Original Article

ESTABLISHING CARNITINE DEFICIENCY IN PATIENTS OF TYPE-II DIABETES MELLITUS WITH EARLY DIABETIC RETINOPATHY

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ABSTRACT

Objective: To compare carnitine levels in patients of Type-II Diabetes Mellitus having early diabetic retinopathy with age matched non-diabetic healthy controls.

Material and Methods: This observational cross-sectional study was conducted at the department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi from October 2019 to March 2020.Known cases of Type-II Diabetes Mellitus of both genders and all ages were included in the study having mild non-proliferative diabetic retinopathy, while healthy non-diabetics were enrolled as controls. Pre-chilled lithium heparin tubes were used for collection of blood samples and were analysed by competitive ELISA technique. Comparison between groups was done by independent sample t test and correlation between high BMI and low carnitine was assessed by Pearson correlation.

Results: Total of 240samples were analysed which included 120 patients of Type-II Diabetes Mellitus having mild non-proliferative diabetic retinopathy and 120 non-diabetic age matched subjects with a male to female ratio of 1:1. Mean level of carnitine was found 35.0±6.54mmol/L in non-diabetics while 28.0±2.0 mmol/Lin patients of diabetes mellitus. Among diabetic population, carnitine was significantly lower as compared to non-diabetics ($p<0.02$). No significant difference was found between males and females ($p>0.05$). BMI had a significant negative correlation with carnitine levels in patients of DR and disease-free groups both (r=-0.76 and -0.69).

Conclusion: This study shows that carnitine levels were low in patients having mild non-proliferative diabetic retinopathy as compared to non-diabetic healthy controls.

Key Words: Carnitine, Diabetes mellitus, Diabetic retinopathy, Insulin resistance.

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INTRODUCTION

Insulin resistance or decreased insulin sensitivity is the hallmark of constellation of symptoms collectively known as type-II diabetes mellitus (DM). Poor glycaemic control results in micro vascular and macro vascular complications. Diabetic retinopathy is one of the early complications of uncontrolled (DM) and is a leading cause of blindness. Insulin is required for the entry of glucose into the cells of human body, where it is used as an energy source. Insulin resistance leads to utilisation of other dietary constituents for energy purposes, fatty acids being the major contributors. In any state of oversupply of dietary fats exceeding the storage capacity of adipose tissues, there is an accumulation of intermediate compounds of fatty acid metabolism. This defective beta-oxidation of fatty acids leads to a state of oxidative stress in skeletal muscle cells, liver, kidneys, heart etc. Progressive insulin resistance is observed in these tissues. One of the theories suggests that over supply of lipids leads to build-up of fatty acyl CoA and diacylglycerol (DAG) in the mitochondria which are detrimental to insulin signaling pathways of the cells. Both long-chain fatty acyl CoAs and DAG activate protein kinase C, which increases serine phosphorylation. This suppresses the tyrosine phosphorylation of the insulin receptor. substrate-1(IRS-1) [1,2]. Another theory is that long-chain Acyl-CoAs are also ceramide precursors which activate a protein phosphatase that dephosphorylates the serine/threonine kinase, also known as protein kinase B (PKB). This results in inhibition of glucose transporter-4 (GLUT4) translocation and glycogen synthesis [3,4]. Ceramide production inhibition has been shown to improve insulin resistance.

Carnitine is one of the essential nutrients involved in fatty acid oxidation. It is required for the efflux of fatty acid metabolites from mitochondria, such as acyl CoA and acetyl groups, reducing the accumulation of intermediate metabolites of fatty acid oxidation. This reduces the metabolic stress and improves insulin resistance by enhancing glucose utilisation in cells via GLUT2 and GLUT4receptors [5]. Carnitine is believed to reduce lipid metabolites within skeletal muscles by their increased oxidation and export. This reduction in lipid toxicity leads to an

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upturn in insulin signaling and increased mitochondrial capacity [6]. In type-II DM, more carnitine is utilized in clearing the intermediates of fatty acid oxidation leading to insulin resistance [7]. So, this is postulated that carnitine levels maybe decreased in type -II diabetes mellitus as compared to healthy non-diabetic persons. Logically, any treatment that can reduce oxidative stress in mitochondria can improve state of insulin resistance.

Current study was aimed to find association of low carnitine as a contributory factor towards insulin resistance leading to micro vascular complication as diabetic retinopathy. The carnitine levels in patients of type-II DM were compared with healthy adults of same age group. This study also aimed to assess the carnitine deficiency among patients of type-II DM, as carnitine is being considered as a novel treatment for insulin resistance in type-II diabetes mellitus.

MATERIAL AND METHODS

This study was sanctioned by institutional review board (IRB vide letter no. Cons-CHP-3/READ-IRB/21/446), the ethical committee of Armed Forces Institute of Pathology, Rawalpindi. All the participants were informed about study and written consent was taken from each participant. In total, two hundred and forty (240) individuals were selected in the study by consecutive sampling according to the inclusion criteria. One hundred and twenty (120) were known cases of diabetes mellitus having mild non-proliferative diabetic retinopathy (NPDR) on fundoscopic examination done by ophthalmologist. The patients having moderate to severe NPDR and proliferative diabetic retinopathy were excluded from the study. The other one hundred twenty (120) were disease-free non-diabetic healthy controls for the purpose of comparison. After age and gender adjustment between cases and controls, as shown in Table-I, male and female were enrolled in ratio 1:1. Two blood samples were taken, 2ml each, through venipuncture. One of the blood sample was collected in pre-chilled lithium heparin tube (green top) and transported on ice to the lab. Plasma was separated within 20 minutes of collection by centrifugation at 1500rpm for 10 minutes in refrigerated centrifuge. The samples were preserved as aliquots at -20^{0}C till analysis. Previous exploratory research has shown that under the collection and storage conditions employed, the stability of L-carnitine and its esters was four weeks [8]. The second sample was transferred to the tube containing potassium EDTA as preservative, for analysis of glycosylated haemoglobin (HbA1C).

Standard height and weight scales were used to measure height and weight, while body mass index (BMI) was calculated by standard BMI calculator. Patients of uncontrolled DM were taken as having HbA1C> 7% indicating poor glycaemic control. For staging of diabetic retinopathy participants underwent complete ocular examination including fundoscopy. Mild NPDR was defined as micro aneurysms and small exudates in less than 4 quadrants of the retina.

Human Bioassay Technology Laboratory ® ELISA kit for total carnitine, having sensitivity up to 0.05mmol/L, was used for analysis of total carnitine using Diagnolab plate reader. Commercially provided control materials, provided with the kit, were used for quality control procedure. For the patients of DM, glycosylated haemoglobin (HbA1c) was assayed by capillary electrophoresis on Sebia ®Octa3 system.

To find association of low carnitine with diabetic retinopathy, the Chi square test was used. Statistical comparisons were performed using 'independent t test' if significance was detected. Significance was set at $p<0.05$. Software SPSS version 23 was used for statistical analysis of data. Univariate regression analysis was used for the prediction of BMI as possible factor for low levels of carnitine in participants. Pearson correlation technique was employed to find association between BMI and carnitine levels in patients of diabetic retinopathy and disease-free participants.

RESULTS

Two hundred and forty (240) subjects participated in the study,120 patients of type-II diabetes mellitus having mild non-proliferative diabetic retinopathy and 120 disease free non-diabetic subjects, with a male to female ratio of 1:1 for both groups. The average age of diabetic group was 55.5±9.95years for males and 56.9±10.4 years for female with their mean BMI calculated as 24.3 ± 0.77 kg/m^2 and 24.5 ± 1.07 kg/m^2 respectively. For non-diabetic population, the mean ages were 51.4 ± 14.44 years and 50.7 ± 16.26 years having mean BMI calculated as 23.7 ± 0.89 kg/m^2 and 23.8 ± 1.06 kg/m^2 for males and females respectively. Mean HbA1c of the diabetic participants was 8.0 ± 0.45%.

Carnitine levels were found significantly low in patients of type-II diabetes mellitus having mild non-proliferative diabetic retinopathy as compared to non-diabetic population (Table-I). There was no significant difference observed among male and female population (Table-II).

Table-I: Carnitine levels in the study population.

Diabetic retinopathy (n=120)	Non-diabetic (n=120)	p value
Carnitine (mmol/l) Mean ± SD	Carnitine (mmol/l) Mean ± SD	
28.0±2.0	35.0±6.54	<0.001

Table-II: Comparison of plasma carnitine levels among male and female population.

	Male (n=120)	Female (n=120)	p value
	Carnitine (mmol/l) Mean ± SD	Carnitine (mmol/l) Mean ± SD	
Non-diabetic	35.2±6.33	34.9±6.80	0.80
Diabetic retinopathy	27.8±1.67	28.2±2.29	0.27

Chi square test was used to find association between low carnitine levels and diabetic retinopathy. p<0.001 indicated a strong association of low carnitine levels with diabetic retinopathy.

Univariate regression analysis was used for the prediction of increasing BMI as possible factor for low levels of carnitine in participants as shown in Figure-I, indicating that there was a significant negative correlation between low carnitine levels in patients having diabetic retinopathy in relation to their increased BMI levels (r = -0.76) as well as non-diabetic group (r = -0.69). This indicates that with increasing BMI, the carnitine levels decrease contributing to possible insulin resistance as an independent factor related to obesity.

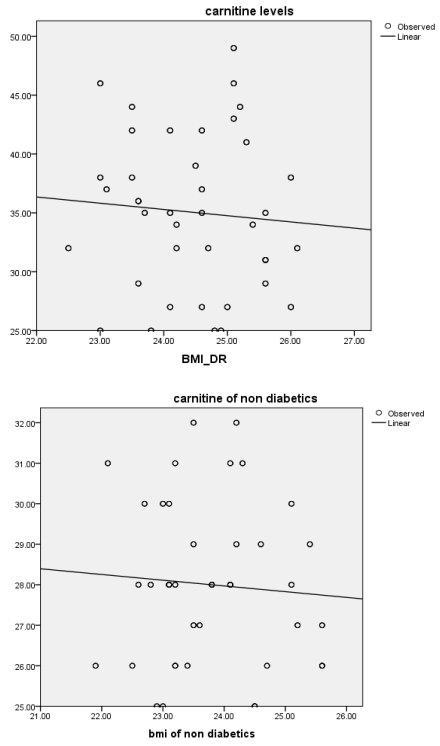

Figure-I. Correlation between carnitine levels and BMI in diabetic retinopathy (left; r = -0.76) and disease free non-diabetic population (right; r = - 0.69).

DISCUSSION

Diabetes is a state associated with disturbed lipid and carbohydrate metabolism (glucotoxicity and lipotoxicity) leading to oxidative stress. Carnitine increases oxidation of long Chain-Acyl Co A in mitochondria, the which is believed to be the cause of insulin resistance in heart and muscle, thus, it has significant effects on glucose metabolism. Carnitine also induces changes in glycolytic and gluconeogenic enzyme activity. Finally, carnitine acts by modifying the expression of genes related to the insulin signaling cascade, thereby improving glucose utilization in cells [9]. As carnitine is involved in beta oxidation of fatty acids, leading to decreased oxidative stress in cells, it can be a promising therapeutic target for improving insulin resistance in diabetics.

Our study has reported carnitine levels 28.0±2.0 mmol/L in patients of diabetic retinopathy and 35.0±6.54 mmol/L in disease free population. These levels are slightly lower as compared to reported in some other reported studies [10].These differences may be attributable to race and ethnicity in different populations. Examination of the effect of gender on plasma carnitine concentrations indicated that no significant difference existed in concentrations of total carnitine between adult males and females in patients of DR and disease-free population (p=0.27 and p=0.80 respectively). In contrast to our study, [11] Lombard *et al.* had previously demonstrated that the males had substantially greater plasma carnitine and total carnitine concentrations than females, with L-carnitine concentrations of 39.94.9 mmol/L and 32.55.3 mmol/L, respectively, and total carnitine concentrations of 49.47.3 mmol/L and 43.35.5 mmol/L. Such differences among male and female population of same age and ethnicity are attributable to difference in race and muscle mass among males and females.

Study results indicated that carnitine levels were significantly low (p<0.05) in the patients of type-II diabetes mellitus having mild non-proliferative diabetic retinopathy as compared to non-diabetic population of same age group with carnitine levels 28.0 ± 2.0 mmol/L and 35.0 ± 6.54 mmol/L respectively. This suggests that carnitine is somewhere involved in causing insulin resistance. These results are consistent with other studies [12]. A number of clinical trials comprising of carnitine supplementation and monitoring of diabetic control have indicated improved diabetic control as a result of carnitine supplementation. One of the explanations of this improved diabetic control in terms of insulin resistance is increased uptake of fatty acyl CoA

(increased accumulation is seen in diabetics), by cell mitochondria and subsequent utilization by cells for energy production [13]. Xu *et al* indicated in his meta-analysis in 2017 that carnitine supplementation had useful effects on HOMA-IR score (indicator of insulin resistance) [14]. Carnitine supplementation at a dosage of 2000mg/day for 28 days in patients with compromised glucose breakdown resulted in a significant decrease of insulin levels and HOMA-IR score. In a clinical trial by Bae *et al*, it was observed that supplementation with carnitine resulted with a significant decrease in fasting glucose, HbA1c, and HOMA-IR score [15], supporting the results of current study that low carnitine could be a cause of insulin resistance.

Early researches suggest that intravenous administration with L-carnitine may increase insulin sensitivity in patients of diabetes mellitus by lowering muscle lipid load and may results in lowering of blood glucose levels in the blood by more quickly increasing its oxidation in cells [16]. A contemporary analysis of a multi-centric clinical trials of patients with either type 1 or type II diabetes established that treatment with Acetyl-L-carnitine (3000milligrams/day orally) for 12 months resulted in significant nerve pain relief and improvement in vibration perception in patients with diabetic neuropathy. The treatment was proved to be effective in patients of newly diagnosed type II diabetes [17].

Ringesis *et al* [18] concluded in his study that a lack of L-carnitine impacts the development of insulin resistance during metabolic load. Administration of L-carnitine is thought to stymie this course. Other tools for L-Carnitine's effect on glucose homeostasis comprise: 1) regulating the intra-mitochondrial acetyl-CoA/CoA ratio and the role of the pyruvate dehydrogenase complex, 2) modifying the manifestation of glycolytic and gluconeogenic enzymes, 3) modifying gene expression in the insulin signaling flow, and 4) exciting the IGF-1 axis [19].

A decreased level of carnitine in diabetics has a significant impact on regulating blood glucose levels by altering glucose utilization in cells. However, no significant impact has been reported on levels of HbA1c; possibly because the short-term changes in glucose metabolism doesn't significantly affect glycosylation of RBCs [20]. Carnitine being a key player in lipid metabolism, also indirectly improves glycaemic control by affecting lipid metabolism [21]. Mierea *et al* concluded in their study that carnitine metabolism end products and intermediates of synthetic pathway should be considered in the metabolic classification of diabetes [22]. This consideration signifies the role of low levels of carnitine in diabetics as shown in our study. Romaine *et al* [23] observed in their study that normalization of impaired lipid metabolism can result in improved glycaemic control by altering insulin resistance in end organs especially in skeletal muscles. Maryam Asadi *et al* [24] concluded in their study that carnitine levels have a cardio protective effect and carnitine levels are reduced in cardiac disease such as in case of diabetes. In current study, carnitine levels were analysed in both diabetics and non-diabetics and were correlated with glycaemic control. Carnitine levels were found low in diabetics that justifies their impaired lipid metabolism, contributing to their poor glycemic control. Carnitine levels should be monitored in patients of type-II diabetes mellitus for insulin resistance and carnitine supplementation should be promptly started for improving insulin sensitivity and glycemic status of diabetics. Our study established that carnitine levels were low in patients having mild non-proliferative diabetic retinopathy as compared to non-diabetic healthy controls. Hence paving the way for future studies that carnitine can be employed as a therapeutic target to improve glycaemic control and prevent early microvascular complications in diabetics.

CONCLUSION

This study established that carnitine levels were low in patients having mild non-proliferative diabetic retinopathy as compared to non-diabetic healthy controls.

AUTHOR CONTRIBUTION

Danya Shaukat: Write up, main research concept.
Zujaja Hina Haroon: Overall supervision.
Usama Bin Khalid: Revisions.
Haroon Javaid: Statistical analysis.
Hassaan Javaid and Farooq Ul Abidin: Data collection and sampling.

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