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Effect of Insulin-Like Growth Factor-1 on Diabetic Retinopathy in Pubertal Age Patients With Type 1 Diabetes

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Purpose: The aims of this study were to correlate diabetic retinopathy (DR) changes with insulin-like growth factor 1 (IGF-1) levels in patients with type 1 diabetes of pubertal age group and to correlate the level of retinopathy with IGF-1 levels.

Methods: This cross-sectional study was done over 2 years and involved patients with type 1 diabetes of age 8 to 25 years. Patients presenting to Ophthalmology OPD and inpatient department along with active recruitment from old pediatrics and endocrinology records were taken for the study. Fasting serum IGF-1 was calculated using enzyme-linked immunosorbent assay technique. Fasting blood sugar levels were taken. Detailed ophthalmic examination was done and DR was noted in all the patients and correlated with IGF-1 levels.

Results: A total of 46 patients with type 1 diabetes were recruited into the study. The mean age of the patients was 14.33 ± 4.36 years, with a female-to-male ratio of 3:2. No relationship of IGF-1 with age of onset of diabetes ($P = 0.7$) or fasting capillary blood glucose (CBG) ($P = 0.6$) was found, but a significant relationship was found with duration of diabetes ($P = 0.001$) and low IGF-1 levels ($P < 0.0001$).

Conclusions: Severity of DR in patients with type 1 diabetes is inversely related to serum IGF-1 levels. Low IGF levels are an indicator for closer follow-up and strict management of diabetes and retinopathy.

Key Words: enzyme-linked immunosorbent aasay, human insulin-like-growth-factor-1 (IGF-1), puberty, type 1 diabetes mellitus, diabetic retinopathy

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Diabetes is the most common noncommunicable disease globally.[1] It is expected that diabetic retinopathy (DR) will cause approximately 4 million people around the world to lose their sight and thus it has become one of the leading causes of blindness.[2] Type 1 diabetes mellitus is the result of interactions of genetic, environmental, and immunologic factors that ultimately lead to the destruction of the pancreatic beta cells and insulin deficiency. It results from autoimmune beta cell destruction and develops mostly before the age of 30 years. Type 1 diabetes accounts for about 5% to 10% of all diagnosed diabetes.[3]

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DR was first described in 1855 by Jaeger just a few years after the invention of the ophthalmoscope. It refers to retinal changes seen in patients with diabetes mellitus and is more common in type 1 diabetes than in type 2.[4]

Interestingly, it has been found that DR rarely develops before puberty[5]; infact children diagnosed before age of 2 years have been found to have negligible risk of retinopathy for first 10 years of life[6] and this has been linked to hormonal changes during puberty.[7]

Recently insulin-like growth factor (IGF)-1 has gained a lot of interest in the pathophysiology of DR. IGF-1 is closely related to insulin, except that its C chains are not separated and it has an extension of the A chain called the D domain.[8] It is the peripheral target hormone for growth hormone (GH). Its levels start increasing during puberty, peak around 16 years, and subsequently decline by >80% during the aging process.[3]

A research done by Ben Mehidi et al[7] in 2003 on children and adolescents showed that DR rarely occurred before puberty and was never proliferative in prepubescent children. On the contrary, puberty and adolescence were found to be high-risk periods for DR progression. The period between 16 and 18 years of age was found to be particularly critical. It was attributed to several factors associated with type 1 diabetes such as diabetes duration, difficulties in achieving glycemic control due to increase in insulin requirements, low compliance to treatment, and most of all hormonal changes related to puberty. Several other researches[9,10] have also shown that the risk of DR was higher in patients who developed diabetes in pubertal age rather than in prepubertal age signifying that puberty is a risk factor for DR changes in patients with type 1 diabetes.

There have been various studies suggesting the role of IGF-1 in DR. Few studies have shown a direct correlation of IGF-1 with DR, whereas some have shown an inverse relationship. A study by Haurigot et al[11] demonstrated that intraocular IGF-1 triggers processes leading to blood retinal barrier breakdown and thus increased retinal vascular permeability. This caused edema and tissue damage resulting in visual impairment in patients with DR.

The low serum IGF-1 levels in infants with retinopathy of prematurity, a condition of severe neovascularization, have long suggested that IGF-1 might prevent angiogenesis.[12,13] IGF has been shown to have a role in retinal neurogenesis and there is some evidence that it may also have neuroprotective effects.[14] Conversely, Payne et al[15] in a large cross-sectional study of 225 subjects did not find any association with serum IGF-1 concentrations and Laron and Weinberger concluded that both GH/IGF-1 excess and GH/IGF-1 deficiency can promote DR.[16]

Thus, majority of previous studies on the role of IGF-1 in the pathogenesis of DR have provided varying results and are indeterminate. This study aimed to establish more supportive data for relationship between DR and serum IGF-1 levels. Furthermore, it

aimed to find a correlation of DR in patients with type 1 diabetes with age, sex, duration of diabetes, and IGF-1 levels to support research studies to consider IGF-1 as a new target factor in the control of DR in patients with type 1 diabetes in younger individuals.

METHODS

This study was conducted at a tertiary hospital in Southern India spanning over a period of 2 years between August 2014 and August 2016. Ethics Committee approval was obtained. Inclusion criteria included all patients diagnosed with type 1 diabetes of age group 8 to 25 years irrespective of duration and type of treatment, patients not known to have any syndromic association such as autoimmune polyendocrine syndromes, Down syndrome, Turner syndrome, and Prader–Willi syndrome. Patients with chronic systemic illnesses such as tuberculosis and kidney disease were excluded. Patients suffering from ophthalmic disorders such as amblyopia, congenital glaucoma, and congenital optic disc abnormality cases were excluded so as to prevent any bias. Patients were actively searched from pediatrics and endocrinology records and patients reporting to the outpatient and inpatient care were recruited into the study. Tenets of Declaration of Helsinki (1964, amended 2013) were adhered to.

The explorative study was carried out for 2 years and 46 blood samples of 46 patients were collected over 3 days as all patients could not present on a single day. All the patients were called in a fasting state of 8 to 10 hours. Before collection, a detailed history, general physical examination, and detailed ophthalmic examination of both eyes including visual acuity, refraction, and fundoscopy were done for all patients and after obtaining written informed consent, blood sample was collected and fasting blood sugar levels were noted. Blood samples were taken in BD vacutainer Acid Citrate Dextrose glass tubes designed for serum collection and kept at room temperature for 2 hours to allow sedimentation of serum and then centrifuged for 10 to 12 minutes at 3000 to 4000 revolutions/minute using a standard centrifugation machine, REMI R-8C BL.

After centrifugation, the samples were transferred to the 1.5-mL microcentrifuge tubes [Eppendorf India Limited Headquarters Plot No. 18, 19, 20 (Part), Ambit Park Road, Sidco Industrial Estate (South), Ambattur, Chennai], labeled properly, and stored at −80°C immediately.

IGF-1 enzyme-linked immunosorbent assay testing kit by Demeditec Diagnostics GmbH, Lise-Meitner-Straße 2, D-24145 Kiel (Germany) was opened at a single setting and detailed instructions of the kit were followed for enzyme-linked immunosorbent assay testing of all samples. The findings of fundus photography were graded for DR according to Early Treatment DR Study revised Modified Airlie House Classification.[17] The grading was done by the first author (P.R.) who is a professor and head of department at the institute where the study was conducted. He is a retina specialist with a vast experience of more than 35 years.

The collected data were analyzed with the aid of SPSS version 22.0 for all the calculations and formulated in the form of tables, figures, graphs, and diagrams whenever necessary, using Microsoft Excel 2013. Both descriptive and inferential statistics were employed for data analysis. Statistical tests used were independent-samples *t* test, Kruskal–Wallis test, Mann–Whitney test, and chi-square rest.

A *P* value of ≤0.05 was considered significant.

TABLE 1. Age Distribution of Patients in Age Groups

Age, y	No. of Patients	Percentage of Patients
8–10	11	23.9
11–13	12	26.1
14–16	8	17.4
17–19	9	19.6
≥20	6	13.0
Total	46	100

RESULTS

This was a cross-sectional study carried out at a tertiary care hospital in southern India. The study spanned over 2 years starting from August 2014 till August 2016 with result analysis performed in September 2016.

A total number of 46 patients were studied after strict application of inclusion–exclusion criteria. Among these, 29 patients who did not have DR were marked as controls. The remaining 17 patients showed various grades of DR, hence marked as cases. All patients underwent testing for fasting serum IGF-1 levels and the values were correlated with the clinical evidence of retinopathy.

The mean age of the patients included in the study was 14.33 ± 4.36 years and the median age was 13.50 years. The maximum number of patients was found to be in the age group of 11 to 13 years (n = 12), with the minimum number of patients older than 20 years (n = 6) as depicted in Table 1. Most of the patients included were females which constituted 61% (n = 28) of total in this study.

Forty-four of the total 46 patients had best-corrected visual acuity of 6/6 in both eyes using standard Snellen distant visual acuity charts. Two patients had low vision, 1 each in the case and control groups. The patient in the case group had proliferative diabetic retinopathy (PDR) changes in both eyes and had undergone laser sittings 3 times in each eye and had best-corrected visual acuity of 6/12. The patient in the control group with low vision had developmental cataract and was advised cataract surgery but was lost to follow-up.

Most patients with retinopathy changes (35.3%) belonged to the 17–19 age group. This was in comparison with patients without DR changes who were mainly concentrated in 8 to 13 yrs (65.5%). This was statistically significant ($P = 0.05$). The mean age of controls (12.9 years) was found to be significantly less than that of the cases (16.7 years; $P = 0.003$)

In both case and control groups, females were more than males. The male-to-female ratio was approximately 2:3 in both groups and was found to be not statistically significant ($P = 0.8$).

Of the total 17 patients with DR-related changes, 12 had mild non proliferative diabetic retinopathy (NPDR), 2 had moderate NPDR, and 1 had severe NPDR whereas 2 cases were found to have PDR.

Only the PDR cases had undergone treatment in the form of laser photocoagulation. No statistical significance was found between age of onset of diabetes and DR ($P = 0.7$).

The cases were found to have a highly significantly longer duration of diabetes (6.88 vs 3.09 years, Table 2) as compared with the controls ($P = 0.001$).

The mean fasting blood sugar levels of both groups were similar, 200 mg/dL (SD 126.98) for the cases versus 206 mg/dL

TABLE 2. Comparison of Different Factors With Retinopathy Changes Among Patients

	No Retinopathy			Retinopathy		
	Mean	**Median**	**SD**	**Mean**	**Median**	**SD**
Duration of diabetes	3.09	2.00	3.34	6.88	5.00	4.73
Serum IGF-1 level ng/ml	102.03	102.37	47.66	82.50	69.06	36.12

DM indicates diabetes mellitus; IGF-1, insulin-like growth factor 1.

(SD 107.63) of the controls. The median fasting blood glucose levels were found to be 50% higher in cases as controls ($P = 0.6$).

Direct comparison of serum IGF-1 levels between cases and controls showed that patients without retinopathy had much higher values of serum IGF-1 than the patients with retinopathy. There was an almost 1.5 times difference in the median values of the 2 groups (Table 2). However, this finding was not statistically significant ($P = 0.1$).

The age and sex distribution of the serum IGF-1 levels in comparison with the normative data showed that 94.1% (n = 16) of the cases had low IGF-1 levels in comparison with 41.4% (n = 12) of the controls. Similarly, 58.6% (n = 17) of the controls had normal IGF levels in comparison with only 5.9% (n = 1) of the cases with normal IGF levels and was found to be highly significant ($P < 0.0001$).

No relationship was found between fasting blood sugar levels and serum IGF-1 levels among the patients ($P = 0.535$).

DISCUSSION

This cross-sectional study was conducted at a tertiary level hospital in south India. Seventeen patients had type 1 diabetes-related retinopathy changes, who were designated as "cases", whereas the remaining 29 were having no retinopathy changes and were designated as "controls".

The mean age of the patients was 14.33 ± 4.36 years. This was similar to 15.54 ± 2.9 years in a similar study on 68 patients by polish scientist Peczynska et al.[18] In our study, the cases (16.7 years) were found to be significantly older than the controls (12.9 years). Thus, it was inferred that patients who are in the peak of puberty were more commonly associated with retinopathy changes rather than the patients who were in prepubertal age group. Fascinatingly, this is the age group wherein IGF-1 levels are expected to be at their highest value in an individual,[3] but as discussed further we found our values to be lowest. Ben Mehidi et al[7] had also found that patients in the age group of 16 to 18 years were most likely to have retinopathy changes.

In both groups, females were found to be about 1.5 times more and were found to have no relationship with DR. In an epidemiological cross-sectional study by Esteves et al,[19] a group of Brazilian researchers found that most patients had mild NPDR (15.1%) and PDR (22.2%) rather than moderate and severe NPDR (7.2% combined). This was in contrast to our study which found that most patients had mild NPDR (26.1% patients) and PDR was only found in 4.3% patients, whereas only 6.5% cases had moderate and severe NPDR. This was probably due to the higher age group (at least 18 years) and longer duration of diabetes (minimum 5 years) in their study, whereas in our study the duration of diabetes was just 3 years. Thus, most patients had probably progressed to PDR changes due to the longer duration of diabetes in their study.

The cases were found to have a mean 2.2 times longer duration of diabetes than the controls (cases 6.88 years vs controls 3.09 years) and this was found to be highly significant ($P = 0.001$). A study by Esteves et al[19] has also found similar findings.

Fasting blood sugar levels were found to have no relationship with DR, a similar finding was found by Esteves et al.[19] Even though cases were found to have almost 1.5 times lower median IGF-1 levels than the controls, the relationship failed to achieve significance ($P = 0.1$). On the contrary, it was found that the patients had an inverse relationship of IGF-1 levels with severity of DR. The patients with proliferative DR had the lowest levels of IGF-1 as compared with patients with nonproliferative retinopathy. An inverse relationship of DR severity was seen with IGF-1 level. Patients with severe NPDR and PDR had significantly lower IGF-1 level as compared with patients with Mild DR changes. Furthermore, Patients with No DR changes had maximum IGF-1 level (Fig. 1). Due to extremely small sample size of patients in different groups, P value was not calculated to avoid bias. It was noted that the mean values of IGF-1 in different subgroups were comparable to the median values; thus, the data were found to be parametric and following a bell curve-like pattern (Fig. 1).

For the normative IGF-1 levels, it was found that 5.9% case patients had normal IGF-1 levels in comparison with 58.6% of the controls. This was a highly significant finding ($P < 0.001$), and is similar to the extensive research done by German scientists Chantelau and Frystyk[20] who also found that DR is inversely related to IGF-1 levels and even further postulated that parenteral GF analogues such as octreotide be administered in patients to prevent DR.

IGF-1 has been found to have a protective role in pregnant patients with type 1 diabetes in a study by Finnish researchers Loukovaara et al.[21] In another study by Janssen and Lamberts,[22] it was found that the decline in serum IGF-1 caused a significant progression of diabetic micro vascular complications.

Polish scientists Peczynska et al[18] who did a similar study on 68 adolescent patients of type 1 diabetes also found that serum IGF-1 levels were reduced in patients with retinopathy in comparison with controls. They concluded that impaired activity of GH/IGF-1 axis may be responsible for the development of diabetic microangiopathy in patients with type 1 diabetes. In fact, Israel scientists Laron and Weinberger[16] have described patients with IGF-1 deficiency (Laron syndrome) to have much more vascular complications of diabetes mellitus (DM) in their case series.

In their extensive research, Chantelau and Kohner[23] found that for normal secretion of IGF-1 by the liver, an adequate insulin level in the portal system is required; thus, in poorly controlled insulin-dependent diabetes, plasma IGF-1 concentrations are low and might be one of the causes of increased retinopathy associated with low IGF-1 levels as in our study.

Higashi et al[24] worked extensively on IGF-1 and found that it not only reduced atherosclerotic burden and was responsible for the increment in atherosclerotic plaque stability in animal models

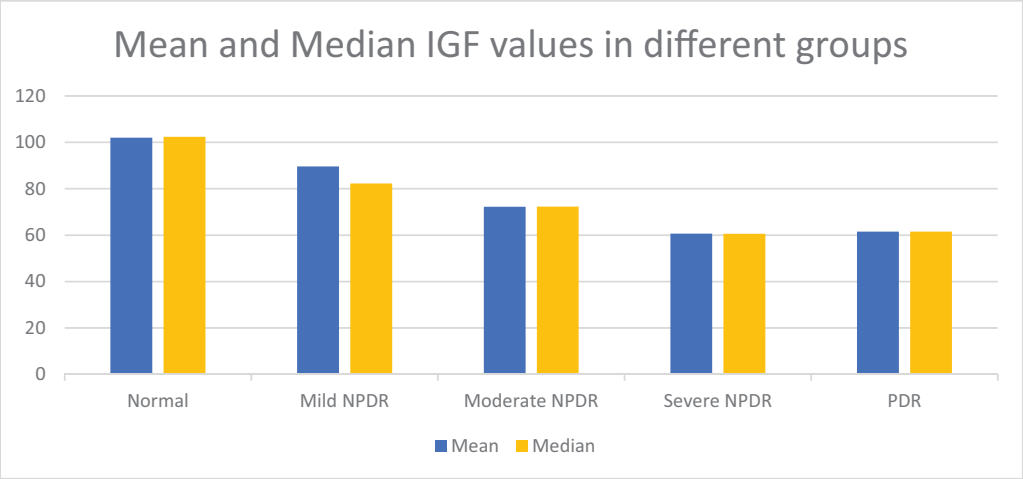

FIGURE 1. Comparison between severity of diabetic retinopathy and serum IGF-1 levels. IGF-1 indicates insulin-like growth factor 1.

but also found that it might help in longevity of cells showing a protective role of IGF-1 like that seen in retinopathy of prematurity, a condition of neovascularization secondary to hypoxia wherein severely low amounts of IGF-1 levels and high levels of vascular endothelial growth factor are seen.[13] It is possible that a similar mechanism works in patients with type 1 diabetes around the pubertal age group who have abnormal IGF-1 axis leading to more retinopathy changes.

Lately, IGF-1 has been postulated to be neuroprotective in various studies and its deficiency might be another explanation for increased retinopathy in our study patients. It has been considered as a potential for the treatment of motor neuron disease[25] and has been used for the treatment of neuroprotection in stroke.[26]

This is one of the few studies that demonstrate inverse association of serum IGF-1 levels with DR around puberty and further depicts the systemic hormonal changes occurring during puberty could be an indirect factor in the development of DR. We observed that patients with decreased IGF-1 values in pubertal age group had more chances of developing DR. It is observed that these patients failed to have a peak of IGF-1 levels in their puberty.

A major limitation of this study was the absence of details on management of diabetes of the patients. Even though all patients were under proper care and follow-up with an endocrinologist, tests such as HbA1c used to monitor blood glucose levels over 3 months were not done in the study.

The results of this study suggests that serum IGF-1 levels can be added to routine investigations in patients with type 1 diabetes and low levels could form a basis for initiating an intense retinopathy management. These findings should encourage further investigation into the mechanism of effect of IGF-1 on DR, its potential as a target for therapy, and the possibility of a new horizon in the clinical care of DR. Further studies on relationship of IGF-1 levels with retinopathy can have an immense impact on patients with type 1 diabetes especially in a larger sample size for further establishment of these results.

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