



Original Research Article

Correlation of serum hyaluronic acid with diabetic indices in type 2 diabetes mellitus patients with diabetic nephropathy

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ABSTRACT

Introduction: Hyaluronic Acid (HA) is produced by the endothelial smooth muscle cells and adventitial fibroblasts of the arterial wall. In conditions of hyperglycemia, there is an accumulation of HA in blood vessels and can contribute to the diabetic micro- and macrovascular complications. The current study aims to determine the association of serum Hyaluronic Acid levels in Type 2 diabetes mellitus with diabetic nephropathy and its correlation with diabetic indices and renal function test parameters.

Materials and Methods: 60 male subjects, 30 cases of Type 2 Diabetes Mellitus patients with diabetic nephropathy and 30 non-diabetic healthy subjects as controls. Fasting venous blood samples were collected from cases and controls and analyzed for serum HA by ELISA and HbA1C, FBS, PPBS, urea, and creatinine by Beckmann Coulter Au 480 automated analyser. 24 hours urine was collected and analyzed for urinary microalbumin by an immunoturbidimetric method. Statistical analysis was done with SPSS package and p-value < 0.05 was considered significant.

Results: Serum HA levels were significantly higher in diabetic patients with nephropathy (75 ± 6 ng/ml) when compared to the normal control groups (25 ± 5 ng/ml). Serum HA also showed a significant positive correlation with FBS, PPBS, HbA1C, urea, creatinine and urine microalbumin.

Conclusion: Highly elevated serum Hyaluronic Acid levels and its positive correlation with diabetic indices and renal function test parameters in Type 2 Diabetes Mellitus patients with diabetic nephropathy suggests its association with poor blood glucose control and may possibly predict the development of diabetes-related vascular complications in Type 2 diabetics without any complications.

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1. Introduction

Diabetes is the most widely prevalent chronic metabolic disease. The macrovascular and microvascular diabetic complications like retinopathy, nephropathy, and neuropathy, occurring as a result of enhanced atherogenesis in diabetes are linked with the increased medical and socioeconomic burden.^{1,2}

HA, a member of glycosaminoglycans (GAGs) is a linear polymer, which comprises the major fraction of carbohydrates in ECM (Extracellular Matrix). Generally, HA is present in low amounts in blood vessels but raises intensely in vascular diseases.^{3–5}

Hyaluronic acid (HA) is a sulfate-free glycosaminoglycan consisting of repeating disaccharide units, D-glucuronic acid, and N-acetyl-D-glucosamine. It is produced by arterial wall cells (fibroblasts, endothelial cells) and participates in the regulation of cellular processes such as adhesion, migration, and proliferation.⁶ The interaction of HA with receptors of cell membrane adds to morphogenesis, tissue remodeling, and inflammation.⁷

The length of HA influences the biological function. High molecular weight HA (106 Da) inhibits angiogenesis, while degradation products of HA of 3–25 disaccharide units stimulate angiogenesis. Along with other glycosaminoglycans (GAGs), HA has a critical role in the interaction of vascular tissue and blood components. GAGs reduces the interaction between endothelial cells and

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leukocytes.⁸

The vascular endothelium is coated by a so-called glycocalyx, one of the factors known to maintain vascular homeostasis.⁹ Hyaluronan glycosaminoglycans, are the major constituents of the glycocalyx which are crucial for preserving the properties of an endothelial barrier for plasma macromolecule.¹⁰

The extracellular network (ECM) gives imperative help to vascular tissue which goes about as a stage for protecting the arrangement of vascular cells into blood vessels, for maintaining the equilibrium, for cell multiplication, migration, and survival. The striking changes in this extracellular grid have been connected to the advancement of atherosclerosis.^{11–13} Angiogenic Hyaluronan oligomers exhibit competition with non-angiogenic native long-chain HA in binding to HA receptor CD44 which are present on endothelial cells.¹⁴ Activation of CD44 is mitogenic and triggers MMP-2(Matrix Metallo -Proteinases) and MMP-9 production which in turn activates transforming growth factor-beta (TGF- β) and cell invasion through ECM to facilitate vascular sprouting and outgrowth.¹⁵ Increased HA fragments in response to hyperglycemia promote activation, migration, and proliferation of cells^{16–22} via interactions with its receptors,²³ including Receptor for Hyaluronan Mediated Motility(RHAMM),²⁴ CD44,²⁵ and Toll-like receptors 2 and/or 4.²⁶

The current study aims to determine the association of serum Hyaluronic Acid levels in Type 2 diabetes mellitus with diabetic nephropathy and its correlation with diabetic indices and renal function test parameters.

2. Materials and Methods

The study included 60 male subjects with 30 cases and 30 controls. The study was led at Victoria Hospital, joined to Bangalore Medical College and Research Institute, Bangalore. Clinically diagnosed Type 2 DM male patients with diabetic nephropathy between age bunch 50-60 years were incorporated into the study. 30 age and sex coordinated non-diabetic healthy people were taken as controls. Patients with alcoholism, patients with liver disease and patients with serious cardiovascular issues were excluded from the study.

2.1. Method of analysis

Ethical committee clearance was obtained from the institution for the study. After obtaining written informed consent from the cases and controls, about 5 ml of fasting venous blood sample was obtained by venepuncture under aseptic conditions and divided into 2 parts, first part of blood was taken in a sterile EDTA tube and was used for measuring-g HbA1C and second in a plain tube, centrifuged and separated serum was used for measuring HA, fasting blood glucose. The postprandial blood sample

was collected at 2 hours in a plain tube for PPBS, HbA1c, FBS and PPBS were measured in automated analyzer BECKMAN COULTER AU480. Serum Hyaluronic acid was measured by ELISA. 24 hours urine sample was collected and analyzed for urinary microalbumin by the immunoturbidimetric method in automated analyser BECKMAN COULTER AU480 The results were tabulated. Results are presented on Mean $\pm$ SD. The Statistical analysis of data was done by using software namely SPSS 20.0. The results of cases and controls were compared by student 't' test. A 'p' value of <0.05 was considered significant. Pearson's Correlation analysis was used for correlation tests. A 'p' value of <0.0001 was considered highly significant.

3. Results

A comparative study with 30 male Type 2 diabetic nephropathy cases and 30 male controls was undertaken and FBS, PPBS, HbA1C, serum urea, serum creatinine, HA and urinary microalbumin levels were studied. The age distribution pattern of controls and diabetic nephropathy cases under study which ranges from 50-60 years with a mean age of 54.67 $\pm$ 2.30 in controls and in cases 58.41 $\pm$ 2.1 (p=0.50).

The study showed significantly higher serum levels of HA (p<0.0001) in diabetic patients with nephropathy (75.17 $\pm$ 6.23ng/ml) than in the normal healthy controls (39.8 $\pm$ 8.4 ng/ml).

The correlation study revealed a significant positive correlation between HA and FBS, PPBS and HbA1c (r=0.8892; r=0.8744; r=0.8544) in diabetic nephropathy cases, whereas there is a negative correlation between HA and FBS, PPBS and HbA1c (r = - 0.0134 ; r = - 0.1903 ; r = - 0.2786) in control group. Correlation study also revealed a positive correlation between HA and blood urea (r = 0.8545) in cases where as control group shows negative correlation between serum SA and blood urea (r = - 0.155). There is a positive correlation between serum SA and serum creatinine in cases (r = 0.9032) and a negative correlation in controls (r = - 0.324). There was a positive correlation between HA and urinary microalbumin (r = 0.3121) in cases and negative correlation in controls (r = - 0.3219). (Table 2).

4. Discussion

This study was conducted to determine the association of serum Hyaluronic Acid levels in Type 2 diabetes mellitus with diabetic nephropathy and its correlation with diabetic indices and renal function test parameters. Our study showed a significantly high levels of serum Hyaluronic acid and also significant positive correlation with short term diabetic indices FBS and PPBS and with long term diabetic index HbA1c in diagnosed cases of type 2 DM with nephropathy as compared to non-diabetic healthy controls

Table 1: Comparison of means of serum HA, FBS, PPBS and HbA1C in two groups studied

Lab Variables	Controls	Cases	p value
Age in years	54.67 ± 2.30	58.41 ± 2.1	0.50
Gender-Males	30	30	1
Serum HA (ng/ml)	38.2 ± 4.86	75.17 ± 6.23	<0.0001
FBS (mg/dl)	86.77 ± 4.66	160.2 ± 27.5	<0.0001
PPBS (mg/dl)	123.93 ± 5.76	250.14 ± 48.12	<0.0001
HbA1C (%)	4.81 ± 0.26	7.3 ± 0.87	<0.0001
Serum Urea	25.6 ± 2.63	69.31 ± 4.64	<0.0001
Serum Creatinine	1.01 ± 0.21	2.94 ± 0.40	<0.0001
Urinary Microalbumin	9.66 ± 1.05	149.79 ± 30.83	<0.0001

Table 2: Correlation of Serum HA with FBS, PPBS, HbA1C, Urea, Creatinine and Urinary Microalbumin in controls and cases studied

S. no	HA vs Other Parameters	Cases r value	p value	Controls r value	p value
1.	HA (ng/ml) Vs FBS (mg/dl)	0.8892	<0.0001	-0.0134	0.9190
2.	HA (ng/ml) Vs PPBS (mg/dl)	0.8744	<0.0001	-0.1903	0.1452
3.	HA (ng/ml) Vs HbA1C (%)	0.8544	<0.0001	-0.2786	0.0311
4.	HA (ng/ml) Vs urea (mg/dl)	0.8545	<0.0001	-0.0169	0.8980
5.	HA (ng/ml) Vs creatinine (mg/dl)	0.9032	<0.0001	-0.3240	0.0115
6.	HA (ng/ml) Vs Urinary Microalbumin (mg/g Cr)	0.3121	<0.0100*	-0.3219	0.0121

suggesting elevated hyaluronic acid levels in response to poor glucose control in accordance with the study conducted by Shinichiro et al²⁷ which showed a significant high levels of serum HA in type 2 diabetes without complications and much higher levels in diabetes with complications.

The current study also demonstrates a highly significant positive correlation between serum HA and renal function test parameters serum urea, creatinine, and urine microalbumin. This is supported by Sano et al study,²⁸ which showed proteinuria increases the accumulation of HA. Another study by Akin et al²⁹ showed that serum HA levels increased as the amount of proteinuria in the 24-hour urine increases. An increased HA production by proximal tubular cells, renal fibroblasts, mesangial cells, glomeruli in diabetic kidneys has been reported. Renal HA expression is increased in some chronic disease states, such as diabetes and nephrolithiasis, which in turn have the potential to lead to chronic renal insufficiency.³⁰

The study conducted by Hansell et al³¹ showed that though HA is not a major component of the normal renal corticointerstitium, it is profoundly expressed around proximal tubular cells (PTC) in diverse renal diseases.^{32–34} The study conducted by Jones et al³⁵ has demonstrated that on exposure to high concentrations of glucose in vitro, HA synthesis by proximal tubular cells was initiated.

The study carried out by Mahadevan et al³⁶ postulated the role of HA in the pathogenesis of diabetic nephropathy and its involvement in the initiation of glomerular hypercellularity in the streptozotocin model of diabetes following the

publication of the observations that increased hyaluronan production. Thus, in conditions of hyperglycemia, elevated HA accumulates in blood vessels and possibly contributes to the complications of diabetes at micro and microvascular levels.

5. Conclusion

From the findings of the present study, it can be concluded that Type 2 diabetes mellitus with nephropathy was associated with a significant increase in serum HA levels as compared to controls. Serum HA levels showed a significant positive correlation with FBS, PPBS and HbA1c and significant positive correlation with renal function parameters in this study reflects its role in diabetic nephropathy. Therefore, it is possible that serum HA levels may indicate general impairment of blood vessels caused by hyperglycemia due to inappropriate blood glucose control in diabetes. This elevated HA levels may further contribute to vascular damage resulting in vascular complications of Type 2 Diabetes and hence can be used as a predictive marker of diabetes-related vascular complications in Type 2 Diabetics without complications.

6. Source of funding

None.

7. Conflict of interest

None.

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