Original Article

Analysis Of Urinary Glycosaminoglycans In Type 2 Diabetes Mellitus Subjects

A. Arviani Desianti Nur[1], Liong Boy Kurniawan[1,2,3], Nurahmi[3,4], Burhanuddin[5], Husaini Umar[6], Tenri Esa[3]

[1]Master of Biomedical Sciences, Graduate School Hasanuddin University, Makassar, South Sulawesi, Indonesia
[2]Hasanuddin University Hospital, Makassar, South Sulawesi, Indonesia
[3]Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia
[4]Makassar City General Hospital, Makassar, South Sulawesi, Indonesia
[5]Department of Nutrition, Faculty of Public Health, Hasanuddin University, Makassar, South Sulawesi, Indonesia
[6]Department of Internal Medicine, Faculty of Medicine, Hasanuddin University, Makassar

Email: liongboykurniawan@yahoo.com
DOI: 10.47750/pnr.2022.13.S07.701

Abstract

Introduction: Diabetes mellitus is known as a non-communicable disease which is a global problem because its incidence continues that increase every year in the world. This condition will continue cause complications which will further increase the morbidity and mortality rates in people with diabetes mellitus. **Methods:** The research design was cross sectional with a sample size of 45 DMT2 patients and 25 non DM patients. The analysis used a comparison test to see a comparison of GAGs levels between the DMT2 and non-DM groups, as well as a correlation test to see the relation between ACR and HbA1c on GAGs levels in T2DM patients. **Results**: The results showed that the results of statistical analysis of comparative tests of urine GAGs levels in T2DM and non-DM were 56.64 ng/mL and 52.36 ng/mL, comparison of urinary GAGs levels in patients with nephropathy and without nephropathy was 59.52 ng/mL and 53.59 ng/mL, the results of the correlation test of urine GAGs and urine ACR levels in DMT2 were 0.55 ($p>0.05$), $r=0.09$, and the results of the correlation test of urine GAGs and HbA1c in DMT2 were 0.56 ($p>0.05$), $r=0.08$. **Conclusion**: It can be concluded that there is no difference in urine GAGs in DM and non-DM patients, as well as differences between DM patients with and without nephropathy. Also, there is no correlation between urine ACR and HbA1c and urinary GAGs in DM patients.

Keywords: Diabetes Mellitus, Urinary Glycosaminoglycans, Nephropathy

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by increased blood glucose levels which over time can cause complications in the heart, blood vessels, eyes, kidneys and nerves. The most common diabetes mellitus is type 2 diabetes mellitus (T2DM) which is generally found in adults which occurs when the body experiences insulin resistance or the body does not produce enough insulin (World Health Organzation, 2022)

The prevalence of DM in the world is reported as many as 537 million adults with age range of 20-79 years old. This number is predicted will increase to 643 million in 2030 and 783 million in 2045 (International Diabetes Association, 2022). The prevalence of DM in Indonesia is reported to be 80.5% or about 20.4 million Indonesians suffer from DM.

Glycosaminoglycans (GAGs) are linear polysaccharides whose disaccharide component consists of an amino sugar (D-glucosamine i.e. N-acetylation, or N-sulfate, or N-acetyl- D-galactosamine) and uronic acid (D-glucuronic acid or L-iduronic acid) or galactose. GAGs are sometimes mentioned as mucopolysaccharides because they are initially characterized by mucous membranes and mucous exudates (Esko et al., 2009).

The relation between diabetes and urinary glicosaminoglycans-proteoglycans (GAGs/PG) excretion has been investigated, however, several investigators have reported no change or even a decrease in GAGs/PG excretion. Some studies have shown an increase in overall excretion (in most cases only quantitative analysis was performed) and especially of heparan sulphate (HS) in both types of diabetes (type 1 and 2) in association with diabetic nephropathy, this is due to GAGs/PG levels. urine increases faster than urinary excretion albumin (UEA) during the early indication of kidney disorder (Lepedda et al., 2017).

Based on the research of Ahn Mi Young et al (2020) stated that glycosaminoglycan treatment effectively lowers blood glucose levels in diseases associated with hyperglycemia. Gambaro et al (1992) suggested that the mechanism of GAGs activity in diabetic nephropathy is unclear. However, their anticoagulant properties, hemodynamic effects, downregulation of several proteases, putative activity on non-enzymatic glycation, and mechanical restoration of glomerular loading by these compounds do not appear to be sufficient to explain the full picture of the preventive effect of GAGs in diabetic nephropathy.

Research related to this matter has not been widely carried out in Indonesia, including in Makassar, so researchers are interested in doing this research.

Methodology

Location and Research Design

This research was conducted at the Endocrine Polyclinic and Clinical Pathology Laboratory at the Hasanuddin University Hospital to recruit T2DM subjects, and the Hasanuddin University Medical Research Center (HUM-RC) laboratory to examine urine GAGs for T2DM sufferers. The design of this study uses experimental analytic methods using a cross sectional approach.

Sample Size and Sampling

The population in this study were all subjects with type 2 diabetes mellitus both outpatient and inpatient at Hasanuddin University Hospital. 45 samples used a non-probability sampling technique, namely purposive sampling, which agreed with the inclusion criteria, namely T2DM subjects who did not suffer from comorbid diseases, and were willing to participate in this study and signed informed consent issued.

Instrument and Data Collection

The data collection used was primary data and secondary data on all T2DM sufferers who able the inclusion criteria and carried out checking at the Endocrine Polyclinic and Clinical Pathology Laboratory, Hasanuddin Hospital Makassar.

Primary data collection in the study used a questionnaire containing questions related to respondents such as name, gender, age, medical history, history of hemodialysis, and others as well as collection of urine samples for examination of urine glycosaminoglycans levels using an ELISA reader instrument using AssayGenie kits from Ireland factories and examination of Albumin Creatinine Ratio (ACR) using the Cobas 311 instrument with the immunoturbidimetric method, while the secondary patient data collected was the results of HbA1c, fasting blood glucose, and/or oral glucose tolerance test examinations contained in the patient's medical record data.

Statistical Analysis

Data analysis was performed using SPSS software. The statistical method used is descriptive statistical calculations and statistical tests. The statistical test uses the Mann-Whitney test if the data is not normally distributed, and the Independent T test if it is normally distributed, the Pearson correlation test if the data is normally distributed or the Spearman test if the data is not normally distributed. The test results are significant if $p < 0.05$.

Ethics

Approval from the Health Research Ethics Commission Hasanuddin University Hospital – Central General Hospital Dr. Wahidin Sudirohusodo Makassar with Letter Number 466/UN4.6.4.5.31/PP36/2022.

Results

Table 1. Characteristics of the T2DM research subjects (n=45)

Characteristics	n	%
Sex		
Male	24	53.3
Female	21	46.7

Age		
30 – 40 years old	3	6.7
41 – 50 years old	10	22.2
51 – 60 years old	14	31.1
61-70 years old	15	33.3
> 70 years old	3	6.6
Urinary Albumin Creatinine Ratio		
< 30 mg/g	22	48.9
≥ 30 mg/g	23	51.1

Source: Primary Data

Table 1 shows the characteristics of the T2DM research subjects, it is known that the sex were mostly male with 24 respondents (53.3%) and women with 21 respondents (46.7%), the highest age distribution was 61–70 years old with 15 respondents (33.3%), ages 41–50 were 10 respondents (22.2%), ages 51–60 were 14 respondents (31.1%), ages 30–40 and >70 were 3 respondents (6.6 successively and 6.7%), the level of the albumin/creatinine ratio in T2DM patients was <30 mg/g in 22 respondents (48.9%) and ≥30 mg/g in 23 respondents (51.1%).

Table 2. Characteristics of non-DM research subjects (n=25)

Characteristics	n	%
Sex		
Man	14	56.0
Female	11	44.0
Age		
30 – 40 years old	20	80.0
41 – 50 years old	5	20.0
Urinary Albumin Creatinine Ratio		
< 30 mg/g	25	100.0

Source: Primary Data

Table 2 shows the characteristics of non-DM research subjects, it is known that the sex were mostly male with 14 respondents (56.0%) and women with 11 respondents (44.0%), the highest age distribution was 30-40 years old with 20 respondents (80.0%), aged 41–50 as many as 5 respondents (20.0%). Albuminuria levels in non-DM subjects were all <30 mg/g in 25 respondents (100.0%).

Table 3. Comparison Test of Urine GAGs Levels in T2DM and Non-DM

	Condition	Mean	Std. Deviation	p-value[a]	p-value[b]
GAGs (ng/mL)	T2DM	56.62	19.01	0.093	0.439
	Non-DM	52.36	26.48		

a: Homogeneity Test

b: Independent T test

Based on Table 7 shows the results of a comparison test for urine GAGs data on the condition of samples suffering from T2DM and Non DM. It was found that the urine GAGs value in samples with T2DM was 56.62 ng/mL with a standard deviation of 19.01 ng/mL while the urine GAGs value in samples without diabetes was 52.36 ng/mL with a standard deviation of 26.48 ng/mL. This shows that the average value of urine GAGs in T2DM patients is higher than non-DM.

Homogeneous test results using the Levene test obtained a p-value of 0.09 which was greater than 0.05, this indicates that the urine GAGs data in T2DM and non-DM sufferers is homogeneous.

The results of the comparison test obtained a p-value of 0.43 which was greater than 0.05 (p-value> 0.05), this indicates that the GAGs value of urine in T2DM and non-DM patients was not significantly different.

Table 4. Comparison Test of Urine GAGs Levels in Patients with Nephropathy and Without Nephropathy

	Condition	Mean	Std. Deviation	p-value*
GAGs (ng/mL)	Nephropathy	59.52	18.36	0.302
	Without Nephropathy	53.59	19.63	

* Independent T test

Based on Table 4 shows the results of a comparison test for urine GAGs data in samples with nephropathy and without nephropathy. It was found that the urine GAGs value in samples with nephropathy averaged 59.52 ng/mL with a standard deviation of 18.36 ng/mL while the urine GAGs value in samples without nephropathy averaged 53.59 ng/mL with a standard deviation of 19.63 ng/mL. This shows that the average value of urinary GAGs in patients with nephropathy is higher than those without nephropathy. The results of the comparison test obtained a p-value of 0.302 which was bigger than 0.05 (p-value > 0.05), this indicates that the GAGs value of urine in patients with nephropathy and without nephropathy was not significantly different.

Table 5. Correlation Test of Urine GAGs Levels with Urinary Albumin in T2DM

Condition	Correlation	Coefficient Correlation (r)	p-value*
T2DM	GAGs-ALB	0.09	0.55

* Pearson Correlation Test

Table 5 shows the results of the relationship test between urine GAGs and urine albumin in T2DM patients. The results of the correlation test showed that the p-value in T2DM patients was bigger than 0.05. This shows that there is no correlation between urinary GAGs and urine albumin in T2DM patients with a value of r = 0.09 which shows a very low.

Table 6. Correlation Test of Urine GAGs Levels with HbA1c in T2DM

Condition	Correlation	Coefficient Correlation (r)	p-value*
T2DM	GAGs-HbA1c	0.08	0.56

* Pearson Correlation Test

Table 6 shows the results of the correlation test between urine GAGs and HbA1c levels in T2DM patients. The results of the correlation test showed that the p-value in T2DM patients was greater than 0.05. This shows that there is no correlation between GAGs and HbA1c levels in T2DM patients with a value of r = 0.08 which shows a very low correlation.

Discussion

This study was to know the comparison of urine GAGs levels in T2DM and non-DM patients, which consisted of 45 T2DM subjects and 25 non-DM subjects.

Based on Table 7, the results of a comparison test of urine GAGs levels in T2DM and non-DM patients. The average value of urine GAGs in T2DM patients is higher than non-DM. However, the results of the comparative test obtained a p-value of 0.439 which was greater than 0.05 (p-value > 0.05), this indicated that the GAGs value in T2DM and non-DM patients was not significantly different. This is not in line with the research of Hiroki Yokoyama, et al (1999) which stated that the level of urinary excretion of GAGs was significantly reduced in diabetic patients when compared to non-diabetic subjects (P<0.001), and levels decreased more in patients with nephropathy.

Another study found total urinary GAGs to be significantly higher in untreated diabetic patients compared to healthy subjects. In addition, it was observed that total urinary GAGs levels in diabetic patients after six months of metformin therapy were significantly decreased compared to the pre-treatment situation (Jura-Półtorak et al., 2022).

Research by Wasty et al (1993) detected significant changes in GAGs in atherosclerosis and diabetes. This study found a decrease in the ratio of GAGs in atherosclerosis and a similar but less pronounced reduction in diabetes. The significance of this ratio is not fully known but F Wasty et al speculate that a lower ratio of GAGs may be an indicator of an increased risk of atherosclerosis. This finding is in agreement with the findings of Ying-Shan et al who detected similar changes in GAGs with an increased risk of atherosclerosis (Wasty et al., 1993).

Narayanan's study reported that there was no correlation between the decline in GAGs with age or duration of diabetes. After nitric acid treatment, the glycosaminoglycan component of diabetic and non-diabetic membranes decreased. This study showed that GAGs, which are the content of the human glomerular membrane, decreased significantly in diabetic patients (Parthasarathy & Spiro, 1982).

The mechanism of GAGs activity in diabetic nephropathy may be complex and remains unclear. GAGs may act through their anticoagulant properties, hemodynamic effects, downregulation of some proteases, their putative role in nonenzymatic glycation, and mechanical restoration of glomerular discharge. The antiproliferative effects of GAGs have also been described (Solini et al., 1997).

Urinary glycosaminoglycans have long been associated with T1DM and T2DM and have been suggested as a promising potential marker for screening, diagnosis, and follow-up, however, despite the abundance of data reported so far, prospective studies are still needed to confirm this clinical utility in daily practice. day. The relationship with other early markers and the detailed structural characterization of GAGs/PGs in diabetes still needs to be explained (Ahn et al., 2020).

Research by Torsten Deckert et al (1991) suggested that there was no significant difference between the HS ratio in the two diabetes groups, namely DM patients without urine albumin and diabetic nephropathy, this might be related to sample size and/or patient genetic heterogeneity. However, it cannot be ruled out that inhibition of heparan sulphate-proteoglycans (HS-PG) production due to hyperglycemia can lead to an increased risk of death and cardiovascular morbidity in diabetic patients with microalbuminuria (Yard et al., 2002).

Extracellular matrix (ECM) that is damaged due to diabetic nephropathy is repaired by glycosaminoglycans (Gambaro & Van der Woude, 2000). Glycosaminoglycans prevent expansion of the kidney ECM, deposition of type III and IV collagen (Gambaro et al., 1992; Ceol et al., 1996) as well as transforming growth factor β (TGF-β) synthesis can be inhibited by GAGs. Therefore, research has been carried out to find new treatments to prevent or slow down the development of nephropathy by using GAGs, because of their activity in treating nephropathy (Masola et al., 2014).

Conclusion

There was no difference in urine GAGs in DM and non-DM patients, as well as differences between DM patients with and without nephropathy. There was also no correlation between urine ACR and HbA1c and urinary GAGs in DM patients.

Acknowledgments

The acknowledgments include the appreciation given by the author to those who have played a role in the research, both in the form of financial support, willingness, consultants, and assistance in data collection.

Conflict Of Interest

There is no conflict interest

References

1. World Health Organzation. (2022). Diabetes. WHO. Available at https://www.who.int/health-topics/diabetes on April 26 2022.
2. International Diabetes Association. (2022). Diabetes around the world in 2021. IDF Diaetes Atlas.
3. Esko, J. D., Kimata, K., & Lindahl, U. (2009). Proteoglycans and sulfated glycosaminoglycans. *Essentials of Glycobiology. 2nd edition*.
4. Lepedda, A. J., De Muro, P., Capobianco, G., & Formato, M. (2017). Significance of urinary glycosaminoglycans/proteoglycans in the evaluation of type 1 and type 2 diabetes complications. *Journal of Diabetes and its Complications*, *31*(1), 149-155.
5. Ahn, M. Y., Kim, B. J., Kim, H. J., Jin, J. M., Yoon, H. J., Hwang, J. S., & Lee, B. M. (2020). Anti-diabetic activity of field cricket glycosaminoglycan by ameliorating oxidative stress. *BMC Complementary Medicine and Therapies*, *20*(1), 1-10.
6. Gambaro, G., Cavazzana, A. O., Luzi, P., Piccoli, A., Borsatti, A., Crepaldi, G., ... & Baggio, B. (1992). Glycosaminoglycans prevent morphological renal alterations and albuminuria in diabetic rats. *Kidney international*, *42*(2), 285-291.
7. Yokoyama, H., Sato, K., Okudaira, M., Morita, C., Takahashi, C., Suzuki, D., ... & Iwamoto, Y. (1999). Serum and urinary concentrations of heparan sulfate in patients with diabetic nephropathy. *Kidney international*, *56*(2), 650-658.
8. Jura-Półtorak, A., Olczyk, P., Chałas-Lipka, A., Komosińska-Vassev, K., Kuźnik-Trocha, K., Winsz-Szczotka, K., ... & Olczyk, K. (2022). Urinary sulphated glycosaminoglycans excretion in obese patients with type 2 diabetes mellitus treated with metformin. *Archives of Physiology and Biochemistry*, *128*(2), 507-513.
9. Wasty, F., Alavi, M. Z., & Moore, S. (1993). Distribution of glycosaminoglycans in the intima of human aortas: changes in atherosclerosis and diabetes mellitus. *Diabetologia*, *36*(4), 316-322.
10. Parthasarathy, N., & Spiro, R. G. (1982). Effect of diabetes on the glycosaminoglycan component of the human glomerular basement membrane. *Diabetes*, *31*(8), 738-741.
11. Solini, A., Vergnani, L., Ricci, F., & Crepaldi, G. (1997). Glycosaminoglycans delay the progression of nephropathy in NIDDM. *Diabetes Care*, *20*(5), 819-823.
12. Deckert, T., Horowitz, I. M., Kofoed-Enevoldsen, A., Kjellén, L., Deckert, M., Lykkelund, C., & Burcharth, F. (1991). Possible genetic defects in regulation of glycosaminoglycans in patients with diabetic nephropathy. *Diabetes*, *40*(6), 764-770.

13. Yard, B., Feng, Y., Keller, H., Mall, C., & van Der Woude, F. (2002). Influence of high glucose concentrations on the expression of glycosaminoglycans and N-deacetylase/N-sulphotransferase mRNA in cultured skin fibroblasts from diabetic patients with or without nephropathy. *Nephrology Dialysis Transplantation*, *17*(3), 386-391.

14. Gambaro, G., & Van der Woude, F. J. (2000). Glycosaminoglycans: use in treatment of diabetic nephropathy. *Journal of the American Society of Nephrology*, *11*(2), 359-368.

15. Ceol, M., Nerlich, A., Baggio, B., Anglani, F., Sauer, U., Schleicher, E., & Gambaro, G. (1996). Increased glomerular alpha 1 (IV) collagen expression and deposition in long-term diabetic rats is prevented by chronic glycosaminoglycan treatment. *Laboratory investigation; a journal of technical methods and pathology*, *74*(2), 484-495.

16. Masola, V., Zaza, G., & Gambaro, G. (2014). Sulodexide and glycosaminoglycans in the progression of renal disease. *Nephrology Dialysis Transplantation*, *29*(suppl_1), i74-i79.