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Serum adiponectin levels in diabetic retinopathy

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ABSTRACT

The present study was carried out with the aim to find out the association between serum adiponectin levels with diabetic retinopathy in order to search for a putative biomarker for development and prevention of Diabetic retinopathy and to co-relate adiponectin levels with the severity of diabetic retinopathy. Patients of Diabetes mellitus attending the outdoor and indoor divisions of the department of Ophthalmology, King George's Medical University, Lucknow, were recruited for the study with their informed consent. Non proliferative and proliferative diabetic retinopathy was assessed by the Ophthalmologist. 63 subjects in each of the two primary groups; diabetic and non-diabetic were taken. Blood samples each from cases and controls were collected under aseptic condition and the sera samples were used for biochemical tests as well as Adiponectin ELISA. Comparisons between the serum adiponectin levels of different groups were studied and it was found that control group has the highest adiponectin levels which is significantly different from that of the diabetic group; Control group adiponectin levels are not statistically different from the PDR sub-group and there is statistically significant difference in adiponectin levels between diabetic without retinopathy and the PDR subgroup.

Abbreviations: APN: Adiponectin, DM: Diabetes Mellitus, DR: Diabetic Retinopathy, PDR: Proliferative Diabetic Retinopathy, NPDR: Non Proliferative Diabetic Retinopathy

1. INTRODUCTION

India leads the world having the largest number of diabetic subjects, earning the dubious distinction of being termed the "diabetes capital of the world" and the so called "Asian Indian Phenotype" referring to certain unique clinical and biochemical abnormalities in Indians which include increased insulin resistance, greater abdominal adiposity *i.e.*,

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higher waist circumference despite lower body mass index, lower adiponectin levels and higher high sensitive C-reactive protein levels (Akihisa Imagawa, et al., 2002; Akiko Higuchi, 2009) phenotype makes Asian Indians more prone to diabetes and its complications.

Diabetic retinopathy (DR), a common complication of diabetes mellitus and is the principal cause of irreversible blindness in patients of working age. Pathological retinal neovascularization is a common feature of these ischemic retinopathies (Akiko Higuchi, 2009). Clinically diabetic retinopathy are diagnosed as; non proliferative and proliferative. Non proliferative retinopathy is the earlier stage which usually is asymptomatic. There may be retinal hemorrhages and exudates resulting in retinal anoxia. Macular edema may lead to diminution of vision. In the early phase of diabetic retinopathy, hyperglycemia initiates endothelial cell injury, retinal vessel loss, and ischemia, as well as changes in leukocyte adhesion to the vascular endothelium.

Second stage is the proliferative retinopathy in which new abnormal vessels develop in the retina and grow towards the center of the eye. These vessels frequently bleed into the vitreous which cause severe visual problems. Small bleeds may clear up on their own but larger bleeds need surgery. The abnormal vessels may also produce large scars in the retina that may cause retinal detachment. If diagnosed and treated promptly, blindness is usually preventable.

These conditions subsequently lead to the overproduction of various proangiogenic factors and proinflammatory cytokines, which, in turn, promotes abnormal neovascular changes. It is now recognized that adipose tissue functions as an endocrine organ by secreting adipocytokines such as leptin, tumor necrosis factor-α (TNF-α), plasminogen activator inhibitor type 1, interleukin (IL)-1β, IL-10, retinol binding protein-and adiponectin , that are directly involved in obesity-linked disorders (Akiko Higuchi, 2009; Dieg et al., 2003).

Studies have shown that an inflammatory cytokine adiponectin (APN) is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism. APN is exclusively secreted from adipose tissue into the blood stream where it accounts for approximately 0.01% of all plasma proteins at around 5-10 microgram/ml. Plasma concentrations reveal a sexual dimorphism, with females having higher levels than males.

Levels of APN are reduced in diabetics as compared to non-diabetics (Kenichi Kato, et al., 2008). The plasma adiponectin concentrations have been found to be lower in patients with type 2 diabetes than in age, BMI and sex-matched controls, lower in patients with diabetic retinopathy than in those without retinopathy and are involved in the generation of diabetes and diabetic retinopathy. Serum APN levels have also been found to be associated with stage of DR, independent of age, gender, BMI, duration of type 2 and type 1 DM, and hypertension . Recent experimental studies provide evidence that adiponectin protects against retinal vessel injury following pathological stimuli through modulation of TNF-α inflammatory responses (Mahmut Ilker Yilmaz et al., 2004; Mohan Rema et al.,2005).

The primary goal for treatment of diabetic ischaemic retinopathy is to preserve vision through the inhibition of abnormal neovascularization and vascular damage. The ease with which the levels of adiponectin can be measured owing to its high abundance, small diurnal variation and high stability in plasma have made it a popular target for measurements in many clinical studies (Nedvidkova et al. 2005). It has emerged as a valuable biomarker for insulin sensitivity, cardiovascular risk and inflammation. Adiponectin levels have been measured in many additional disease states (Rei Shibata et al., 2009). Preclinical studies not only have implicated adiponectin as an outstanding biomarker but also have demonstrated direct cardio-protective and insulin-sensitizing properties to be associated with the protein (Rei Shibata et al., 2009; Renaldi O et al., 2009). Adiponectin might, therefore, be a viable protein therapeutic that could be supplied in a recombinant form.

2. AIM

The aim of this study was to find out the association between serum APN levels with diabetic retinopathy in order to search for a putative biomarker for development and prevention of Diabetic retinopathy.

3. HYPOTHESIS

Total serum APN levels are significantly decreased in type 2 Diabetes mellitus as compared to healthy non diabetic controls.

4. MATERIAL AND METHODS

This study was tertiary care hospital based case control study. Patients of Diabetes mellitus attending the outdoor and indoor divisions of the department of Ophthalmology, King George's Medical University, Lucknow, UP were recruited for the study with their informed consent and subjected to a detailed questionnaire on diabetes. Physical examination, blood pressure and BMI measurement, tests for retinopathy (fundus examination and fluorescein

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ADIPONECTIN

AviBion Human Adiponectin (Acrp30) Elisa Kit (90 cells plate) by Orgenium laboratories, Finland was used for performing the test on serum adiponectin. This kit was meant for Research Use Only (RUO). First the serum samples containing adiponectin and standard solution were diluted in the test wells as per the manual's instructions; biotinylated antibody was then added followed by washing. Ready-to-use streptavidin-horse peroxidase was then added followed by half an hour incubation and tetramethylbenzidine (TMB) substrate addition with a 20 minute incubation following it. Lastly Stop solution was put in to end the reaction and colour read at 450 nm within 15 minutes on ELISA microplate reader and the results obtained by multiplying the serum value by the dilution factor.

angiography) and serological tests were done. Non proliferative and proliferative Diabetic Retinopathy was assessed by the Ophthalmologist based on fundus examination and fluorescein angiography.

Definitions of cases-

1. **Diabetes mellitus without diabetic retinopathy-** no evidence of diabetic retinopathy on fundus examination
2. **Diabetes mellitus with Non-proliferative diabetic retinopathy-** presence of at least one retinal microaneurysm with one or more of the following: retinal haemorrhage, exudates, venous beading or IRMA (intra retinal microvascular abnormality)
3. **Diabetes mellitus with Proliferative diabetic retinopathy-** presence of one or more of the following: NVE (neo vascularisation elsewhere), NVD (neo vascularisation disc) or tractional retinal detachment or vitreous haemorrhage obscuring ability to grade NVE/NVD

BMI (Body Mass Index) done using Broca's index (weight in kilogram/ height in meter square)

Hypertension – considered as Systolic blood pressure >/140 mm Hg and/or Diastolic blood pressure > 90 mm Hg and/or patients under medication with antihypertensive drugs.

Controls : Age, sex and BMI matched non-diabetic healthy persons were taken as controls.

Sample size: 63 subjects in each of the two primary groups; diabetic and non-diabetic. STAT10.1 software has been used to test the hypothesis that the mean adiponectin level in diabetic subjects is 0.5 units lower than that in healthy non-diabetic controls.

Inclusion criteria: Controlled type 2 diabetes mellitus patients, with/without retinopathy, with HbA1c<7%, age >40 years.

DM with PDR
Presence of one or more of the following: NVE (neo vascularisation elsewhere), NVD (neo vascularisation disc) or tractional retinal detachment or vitreous haemorrhage obscuring ability to grade NVE/NVD.

Exclusion criteria: Patients of diabetes mellitus with any/all of the following : sepsis, nephropathy, neuropathy, age <40 years, hypertensive retinopathy, and patients on insulin

Procedure:

Fasting 10 ml blood samples each from cases and controls were collected under aseptic condition and the sera separated. A part of the sera was used for biochemical tests as detailed below and remaining sera was stored at minus 80 degree Celsius for Adiponectin ELISA (Enzyme Linked Immuno Sorbent Assay). Biochemical tests for blood sugar, urea, creatinine, and lipid profile were done as per standard protocols and HbA1c by Glycohemoglobin-Ion-Exchange resin method used in the chemical pathology laboratory of the Department .Serum adiponectin estimation was carried out using a commercially available ELISA kit (as per manufacturer's instructions).

DM with NPDR
Presence of at least one retinal microaneurysm with one or more of the following: retinal haemorrhage, exudates, venous beading or IRMA (intra retinal microvascular abnormality.

5. RESULTS

Comparisons in serum adiponectin levels were made between the following groups:

1. control and patients with diabetes type 2, diabetes without retinopathy, NPDR and PDR
2. diabetic patients without retinopathy and those with NPDR
3. diabetic patients without retinopathy and those with PDR
4. diabetic patients with NPDR and those with PDR

The adiponectin values for the various groups were obtained by the ELISA (Tables 1 & 2; Figures 1 & 2).

6. DISCUSSION

Studies on serum total and high molecular weight adiponectin levels (Kato et al., 2008) have shown an increasing trend with the advanced stages of diabetic retinopathy and nephropathy. Total adiponectin was shown to be inversely correlated to a number of factors involved in the metabolic syndrome, including high blood pressure, obesity, low levels of high density lipoprotein (HDL), high triglycerides and impaired glucose tolerance. However prospective studies have been suggested as required to determine the cause of the observed correlation (Kenichi

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Table 1

CONTROL (n=63)		DIABETES MELLITUS type 2 (n=63)	
10.58± 2.7		8.83±2.2	
CONTROL (n=63)	Diabetics without Retinopathy(n=21)	Diabetics with NPDR (n=21)	Diabetics with PDR (n=21)
10.58± 2.7	7.74 ± 1.5	8.99±2.6	9.77±2.0

Table 2

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Label	Control (n=63)	Diabetic (n=63)	p
	Mean±SD	Mean±SD	
Adiponectin	10.58±2.7 (10.34)	8.83±2.2 (8.41)	**0.0001**[1]

Label	Control (n=63)	Diabetic (n=21)	NPDR (n=21)	PDR (n=21)	p
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Adiponectin	10.58±2.7 (10.34)	7.74±1.5 (7.48)	8.99±2.6 (8.42)	9.77±2.0 (9.31)	<**0.0001**[1]

Label	Control (n=63)	Diabetic (n=21)	p
	Mean±SD	Mean±SD	
Adiponectin	10.58±2.7 (10.34)	7.74±1.5 (7.48)	<**0.0001**[1]

Label	Control (n=63)	NPDR (n=21)	p
	Mean±SD	Mean±SD	
Adiponectin	10.58±2.7 (10.34)	8.99±2.6 (8.42)	**0.0195**

Label	Control (n=63)	PDR (n=21)	p
	Mean±SD	Mean±SD	
Adiponectin	10.58±2.7 (10.34)	9.77±2.0 (9.31)	0.1793[1]

Label	Diabetic (n=21)	NPDR (n=21)	p
	Mean±SD	Mean±SD	
Adiponectin	7.74±1.5 (7.48)	8.99±2.6 (8.42)	0.1249[1]

Label	Diabetic (n=21)	PDR (n=21)	p
	Mean±SD	Mean±SD	
Adiponectin	7.74±1.5 (7.48)	9.77±2.0 (9.31)	**0.0005**

Label	NPDR (n=21)	PDR (n=21)	p
	Mean±SD	Mean±SD	
Adiponectin	8.99±2.6 (8.42)	9.77±2.0 (9.31)	0.2751

Note:

1= Non-parametric tests used (Kruskall Walli's/ Mann-Whitney test).
Figures in parenthesis are the corresponding median.

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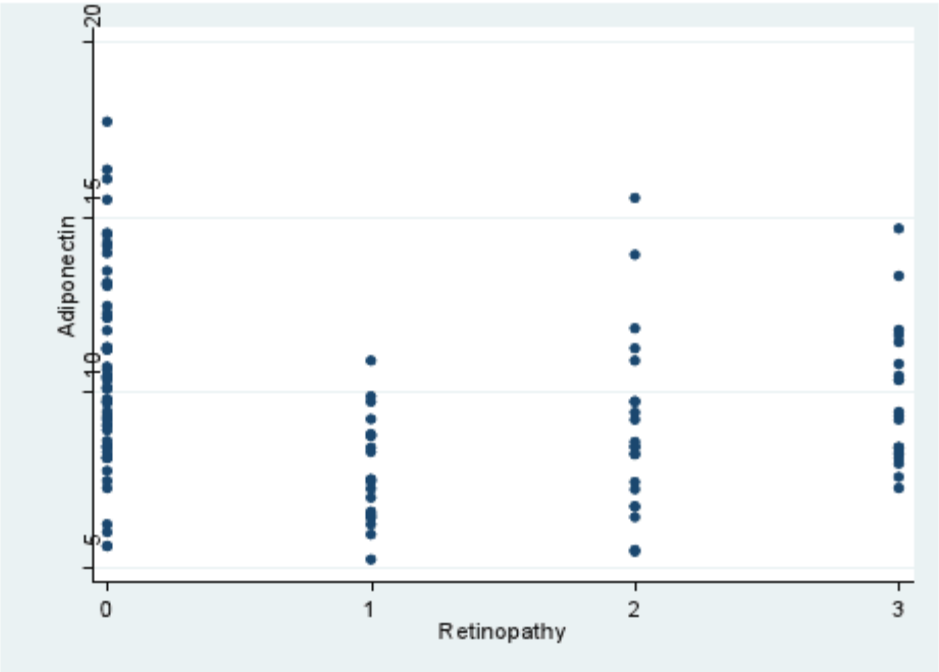

As evident from the various subgroup analysis, the control group has the highest ADPN level but not statistically different from PDR group. However, we have found statistical difference in ADPN levels between diabetic without retinopathy group VS PDR group.

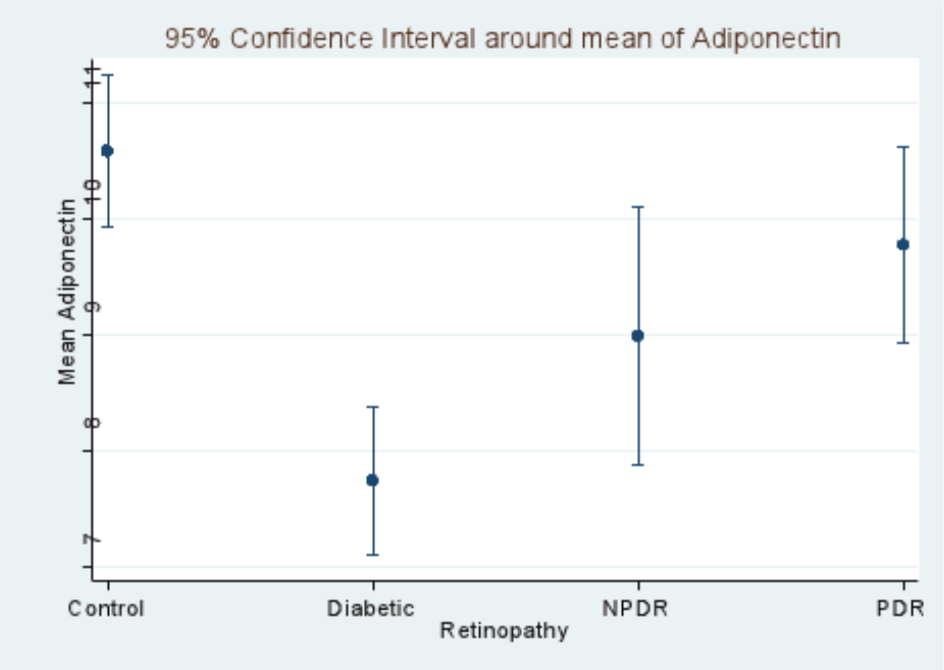

Figure 1 & 2

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Kato, et al., 2008). Plasma concentrations of adiponectin are decreased in obesity, coronary disease, hypertension, insulin resistance and type 2 diabetes. Experimental studies have shown that adiponectin reduces the plasma concentrations of free fatty acids and triglycerides and improves insulin resistance (Yamuchi et al., 2001). Another study (Mahmut Ilker Yilmaz et al., 2004) has reported its major finding as that fasting plasma concentrations of adiponectin were decreased in diabetic patients, proportionate to the severity of retinopathy; with the general agreement that the duration of diabetes and the severity of hyperglycemia are major risk factors for its development. However, ROC (Receiver Operating Characteristics) curve analysis did not reveal a threshold concentration of adiponectin for the existence of diabetic retinopathy and thus a predictive value cannot be assigned to adiponectin concentrations in relation to the development of the condition.

7. CONCLUSION

Statistically significant differences between the groups were thus studied using Kruskall Walli's / Mann-Whitney test

1. Control group has the highest adiponectin levels which are significantly different from that of the diabetic group.
2. Control group adiponectin levels are not statistically different from the PDR sub-group.

There is statistically significant difference in adiponectin levels between diabetics without retinopathy and the PDR sub-group.

FUTURE ISSUES

The study may provide a lead as to what an extent the adiponectin levels in diabetic retinopathy can be found useful as a screening or a therapeutic biomarker and the association of adiponectin with the severity of diabetic retinopathy may lead to future projects on the early detection and prevention of the condition.

DISCLOSURE STATEMENT

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