Correlation between Serum Sialic Acid Level and frequent risk factors of Diabetic Retinopathy

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Summary:

Background: Diabetic retinopathy is an important complication of diabetes mellitus. It is supposed that elevated sialic acid in diabetes mellitus plays an important role in diabetic retinopathy. This study investigated serum total sialic acid levels related to glycemic control, blood pressure, retinopathy, and serum lipid level in diabetic retinopathy patients.

Patients & Methods: Type 2 diabetic patients aged (56.47±10.68) years were recruited for the study. Fasting venous blood samples were collected from 132 diabetic subjects of whom 79 without retinopathy and 53 were diabetic with retinopathy. All the blood samples were processed for serum total sialic acid (TSA), fasting serum glucose (FSG), HbA_{1c}, and lipid profile. Systolic and diastolic blood pressure was recorded by standard mercury sphygmomanometer.

Results: Serum (TSA), FSG, HbA_{1c}, triglycerides (TG), and LDL-cholesterol were increased significantly ($P<0.01$ for TSA, FSG, HbA_{1c}; $P<0.05$ for TG, and LDL) in patients with diabetic retinopathy (DR) compared to diabetics without retinopathy. Duration of diabetes and blood pressure were also significantly higher in DR patient compared to those without retinopathy. Correlation analysis showed a significant positive correlation between serum TSA and several risk factors of diabetic retinopathy: diabetic duration, FSG, HbA_{1c}, systolic blood pressure SBP, diastolic blood pressure DBP, and LDL in diabetic patients with retinopathy.

Conclusion: It is concluded that elevated serum total sialic acid level is strongly associated with the presence of diabetic retinopathy, a microvascular complication of diabetes. Increasing concentration of sialic acid is clinically correlated with several risk factors of diabetic retinopathy including glycemic control (blood sugar and HbA_{1c}), hypertension, and duration of diabetes, triglycerides, and LDL. These findings strengthen the hypothesis that the increase in serum sialic acid is early manifestation of diabetic retinal disease.

Keywords: Type 2 diabetes, sialic acid, diabetic retinopathy, risk factors

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Introduction:

Sialic acid (SA) is a component of cell membranes, the preferred localization of SA is in the outer cell membranes where those sugars often occur in high concentration and are component of glycoproteins, gangliosides, or polysaccharides(1). Serum sialic acid is a protein bound carbohydrate and occurs in combination with monosaccharides like galctose and mannose. Ninety percent is bound and almost none are free (2); in serum they are generally bound to acute phase proteins (3). Elevated levels may indicate excessive cell membrane damage, but more specifically to the cells of vascular tissue. If there is damage to vascular tissue, this leads to ischaemia and this ischaemia is most visible in the smallest blood vessels, including those of the retina, kidneys, heart, and brain (1, 4). Diabetes Mellitus (DM) is a chronic metabolic disorder that can lead to severe cardiovascular, renal, neurologic, nephropathy, and retinal complication (5). Diabetic retinopathy (DR) affects the blood vessels of retina and leads to blindness, for over 10,000 people with DM every year (6), the progression of retinopathy is gradual, advancing from mild abnormalities, characterized by increased vascular permeability, to moderate and severe non-proliferative diabetic retinopathy, characterized by the growth of new blood vessels on the retina and posterior surface of the vitreous (8, 7). A number of risk factors have been associated with the metabolic syndrome, including hypertension, poor glycemic control, obesity, duration of diabetes, dyslipidemia and glycated end products(8). Within resent years, there has been considerable interest and research into sialic acid and its use as a potential inflammatory marker for diabetes mellitus (9). Previous reports have also indicated that serum sialic acid (SSA) concentrations were associated with increase risk of cardiovascular disease in the diabetic population complications (10). The study primarily focuses on the determination whether SA concentration was increased in diabetic patients with and without retinopathy, and the relationship between SSA and metabolic variable.

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Patients and Methods:

A total of 132 previously diagnosed type 2 diabetic patients, their mean age was 56.47±10.68 years, who attend to the National Diabetic Center (Al-Mustansiria University) carried out ophthalmoscopic examination. According to the ophthalmoscope examination diabetic subjects were divided into two groups: (group1)-79 patients without retinopathy; (group2)-54 patients with diabetic retinopathy. Patients suffering from type 1 diabetes mellitus, gestational diabetes, and any known mental illness, macrovascular disease were excluded. All patients were reported in the morning after overnight fast, and underwent physical examination and laboratory tests. Height and weight were noted for Body Mass Index (BMI), it was calculated as weight (Kilogram)/height^2 (meter^2). Blood pressure was

measured twice on the right arm using standard mercury sphygmomanometer while the patient was sitting after resting for 10 min. Laboratory evaluations consisted of measuring serum total sialic acid (TSA), fasting serum glucose (FSG), glycated hemoglobin (HbA_{1c}), and lipid profile. The method for TSA was essentially as described by Katopoidis and coworkers (11) with slight modification in the volume of sample used. Hemoglobin A_{1c} program intended for the determination of Glycated hemoglobin ($A1_c$) in human depended on high performance liquid chromatography and who supplied by Variant Company, USA. Glucose level was determined using kits supplied by Randox, UK. Total cholesterol TC, Triglycerides, High density lipoprotein (HDL) was determined using kits from (biomaghreb, Sa, France). Low density lipoprotein (LDL) was calculated mathematically using the Friedwald formula.

Statistical analysis:

Data were analyzed using computer facility-the available statistical packages of SPSS-11 (statistical packages for social sciences-version 11.0). Data were present in simple measures of Mean±SD. The significance of difference between quantitative variables was tested using student t-test for comparing between two means of independent groups. P value equal and less than 0.05 was used as the level of significance, and P value equal and less than 0.01 was used as the level of a highly significant. Pearson correlation coefficient is significant at the 0.05 level (2-tailed)

Results:

The level of TSA was studied in diabetic subjects with retinopathy to be compared with diabetic patients without retinopathy, thus TSA was found to be higher in diabetic retinopathy (p value< 0.01) as shown in table1. The degree of glycemic control represented by glycated hemoglobin level (HbA_{1c}), and FSG was studied in diabetic patients with retinopathy to compare with their uncomplicated patients. HbA_{1c}, and FSG found to be significantly elevated in those diabetic retinopathy versus those without retinopathy ($p<0.01$) as shown in table1. Systolic and diastolic blood pressures were raised significantly ($P<0.001$, $p<0.01$) respectively in patients with diabetic retinopathy compared to DM without retinopathy. Although, diabetic duration found to be increased significantly in diabetic retinopathy compare to diabetic patients without retinopathy as shown in table1. The study showed that TG and LDL were significantly increased in diabetic patients with retinopathy compare with diabetic subjects without retinopathy, but there was no significant difference in TC, and HDL level as shown in table1. Correlation analysis showed a significant positive correlation between serum total sialic acid (TSA) and FSG ($r=0.51$, $p<0.01$); HbA_{1c} ($r=0.49$, $p<0.01$); systolic blood pressure ($r=0.37$, $p<0.05$); diastolic blood pressure ($r=0.34$, $p<0.05$); duration ($r=0.48$, $p<0.01$); and LDL-cholesterol ($r= 0.36$, $p<0.05$) in diabetic patients with retinopathy as shown in table2. Another, finding of the study was that serum TSA was not correlated to age, sex, cholesterol, triglycerides, and HDL levels.

Table1: unvaried analysis of various factors that could be risk in the presence of diabetic retinopathy.

Variables*	Group 1: (n: 79) DM without retinopathy	Group 2: (n: 53) DM with retinopathy	P value
Age (years)	55.6±11.3	57.04±9.07	N.S
Sex (m:f)	(41:38)	(28:25)	N.S
Duration of DM	6.99±5.7	13.67±6.9	<0.001
BMI (kg/m^2)	29.26±5.5	28.7±5.5	N.S
SBP (mmHg)	123.8±8.2	162.5±15.7	<0.001
DBP (mmHg)	79.4 ±6.2	92.6±8.0	<0.01
TSA (mg/dl)	114.7±16.2	122.7±15.6	0.01
FSG (mg/dl)	162.7±69.5	215.4±61.5	<0.01
HbA_{1c}	7.41±1.6	9.31±1.7	<0.01
TC (mg/dl)	215.9±46.7	230.8±68.2	N.S
TG (mg/dl)	122.4±72.3	194.2±98.2	<0.05
HDL (mg/dl)	46.3±10.27	45.6±10.5	N.S
LDL (mg/dl)	133.32±42.8	152.5±60.2	<0.05

***Values are expressed as Mean±SD, NS: not significant.**

Table (2): The correlation between serum TSA and variables.

Parameters	DM with retinopathy	
	R	P
TSA – FSG	0.51**	<0.01
TSA – HbA_{1c}	0.49**	<0.01
TSA – Duration	0.48**	<0.01
TSA – SBP	0.37*	<0.05
TSA – DBP	0.34*	<0.05
TSA – LDL	0.36*	<0.05
	DM without retinopathy	
	R	P
TSA – HbA_{1c}	0.31*	<0.05
TSA – Duration	0.29*	<0.05

***correlation is significant at the 0.05 level (2-tailed)**
****correlation is a highly significant at the 0.01 level (2-tailed)**

Discussion:

Within recent years, there has been considerable interest and research into sialic acid and its use as a potential inflammatory marker for diabetes mellitus (9). Previous studies have indicated that serum sialic acid concentrations are elevated in diabetics (both type 1 and type2) with and without complications, while other reported no such correlation (12, 13). Studies have also found that the presence or absence of this trend may relate to ethnicity (14). Our research indicates that SSA concentration was elevated in DR when compared to diabetic without retinopathy. There are several possible explanations for the increase in SSA concentrations, several research studies have shown that the concentration of sialic acid in serum is elevated in pathological states when there is damage to tissue, tissue proliferation and inflammation(2). Other study has also indicated that vascular permeability is regulated by sialic acid moieties; the vascular endothelium carries a high concentration of sialic acid and hence extensive microvascular damage associated with

type 2 diabetic patients could account for its shedding into the circulation, this lead to an increase in vascular permeability and overall increase SSA concentrations (10). Although, tissue injured caused by diabetic vascular complications stimulates local cytokine secretion from cells involved in the complications such as macrophage and endothelium, this induces an acute phase response which involves the release of acute phase glycoproteins with sialic acid from the liver into the general circulation leading to increase SSA concentrations (9). Korte, (15) observed that plasma membrane or regenerating retinal pigment epithelium contained SA-N-acetyl glucosamine residues. Although, in diabetic retinopathy there is a further increase in microvascular damage which may have resulted in the greater increase in the SSA concentrations observed, diabetic retinopathy is characterized by a wide spectrum of different features. Thrombolytic vascular interactions are characterized by platelet spreading and capillary fragility which are significant for development diabetic retinopathy (16). The study showed that serum TSA was related with several risk factors, notably FBG, HbA_{1c}, duration, hypertension, and LDL in diabetic retinopathy. Serum TSA is a marker for inflammation, so its serum concentrations have been commonly increased during acute inflammation (4). Results from prospective studies suggested that inflammation involved in the pathogenesis of diabetes (17). Inflammation could be common antecedent for both diabetes and cardiovascular disease. Hyperglycemia and insulin resistance could also promote inflammation and may be factor linking diabetes to the development of atherosclerosis; elevated glucose levels could promote inflammation by increased oxidative stress (18). The degree of glycemic control represented by glycated hemoglobin level (HbA_{1c}), and FSG was studied in diabetic patients with and without. They were significantly higher in diabetic retinopathy. This situation could be explained by the fact that hyperglycemia damages retinal vascular in several ways and progression of DR is generally related to the severity and duration of hyperglycemia. The exact mechanism by which raised glucose levels lead to vascular disruption seen in retinopathy is poorly defined. However, various biochemical pathways have been suggested to demonstrate a correlation between hyperglycemia and microvascular complications of retinopathy. Among these pathways increasing the activity of protein kinase C (PKC) and glycation of key proteins that lead to formation of advance glycation ends (AGEs) products are more important than polyol accumulation or oxidative stress (19). In this study, the duration of the disease has been demonstrated to be increased significantly in diabetic retinopathy. Increased duration influencing the occurrence of diabetic retinopathy and its severity was probably related to the magnitude or prolonged exposure, or both to hyperglycemia coupled with other risk factors (20), and the prevalence of DR is highly related with the increasing HbA_{1c}. So the duration of diabetes is an important factor for the incidence and development of DR. similar conclusions were also found in several studies, which demonstrated that the duration of diabetes and hyperglycemia contributed to the development of DR (21, 22). The present study found a statistically significant association between hypertension and DR, which accordance with previously published reports (23, 24). Though, a lot of published studies have shown the association between hypertension and DR, the exact pathogenesis is not known. However, the available evidence support the theory that raised blood pressure causes endothelial stress with release of vascular endothelial growth factors (VEGF) altering retinal auto regulation leading to increase perfusion pressure and injury (23) other results (24, 25) have demonstrated that hypertension is determinal to each stage of DR and a tight blood pressure control strategy can reduce the risk of eye complications from diabetes. Our data suggest that only serum triglyceride was associated with diabetic retinopathy and not cholesterol; one explanation would be that raised triglycerides and not cholesterol is associated with insulin resistance (26). An increased triglycerides level is a common feature of diabetes mellitus, research has suggested that this is a result of reduced action of insulin on adipocytes resulting in suppression of lipolysis, this results in reduced hydrolysis of stored triglycerides and so a greater increase in non esterified fatty acids(27). Our data also suggest that serum LDL-cholesterol increased significantly in diabetic retinopathy, this result consistent with other observation in early treatment diabetic retinopathy study (28, 29). In conclusion, the present study suggests that increased serum TSA levels are associated with the development of diabetic retinopathy. The markers of glycemic control (blood sugar and HbA_{1c}), hypertension, and duration of diabetes, triglycerides, and LDL are clinically correlated with increasing concentration of sialic acid. These findings strengthen the hypothesis that the increase in circulating serum sialic acid is early manifestation diabetic retinal disease. Further research would be help to clarify the role of sialic acid in development of retinopathy.

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