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INFLAMMATORY MARKER (SERUM SIALIC ACID) AND HbA1c ASSOCIATION IN PATIENTS HAVING TYPE 2 DIABETES WITH AND WITHOUT DIABETIC RETINOPATHY

MAJEED N[1], MAHMOOD R[*2], BATOOL M[3], SHOUKAT Z[4], SADIQ MI[5] AND DASS NH[6]

1: MBBS, MPhil, Assistant Professor Department of Biochemistry, Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur, Kashmir Pakistan

2: MD, MPhil, PhD, Associate Professor Department of Physiology, Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur, Kashmir Pakistan

3: MBBS, M. Phil, Assistant Professor Department of Physiology, Poonch Medical College, Rawalkot, Kashmir Pakistan

4: MBBS, Post Graduate Trainee Physiology, Foundation University Medical College Islamabad Campus

5: MBBS, FCPS, Assistant Professor Department of Ophthalmomolgy, Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur, Kashmir Pakistan

6: MPhil (Biochemistry) Assistant Professor, Department of Biochemistry, Edwards College Peshawar, Pakistan

[*]Corresponding Author: Dr. Rashad Mahmood: E Mail: rashadmahmood74@yahoo.com

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ABSTRACT

Background/aim: Diabetes Mellitus is a major metabolic disorder leading to macro as well as microvascular complications including retinopathy, which is a major cause of blindness worldwide. The study was conducted to find and compare the levels of total serum sialic acid with HbA1c levels in diabetic patients with and without retinopathy.

Methods: For this study, one hundred eighty patients having type 2 diabetes aged forty and above were selected based on the inclusion and exclusion criteria set for the study. Patients were divided into two groups: Group A (ninety diabetic patients having diabetic retinopathy) and Group B (ninety diabetic

patients without having diabetic retinopathy). Data was collected after informed written consent of the individuals.

Findings: The mean ± SD sialic acid and HbA1c for both groups, A and B were 464·74 ± 348·18 mmol/l and 7·43 ± 1·42%, and 433·49 ± 408·64 mmol/l and 8.08 ± 1.62% respectively. There was no difference seen in serum sialic acid levels in both groups (t = 0·55, p = 0·581). However, HbA1C was found significantly lower in the group without diabetic retinopathy (t = -2·87, p = 0·005).

Interpretation: Although serum sialic acid levels do increase in diabetes, there were no significant links between rising levels of serum sialic acid and the development of diabetic retinopathy. However, the opposite was found to be true for glycosylated hemoglobin.

Keywords: Inflammatory Marker, Sialic Acid Diabetic Retinopathy, HbA1c Association

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder described by persistently elevated glucose levels and impaired carbohydrate, fat, and protein metabolism. There are two common types of diabetes: Type 1 diabetes or Insulin Dependent Diabetes resulting from the destruction of beta cells in the pancreas leading to a deficiency of insulin; Type 2 diabetes or Non-insulin Dependent Diabetes caused by reduced sensitivity of the tissues to insulin **[1]**.

Global estimates suggest that the number of people having diabetes rose from 108 million in 1980 to 422 million in 2014 with a higher prevalence in low- and middle-income countries than in high-income countries **[2]**. According to the most recent estimates, the prevalence of diabetes in Pakistan is 9·8% and attributes to about 3% of the mortalities **[3]**. The prevalence of type 2 diabetes in Pakistan ranges from 7·6% -11% and is expected to reach up to 15% by the year 2030 **[4, 5]**.

As cited, several risk factors such as family history, increased body mass index (BMI), high diastolic blood pressure (DBP) and higher systolic blood pressure (SBP); increased waist circumference (WC), higher waist-hip ratio (WHR), and higher fasting blood glucose are linked to diabetes **[6, 7]**.

Diabetic retinopathy is one of the major complications of DM **[8]** causing gradual loss of vision and blindness in some diabetics, and its prevalence increases with the age of the patient and duration of diabetes mellitus **[9]**. The incidence of diabetic retinopathy has been associated with the use of insulin and the duration of treatment **[10-12]**. There is also an association found between blood pressure and the level of glycemic control with diabetic retinopathy **[13]**.

Sialic acids are a group of nine-carbon sugars and are an essential part of

glycoproteins and glycolipids. The most important sialic acid present in human tissues is N-acetyl Neuraminic acid **[14]**. Increased serum sialic acid (SSA) levels are strongly associated with the presence of microvascular complications e.g., retinopathy and nephropathy **[15]**. In individuals having diabetes, sialic acid increases due to abnormalities of the erythrocyte cell membrane resulting in increased levels **[16]**. Some studies reported that there may be no significant correlation between SSA, age, diabetes duration, and complications of diabetes.[15] Whereas another study reported that increased serum sialic acid was seen in diabetic groups, with or without complications **[17]**.

HbA1c is a type of hemoglobin that is non-enzymatically linked to sugar in a process called glycation. During this process, glucose gets bound to the amino groups of the N- terminal valine of the β-globin chains **[18]**. Glycated hemoglobin reflects the glycemic control of a person with diabetes in the past 2-3 months. An average HbA1c level is below 5·7% while for pre-diabetics it is 5·7%-6.4%. HbA1c of 6·5% or above-indicated diabetes **[19]**. The measurement of glycated hemoglobin can be used to figure out the risk of the development of different vascular complications of diabetes **[20]**. In addition, raised levels are associated with elevated serum levels of C-reactive protein (CRP) suggesting an association between systemic inflammation and glycemic control in diabetics **[21, 22]**. Some studies reported that there was no significant difference seen in the levels of fasting plasma glucose, glycosylated hemoglobin, and serum sialic acid in diabetic patients with and without chronic complications like retinopathy **[23, 24]**.

The study determined the role of serum sialic acid in diabetes and compared its levels with HbA1c. It also helped us understand their roles in the progression of diabetes into its microvascular complications such as diabetic retinopathy.

MATERIALS AND METHODS

Experimental Design

This was a Cross-sectional Comparative study in which one hundred eighty diabetic patients aged forty and above were divided into two groups i.e., Group A: having no diabetic retinopathy and Group B: having diabetic retinopathy.

Sample Selection

Samples were collected through non-probability consecutive sampling technique by considering the following criterion:

Inclusion Criteria

Group A. Patients diagnosed with Type 2 Diabetes Mellitus without retinopathy.

Group B. Patients diagnosed with Type 2 Diabetes Mellitus with retinopathy.

Exclusion Criteria

Group A. Diabetic patients with any known rheumatic disease, hypothyroidism, neoplastic disorders, uremia, and diabetic retinopathy.

Group B. Diabetic patients with any known rheumatic disease, hypothyroidism, neoplastic disorders, and uremia.

Sample Collection

Five ml of blood (150 μl for SSA, 100 μl for HbA1c and 50 μl for fasting blood sugar level per test) was drawn from antecubital vein of study participants under strict aseptic conditions after twelve hours of fast to determine the serum or blood levels of glucose, sialic acid, and HbA1c. To prepare the serum, 2·5 ml sample was left to coagulate and then centrifuged at 3000 rpm for 15 minutes. Samples were stored at -20°C until its use.

Screening and Chemical Analysis

Patients were screened for diabetic retinopathy by fundoscopy after assessing visual acuity in both eyes using Snellen's Chart. Blood pressure was measured by a mercury sphygmomanometer. Samples were used for the analysis of:

- Serum sialic acid by ELISA Kit method using GLORY BIO
- Fasting blood glucose by auto analyzer using Chroma Test Kit through GOD-PAP method.
- HbA1c by chromatography and read spectrophotometrically in the whole blood sample.

Statistical Analysis

All the data were statistically analyzed by using Statistical Package for Social Sciences (SPSS) software version 20.0. All qualitative variables were reported by using frequencies and percentages as shown in **Tables 3 and 4**, while quantitative variables were reported by using ± SD. Student t-test was then applied to figure out the mean difference in serum sialic acid and HbA1c in both diabetic groups. A p-value of <0·05 was considered statistically significant. BMI was calculated in accordance with the formula BMI= weight/ height^2 (kg/m^2) **[6]**.

RESULTS

For the study, 180 diabetic patients were selected and divided into two groups: Group A without having diabetic retinopathy and Group B having diabetic retinopathy. They have been biochemically assessed for their serum sialic acid and blood HbA1c levels. The mean ± SD sialic acid and HbA1c for both groups, A and B were 464·74 ± 348·18 mmol/l and 7·43 ± 1·42% and 433·40 ± 408·64 mmol/l and 8·08 ± 1·62% respectively. Then a two-sample t-test was applied to determine the relationship between serum sialic acid and HbA1c in the two groups. There was no difference seen in serum sialic acid levels in both groups (t = 0·55, p = 0·581).

However, HbA1c was found significantly lower in the group without diabetic retinopathy (t = -2·87, p = 0·005).

Table 1: Anthropometric and Characteristics of Patients in Both Study Groups

Variable	Group	Mean	Standard Deviation	Minimum	Maximum
Age of patients	Group A	53·83	9·55	30	76
	Group B	53·67	8·97	40	78
Duration of Diabetes	Group A	7·79	3·31	5	17
	Group B	14·16	8·24	5	56
Body Weight (Kg)	Group A	71·82	11·74	45	110
	Group B	72·19	14·39	44	114
Height (cm)	Group A	160·67	8·48	142	180
	Group B	161·131	17·92	160	187
Body Mass Index (kg/m^2)	Group A	27·99	4·75	18	44
	Group B	27·33	5·31	19	45
Systolic BP (mmHg)	Group A	133·78	20·03	90	190
	Group B	143·27	28·94	90	240
Diastolic BP (mmHg)	Group A	82·20	12·82	50	130
	Group B	83·67	17·46	50	150

***Note*: For group A (patients without diabetic retinopathy) the mean ±SD age was 53·83±9·55 years with a range of 30-76 years. The mean ±SD duration of diabetes mellitus was 7·79±.3·31 years with a range of 5-17 years. The mean ±SD BMI was 27·99 ±4·75. The mean ±SD was 133·78 ±20·03 and 82·20 ±12·82 for Systolic and Diastolic BP, respectively.**

For groups B (patients with diabetic retinopathy) the mean ±SD age was 53·67±8·97 years with a range of 40-78 years. The mean ±SD duration of diabetes mellitus was 14·16±.8·24 years with a range of 5-56 years. The mean ±SD BMI was 27·33 ±5·31. The mean ±SD was 143·27 ±28·94 and 83·67 ±17·46 for Systolic and Diastolic BP, respectively.

Table 2: Biochemical Characteristics in Both Study Groups

Variable	Group	Mean	Standard Deviation
Serum Sialic Acid (mmol/l)	Group A	464·74	348·18
	Group B	433·49	408·64
Fasting Blood Sugar (mg/dl)	Group A	177·31	58·79
	Group B	199·76	78·06
HbA1c (%)	Group A	7·43	1·41
	Group B	8·08	1·62

***Note*: The mean ±SD serum sialic acid and HbA1c concentrations were 464·74±348·18 mmol/l and 7·43±1·41% for Group A, respectively. While the mean ±SD serum sialic acid and HbA1c concentration were 433·49±408·64 mmol/l and 8·08±1·62% for Group B, respectively.**

Table 3: Frequency of Family History of Diabetes Mellitus in Whole Study Population

	Group A	Group B	Total
Positive Family History	64	64	128
Negative Family History	26	26	52

***Note*: One hundred twenty-eight (71·11%) patients had a positive family history of diabetes while fifty-two (28·88%) had a negative family history of diabetes. In group A, sixty-four (71·11%) patients reported a positive family history of diabetes and it appeared to be the same in group B, interestingly.**

Table 4: Mode of Treatment of Diabetes Mellitus in Whole Study Population

Variable	Group A	Group B	Total
Oral Hypoglycemic Drugs	79	50	129
Insulin	3	19	22
Oral Hypoglycemic Drugs + Insulin	8	20	28
Dietary	0	1	1

***Note*: Overall, fifty (27·78%) study participants were on insulin therapy either alone or as in combination with oral hypoglycemic drugs. One hundred twenty-nine (71·66%) participants used oral hypoglycemic drugs as a sole agent for the treatment of diabetes. Whereas only one (0·56%) participant was on dietary measures to control diabetes. In group A seventy-nine (87·77%) patients were on oral hypoglycemic drugs, three (3·33%) were on insulin, and eight (8·88%) were receiving insulin in addition to oral hypoglycemic drugs for the management of diabetes.**

In group B fifty (55·55%) patients were on oral hypoglycemic drugs, nineteen (21·11%) were on insulin, and twenty (22·22%) were receiving insulin in addition to oral hypoglycemic drugs for the management of diabetes. Only one (0·56%) patient in group B was managing diabetes through dietary measurements.

DISCUSSION

Numerous inflammatory markers are believed to be increased in diabetes and its various complications **[25, 26]**. According to Nayak B, there has been a significant increase in serum sialic acid levels in diabetics with or without complications of diabetes **[26]**. Diabetic retinopathy has been shown to be affected by the concentrations of serum sialic acid and there is a link between the two of them **[15, 26-28]**. However, at present, there is no consensus present about the association of serum sialic acid with various complications of diabetes **[14, 15, 27-32]**. In this study, we were not able to find any statistical link between increased serum sialic acid concentrations and the occurrence of diabetic retinopathy. Comparable results have been reported by Deepa *et al*., **[33]** who observed that there was no significant difference in the serum sialic acid levels between type 2 diabetes patients having no retinopathy, type 2 diabetes patients with proliferative diabetic retinopathy, and type 2 diabetes patients with non-proliferative diabetic retinopathy **[33]**. Crook *et al*., also reported no correlation between diabetic retinopathy and total serum sialic acid in UAE and the trend was present in Arab as well as South Asian populations **[15]**. Since Pakistan is in the same region, unsurprisingly our results are in line with the results reported by the aforementioned researchers. However, a few studies from Pakistan do report otherwise.

We did not include any of the healthy controls in the study, therefore no comments can be made on the increment of serum sialic acid in diabetes as they were not compared with non-diabetics. However, inflammatory markers such as sialic acid do increase in diabetes **[15]**.

In this study, we found a statistical link between HbA1c concentrations and the development of diabetic retinopathy with mean ± levels of HbA1c being 8·08±1·62%. This observation has also been reported by Memon and his colleagues from Pakistan **[34]** that an increased prevalence of diabetic retinopathy was seen in patients with HbA1c levels of 8% or more. Numerous studies reported the association of increased HbA1c concentrations with positive family history, longer duration of diabetes, and development of diabetic retinopathy **[35-37]**. consistent with our study. A recent study from Shanghai reported that the mean HbA1c of diabetic patients and mean systolic blood pressure are remarkably associated with the

incidence of diabetic macro-vascular and microvascular complications, suggesting that diabetic retinopathy is associated with poor glycemic control **[38]**.

CONCLUSION

There is no significant difference in the levels of serum sialic acid in both study groups i.e., 464·74 ± 348·18 in group A and 433·49 ± 408·64 in group B, respectively. The presence of conflicting evidence regarding the inflammatory markers in the development of complications of diabetes makes it difficult to determine their role in the pathogenesis of the disease. However, studies with a larger sample size can help us determine the true nature of this association. The recorded observations relevant to HbA1c conclude that diabetic retinopathy is associated with poor glycemic control which is usually manifested by an increased HbA1c level.

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Author's Disclosure

We have no conflicts of interest to disclose and ICMJE form has been filled and signed by both authors. We have no support in financial or other manner to disclose. No copyright infringement is intended.

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