccharides. Except for keratan, where the uronic sugar is galactose, these rehashing two-sugar units consist of uronic sugar and amino sugar. Changes in these monosaccharide units inside the GAG backbones, such as heparin and keratan sulfate, give rise to different GAG families. There is no protein core in hyaluronan or hyaluronic acid (HA). GAGs' structural differences and heterogeneities, as well as their high sulfation content (except HA) and widespread appearance in the extracellular matrix (ECM) or on the surface of cells, all contribute to the diversity of their biological functions by allowing GAGs to bind to a variety of extracellular proteins whose activities are distributed through various pathophysiologic pathways. GAGs are highly polar and hydrophilic, retaining a huge volume of water in the interstitial space and serving as protection/buffer in the body. Mucopolysaccharidoses are metabolic disorders characterized by abnormal accumulations of glycosaminoglycans caused by protein deficiency. Cell binding, cell growth and expansion regulation, formative cycles, cell surface influence of lipoprotein lipase and other proteins, angiogenesis, viral attack, and tumor metastasis are just a few of heparan's natural applications and capabilities of sulfate (HS).

Plasma GAGs are becoming more widely At initial consultation, the social economical used as a diagnostic and monitoring tool for renal and demographical (age, gender, marital cell carcinoma (RCC)). GAG plays an important status, schooling years) characteristics were part in the cell signaling process, which includes assessed as independent variables cell growth, proliferation, cell adhesion Enzyme-linked immunoassay enhancement, anticoagulation, and wound repair (ELISA), which is based on antigen-antibody These characteristics can be used to closely reaction via sandwich technique, was used to monitor GAG as a neoplastic marker, particularly determine plasma GAG levels. GAG levels RCC. Plasma GAG measures can assist in were measured in blood samples collected distinguishing RCC from normal cells and give from selected study participants from the detailed diagnostic particulars that are helpful antecubital vein in an EDTA (Ethylene for disease control. The GAGs sensitivity and diamine tetra-acetic acid) tube. Within two physisibility by which serum or urine-based hours, the material was delivered to the

Materials and Methods:

AFIP and Armed Forces Institute of Urology (AFIU) collaborated on this cross-sectional research. After receiving Institutional Review Board (IRB) approval, the study lasted one year, from January 2020 to December 2020. The sample size was expected after a in-depth study of literature with a 90 percent confidence interval & working the global occurrence of renal cell carcinoma as 2 percent. For reference interval study, the sample size was taken as 120 from the healthy population. To study the reference interval of GAG levels, disease-free individuals were enlisted. The study excluded patients with endocrine diseases, bone abnormalities such as osteoarthritis, osteosarcoma, small cell carcinoma, and bladder cell carcinoma. The non-apprehension appropriate sampling strategy was applied for selection of the samples. A structured, consistent, and pre-tested inquiry form was used in a pilot study.
laboratory and centrifuged. Human GAG capture antibody was pre-coated on the plate. The GAG in the sample binds to the antibody on the well. A substrate containing the detecting antibody was added. A sandwich was produced between the capture antibody, sample antigen, and detection antibody. The analyte concentration was proportional to the detection signal.

SPSS version 21 was implemented for data analysis. For computation of Quantitative variables means and SD was employed, while for qualitative variables it was frequency and percentage. Test of normality (Kolmogorov-Smirnov test) was applied to check for data distribution. For reference intervals, mean and SD were calculated. The following formulas calculated the percentiles:

\[ \text{2.5 percentile} = X - 1.96 \times SD \]
\[ \text{97.5 percentile} = X + 1.96 \times SD \]

where \( X \) represents the mean.

**Results:**
One hundred twenty participants were disease-free and included for the estimation of reference interval. The mean age of the healthy population (\( n=120 \)) was \( 24.67 \pm 4.35 \) years. 62 (54%) were male, and 58 (46%) were female. The characteristics of the study population are listed in table 1.

The Kolmogorov-Smirnov test yielded \( p>0.05 \), which implied that the data was parametric. As our data were parametric, reference interval was computed based on mean and SD. Reference interval was computed in both genders separately and overall population as shown in table 2.

**Discussion:**
Glycosaminoglycans (GAGs) are considered a biochemical marker for diagnosing and monitoring renal cell carcinoma (RCC). Their association with RCC, especially clear cell renal cell carcinoma (ccRCC), is well established. Many studies are being carried out to see its role as a reliable biochemical marker in patients of RCC both for diagnosis and post-operative monitoring for recurrence and surveillance. Blood and urine are appropriate biomarker sources for theoretical, methodological, and practical reasons. 10 Consequently, one of the utmost tough tasks in research for oncology is recognizing molecular markers resides in plasma and urine that perhaps needed for diagnosis, screening, follow-up, and drug-based therapeutic monitoring in patients with RCC.11 For RCC sufferers the clinical workout, histological examination, TNM staging, and the Fuhrman nuclear grade are at present engaged prognostic variables. Presently, the use of plasma biomarkers is not in use for diagnosing RCC. As a result, the current study was created to calculate the reference range for plasma GAG levels and validate the diagnostic significance of biomarkers and their predictive value.

In our study, a disease-free, relatively young population (mean age 24.6 years) was taken to determine reference intervals for plasma GAG levels. The total male population was 62 (51.7%) while female was 58 (48.3%) in disease-free population (\( n=120 \)). The overall GAG levels were calculated as \( 24.12 \pm 7.9 \) mg/l in blood samples against the reference range of \( 11.48-36.76 \) mg/l. Furthermore, the plasma GAG levels in both genders were ascertained separately to determine a sex-specific reference range for plasma GAG levels. For the male population, the mean GAG levels were \( 24.67 \pm 6.65 \) mg/l with a corresponding reference interval of \( 11.67-37.7 \) mg/l. While for the female population, these were \( 22.22 \pm 5.36 \) mg/l with a reference range of \( 11.71-32.73 \) mg/l.

The GAG levels are found higher in children or lower age groups. In a recent study in Japan in 2018 by Khan et al.,12 the mean plasma GAG levels in healthy individuals were found \( 31.1 \pm 22 \) ng/ml. These are slightly higher than found in our population, probably because the study population had lower age than our study population. In another study carried out by Shunji et al. 13 on newborn screening, the control group had mean plasma levels on dried blood spots (DBS) of \( 67 \pm 28 \) ng/ml.

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The measurement of plasma GAG levels is of great importance due to its use in diagnosing disorders like Mucopolysaccharidoses and renal cell carcinoma. The population-based reference values are a valuable addition in diagnosing these disorders. Although gender-based reference intervals are determined in the current study, more research is needed for age-based reference intervals with larger sample sizes.

Conclusion:
Our study concluded that the reference range for plasma GAG was 11.48-36.76 mg/l in our study population. GAG levels differ significantly among males and females with reference ranges of 11.67-37.7 mg/l and 11.71-32.73 mg/l, respectively.

References:

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### Table 1: Demographics of the study population

<table>
<thead>
<tr>
<th>Age (mean SD)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.67±4.35 years</td>
<td>24.53±4.23</td>
<td>25.15±4.76</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>62 (54%)</td>
</tr>
<tr>
<td>Female</td>
<td>58 (46%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rural</th>
<th>Urban</th>
</tr>
</thead>
<tbody>
<tr>
<td>63 (53%)</td>
<td>57 (47%)</td>
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</tbody>
</table>

### Table 2: Reference intervals for plasma GAG levels.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Reference Interval (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosaminoglycan Level</td>
<td>24.12±6.45 mg/L</td>
<td>11.48-36.76</td>
</tr>
<tr>
<td>Male</td>
<td>24.67±6.65 mg/L</td>
<td>11.67-37.7</td>
</tr>
<tr>
<td>Female</td>
<td>22.22±5.36 mg/L</td>
<td>11.71-32.73</td>
</tr>
</tbody>
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