LEPTIN LEVELS IN VITREOUS FLUIDS OF PATIENTS WITH DIABETIC RETINOPATHY

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ABSTRACT

Introduction: Leptin, a 16 KDa protein hormone, plays a key role in regulating energy intake and energy expenditure, including the regulation of appetite and metabolism. Abnormalities of leptin secretion and regulation are implicated in obesity, the metabolic syndrome and diabetes mellitus type 2 along with its complications such as neovascularization. The present study aimed to compare the levels of leptin in vitreous fluids of diabetic patients suffering from non-proliferative and proliferative diabetic retinopathy.

Materials and Methods: The comparative study was carried out from June to November 2010 on inpatients of Al-Shifa Eye Trust Hospital, Rawalpindi, Pakistan. In this study, 45 patients with confirmed diabetic retinopathy were selected, comprising 22 female and 23 male patients. Retinopathic groups were designated as non-proliferative diabetic retinopathy (NPDR = 20) and proliferative diabetic retinopathic (PDR = 25). Vitreous samples were obtained after vitrectomy indicated for retinopathy. The enzyme immunoassay test followed a typical two-step capture or "sandwich" type assay by using AssayMax Human Leptin EliSA Kit (Assaypro, Belgium). Data were analyzed using SPSS version 14.0.

Results: The vitreous leptin concentrations of patients did not differ significantly between NPDR and PDR patients (53.42 ± 18.20 ng/ml and 53.18 ± 19.60 ng/ml; p=0.915); moreover there were no significant differences by gender.

Conclusion: Vitreous leptin levels in diabetic patients appear not to contribute to proliferative retinopathy.

Key words: Leptin; Diabetes Mellitus; Diabetic Retinopathy; Proliferative Vitreoretinopathy; Vitrectomy.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by insufficient insulin secretion, often combined with impaired insulin sensitivity.[1] Both types I and II diabetes mellitus are characterized by the lack of insulin-stimulated glucose transport from blood to tissue, a consequence of β-cell failure in the pancreas.[2] Simply, type I diabetes mellitus is a result of β-cell apoptosis activated by cytokines produced by invading immune cells. Type II diabetes is characterized by insulin resistance, a condition in which the body is able to produce insulin but the adipose, liver, and muscles are unable to absorb glucose. This results in an elevated level of blood glucose, signaling β-cells to increase insulin production. Over time, the β-cells cannot maintain the amount of insulin necessary for glucose uptake.[3-5]

Leptin is a 16 KDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including the regulation of appetite and metabolism.[5,6] Leptin also affects cytokine production during the activation of monocytes /macrophages, wound healing, angiogenesis and hematopoiesis.[7] Leptin directly regulates the production of several cytokines in vitro. Leptin displays proliferative and anti-apoptotic activity in a variety of cell types. It stimulates proliferation of tracheal epithelial cells, squamous cells of lungs and has a role in glomerulosclerosis. Leptin causes proliferation of endothelial cells and causes angiogenesis. Both in vitro and in vivo assays show that leptin has angiogenic activities including neo-vascularization and formation of capillary like structures.[6]

Research on obese humans has highlighted the role of Leptin in energy balance, neuroendocrine functions and immunity.[8] Mutations in the Ob/Ob genes and/or receptors are thought to underlie many clinical conditions associated with Leptin, such as appetite disorders, obesity,

metabolic syndrome and disorders of immunity. Similar alterations have been described in leptin-deficient humans. In diabetes there is resistance to leptin and not deficiency of leptin.[9,10]

MATERIALS & METHODS

The comparative study was carried out over a period of from June to November 2010 on inpatients of Al-Shifa Eye Trust Hospital, Rawalpindi, Pakistan.

Written informed consents were obtained from all patients who presented with complaints of diabetes. Patients' examinations were carried out in the presence of qualified diabetologist and ophthalmologist. Retinopathic groups were designated as non-proliferative diabetic retinopathic (NPDR) and proliferative diabetic retinopathic (PDR).

Performa was designed to record anthropomorphic and clinical case histories such as age, weight, height etc. Exclusion criteria were systemic diseases, kidney or heart disease, liver malfunctioning, respiratory or gastrointestinal disorders. Inclusion criteria were marked diabetes, adult onset of the disease and visual symptoms. Only those patients who had hypertension related with diabetes were included, and were excluded otherwise. Detailed history was followed by standard physical examination including the measurement of blood pressure, testing of visual acuity and fundus examination.

Samples of vitreous humor were obtained for measuring vitreous Leptin levels after vitrectomy was performed in these patients for the vitreoretinal disorders.

Leptin measurement

Leptin concentration in vitreous samples was estimated through a standard solid phase sandwich enzyme linked-immunosorbant assay (ELISA) using a commercial kit obtained from Immunotech SAS (France). The manufacturer provides the data for Sensitivity as the limit of detection (loD) for leptin is 0.05 ng/ml, as determined by use of a Clinical & Laboratory Standards Institute (CLSI, formerly NCCLS) protocol and with proportions false positives (α) less than 5% and false negative (β) less than 5% based on 82 blank determinations LoB= 0.42 ng/ml. The data for Specificity is given as the following substances tested at 1000 mg/ml and exhibited no cross-reactivity: Mouse leptin, TNF-α, IL-2, II-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-16, GM-CSF, CSF and EGF.

Calculations

The mean optical density of each calibrator was calculated at 450 nm. A calibrator curve was drawn on a semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on X-axis. The mean optical density of each unknown was calculated. The values of the unknowns were read directly off the calibrator curve. If a sample read more than100 ng/ml then it was diluted with assay buffer at a dilution of no more than 1:8. The results obtained were multiplied by the dilution factor. Reference value: Vitreous: < 2.0 pg/ml.

Data are presented as mean ± SD (standard deviation). Analyses were done using the SPSS version 14.0 (Chicago, Illinois, USA).

RESULTS

A total of 45 patients of diabetic retinopathy (males=23, females=22) were included for the study based on adequacy of their Vitrectomy samples. Of these 45, 20(44.44%) belonged to the Non Proliferative Retinopathy (NPDR) group and 25(55.56%) were in the Proliferative Diabetic Retinopathy (PDR) group. Vitreous leptin concentration in male diabetic patients of PDR did not differ significantly (P = 0.125) from NPDR patients (Table.1). Median values for male NPDR and PDR patients were 36.95 and 65.0 ng/ml.

Vitreous leptin concentration in female diabetic patients was non-significantly different (P =

0.086) between NPDR and PDR patients (Table 1). Median value of 59 ng/ml was greater in female NPDR patients as compared to PDR patients.

While for overall diabetic patients, Vitreous leptin concentrations did not differ significantly (P = 0.915) between NPDR and PDR patients (Table 1).

Table 1: **Levels of Vitreous Leptin in Retinopathy groups by gender (n=45).**

S #	Groups	Vitreous Leptin levels (Mean ± SD; ng/ml)		Overall ng/ml	Range ng/ml	p value
		Males	Females			
1.	NPDR (n=20)	45.60±16.51	61.25±19.89	53.42±18.20	27.20-88.30	0.915
2.	PDR (n=25)	59.02±23.42	47.35±15.78	53.18±19.60	18.30-88.90	

DISCUSSION

Gariano et al. (2000)[7] found higher levels of leptin in the serum and vitreous samples of patients with diabetes than those without, while vitreous leptin was specifically elevated in patients with PDR or retinal detachment. Leptin is an angiogenic cytokine. In advanced diabetic retinopathy, retinal angiogenesis and growth of fibrotic tissue may result in vitreous hemorrhage and traction retinal detachment, the principal causes of severe vision loss in diabetics.

In the same study by Gariano et al. (2000),[7] BMI was shown to be significantly higher in diabetics than non-diabetics. Leptin correlated positively with BMI, its levels were higher in females than males, but there was no relationship with age. Leptin levels were shown to higher in PDR, intermediate in NPDR and lower in DNR patient.

Similarly, Uckaya et al. (2000)[11] also showed higher leptin levels in advanced diabetic retinopathy. Leptin is known to induce promotion of angiogenesis and neovascularization.

The present study showed a linear but non-significant increase in the levels of leptin in NPDR and PDR patients. These data also correlated with the ophthalmoscopic observations and fundus examination. PDR patients frequently showed retinal hemorrhage, ischemia, excessive blood vessel growth and a tendency toward blindness. However, in none of the patients visual acuity of both eyes was more than 6/36. Olmos et al. (2009)[12] showed no difference between serum and vitreous leptin concentrations in PDR patients. Further they did not observe any relationship between intravitreous leptin levels and PDR activity. In contrast presently, vitreous leptin concentrations were found significantly elevated in vitreous fluid of both NPDR and PDR patients.

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Submitted for Publication: May 15, 2015.
The authors have no conflict of interest. All authors contributed substantially to the planning of research, questionnaire design, data collection, data analysis and write-up of the article.

This article may be cited as:
Parveen N, Bukhari AAS, Khan Q, Khan K. Leptin levels in vitreous fluids of patients with diabetic retinopathy. JRMI. 2015;1(1):17-20.