ASSOCIATION OF SERUM SPARC WITH INSULIN RESISTANCE IN TYPE-2 DIABETES MELLITUS

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ABSTRACT

Objective: To determine the association of serum SPARC with insulin resistance in type-2 diabetes.
Study Design: Descriptive study.
Place and Duration of Study: Physiology department and CREAM lab, Army medical college, Rawalpindi, in collaboration with Military Hospital Rawalpindi, from Feb 2016 to Oct 2016.
Material and Methods: Sixty individuals were recruited in this descriptive study. Thirty diagnosed cases of type-2 DM were included, while thirty age and gender matched healthy individuals were included as controls through non-probability purposive sampling. Controls were labelled as group A, while cases were labelled as group B. Patients with type-1 DM, type-2 DM on insulin therapy, hyperglycemic states other than DM and inflammatory disorders were excluded from the study. Data were collected after informed and written consent. Blood samples were withdrawn under strict aseptic measures and serum was stored at -20^{o}C. Serum insulin levels and serum SPARC levels were analyzed by enzyme linked immunosorbent assay (ELISA). Insulin resistance was determined using homeostasis model assessment of insulin resistance (HOMA-IR), and its value >1.5 was considered significant.
Results: Fasting insulin levels were significantly higher in group B as compared with group A, supporting the diagnosis of type-2 DM. HOMA-IR values were greater than 1.5 in group B, thus establishing significant insulin resistance. Serum SPARC levels were significantly higher in group B than group A (17.7 ± 1.14 vs 8.7 ± 1.08 ng/ml) with *p*-value<0.001. Serum SPARC levels showed positive correlation with fasting insulin levels and HOMA-IR values.
Conclusion: Our study showed a positive correlation between serum SPARC levels and insulin resistance, which indicates that SPARC plays an important role in the development of insulin resistance in type-2 diabetes mellitus.

Keywords: Insulin resistance, SPARC, Type-2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders which results due to insulin deficiency, its defective mechanism of action, or insulin resistance[1]. It is considered one of the largest non-communicable epidemics because of its high prevalence and increasing disease burden. Pakistan is the 7th largest country in terms of diabetic population, and it will be 4th by the year 2030[2]. This highlights the importance of studying the molecular mechanisms, and to develop targeted therapies for better control of diabetes and its associated complications[3].

Type-2 diabetes mellitus (DM) accounts for nearly 90% of total diagnosed cases of DM[4]. Type 2 DM is characterized by insulin resistance in insulin target tissues, along with increased insulin demand. A wide range of cellular disturbances, inflammatory processes and environmental factors lead to the development of insulin resistance, mainly by causing defects in the insulin signalling pathways. Among other causes, obesity-linked insulin resistance is most prevalent which directly alters the cell response to insulin[1,3,4]. Adipose tissue fibrosis and abnormal lipid accumulation in skeletal muscle and liver lead to insulin resistance in these

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tissues, leading to development of impaired glucose tolerance and type 2 diabetes mellitus[5].

Secreted Protein Acidic Rich in Cysteine (SPARC) is a matricellular glycoprotein and an adipokine. It is expressed by the adipose tissue, liver, skeletal muscle, bone and extracellular matrix (ECM). It is also known as Osteonectin or basement membrane – 40 (BM40). It has diverse roles in ECM assembly, tissue remodeling, and tissue renewal, regulation of matrix metalloproteinases, synaptogenesis, angiogenesis and adipogenesis. It is also proposed to have role in cancer cell survival, cataractogenesis and wound healing[6-8].

SPARC is proposed to have a role in the development of insulin resistance by regulating adipogenesis. It inhibits adipogenesis by multiple mechanisms: a) by causing increased deposition of fibronectin and enhanced accumulation of beta-catenin, thus suppressing transcriptional cascade of collagen formation in basement membrane of adipose tissue[9]. b) It prevents hypertrophy of adipocytes, and promotes adipose tissue fibrosis[10]. c) It inhibits adipogenesis by regulating angiogenesis of adipose tissue[8]. These profibrotic effects of SPARC may lead to metabolic dysregulation. This may lead to decrease in insulin signal transduction and promote insulin resistance. SPARC is also proposed to have positive correlation with inflammatory markers such as C - reactive protein and macrophage migration inhibitory factor. The inflammatory process leads to insulin resistance by causing oxidative stress and development of type 2 diabetes mellitus[3,11,12]. Thus SPARC may play a key role in the development of obesity-linked insulin resistance[5] and is related to multiple diabetic complications, including nephropathy, retinopathy and cardiovascular disorders[12].

The present study was planned to evaluate the levels of serum SPARC in patients of type 2 diabetes mellitus, in order to identify SPARC as another predictive marker for development of insulin resistance.

PATIENTS AND METHODS

This descriptive study was conducted in Military hospital, Rawalpindi in collaboration with department of Physiology, Army Medical College, Rawalpindi. The study was carried out after formal approval from ethics review committee of Army Medical College and Military Hospital Rawalpindi. Informed written consent was taken from all the patients and healthy controls. Thirty diagnosed cases of type-2 DM were recruited through non-probability purposive sampling. Thirty controls were recruited, who were healthy individuals without type-2 DM. Controls were labelled as group A, while cases were labelled as group B. History and general physical examination was carried out for all the subjects. Height and weight of all the subjects was measured and BMI was determined.

BMI = Weight (kg)/Height (m^2)

The subjects having BMI >25 kg/m^2 were considered as overweight/obese, and were excluded from the study.

Blood samples were collected under strict aseptic measures as per protocol. Five ml of blood was taken by venipuncture. Sample was transferred to the gel separator tube and was centrifuged at the speed of 2000-3000 rpm for 20 min. Serum was pipetted out and transferred to the polypropylene tubes for storage at -20°C for analysis at CREAM lab, Army Medical College, Rawalpindi. Plasma insulin was measured with a commercial assay kit (Chemux Bioscience, Inc, USA). Insulin resistance was calculated from fasting insulin and glucose levels with following equations:

HOMA-IR = insulin (uU/ml) x glucose (mmol/l)/22.5[13].

Homa-IR value >1.5 was considered significant. Serum SPARC was evaluated using a commercially available ELISA kit (Glory Science Co. Ltd, USA). The linear range of the assay was 10ng/ml -500ng/ml for SPARC (all data and specifications of the SPARC ELISA were derived from the manufacturer).

The statistical data were analyzed by using computer software IBM- statistical package for social sciences (SPSS) version 21. Quantitative variables were expressed as mean and standard deviation (SD). Qualitative variables were expressed as frequency and percentage. The statistical significance of differences across the groups was determined by applying independent t-test to find the difference in various pairs of groups. Correlation between different groups was assessed by use of Pearson correlation coefficients. A *p*-value <0.05 was considered significant.

RESULTS

A total of sixty subjects were recruited (N=60) out of which, thirty were diagnosed cases of type -2 DM with mean age of 58.2 ± 13.3 and thirty age and gender matched controls with mean age of 46.9 ± 9. There were 22 males (73.3%) and 8 females (26.7%) in both the groups. The mean values of BMI in group A was 23.6 ± 2.82, while in group B these were 22.7 ± 2.24. The mean fasting glucose levels were 4.65 ± 0.56mmol/l in group A, while in group B, they were found to be 12.2 ± 5.65mmol/l. The values in group B were significantly higher, with *p*-value 0.001, as shown in table-I.

HbA1c was significantly higher in group B (7.4 ± 1.29) compared with group A (5.13 ± 0.78). The *p*-value was 0.001, exhibiting suggestive difference between both groups.

Similarly, fasting levels of insulin were significantly higher in group B, the values being 13.66 ± 1.8 µlU/ml, while in group A, the values were 4.6 ± 2.8 µlU/ml. The *p*-value was 0.001 showing significant difference between the two groups (table-I).

HOMA-IR was calculated to be 7.41 ± 3.74 in group B whereas its value is 0.94 ± 0.5 in group A. It was significantly higher in group B, the *p*-value being 0.001. As the Homa-IR >1.5 is considered significant, the values of group B were considered consistent with significant insulin resistance (table-I).

Serum SPARC levels, as measured by ELISA, were found to be much higher in group B, the values being 12.27 ± 1.64 as compared to 8.7 ± 1.08 ng/ml in group A. The *p*-value was significant at 0.001 (table-I).

All the metabolic parameters for both the groups are summarized in table-I.

Serum SPARC levels were directly correlated with fasting glucose levels and HbA1c (table-II). There was positive correlation between serum SPARC and serum insulin levels at *p*-value 0.001. Serum SPARC also showed direct correlation with insulin resistance (HOMA-IR) which is

Table-I: Comparison of metabolic characteristics of subjects.

	Group A (n=30)	Group B (n=30)	*p*-value
Fasting glucose (mmol/l)	4.65 ± 0.56	12.2 ± 5.65	0.001*
HbA1c	5.137 ± 0.78	7.4 ± 1.29	0.001*
Fasting insulin (ulU/ml)	4.6 ± 2.8	13.6 ± 1.84	0.001*
Homa-IR	0.94 ± 0.5	7.41 ± 3.74	0.001*
Serum SPARC (ng/ml)	8.7 ± 1.08	12.27 ± 1.64	0.001*

*Significant *p*-value<0.05.

Table-II: Correlation between serum SPARC and parameters.

Pearson's correlation	Parameter	r-value	*p*-value
	Fasting glucose (mmol/l)	0.42	0.001*
	HbA1c	0.408	0.001*
	Serum insulin	0.82	0.001*
	HOMA-IR	0.823	0.001*

*Significant *p*-value<0.05.

statistically significant at r-value 0.823 and *p*-value 0.001 (table-II).

DISCUSSION

Type 2 DM is associated with tissue non-responsiveness to insulin. Thus a higher value of insulin in blood along with high Homa IR value is predictable in patients with type 2 DM. This was also observed in our study, as Homa values were much above the cut-off value of 1.5 in group-B.

The results of our study demonstrated a positive correlation between SPARC and insulin resistance. We observed that serum SPARC levels were significantly higher in patients with type-2 DM as compared with healthy controls. As mentioned in literature, serum SPARC levels inhibit the expansion of subcutaneous adipose tissue. The inhibition of adipogenesis results in adipose tissue fibrosis, which leads to ectopic adiposity and acts as a causative factor for the development of insulin resistance in the various target organs. Some studies postulate SPARC-induced modulation of collagen in the ECM of adipocytes, causing excess deposition of fibronectin in the basement membrane and modulating transcriptional cascade for collagen development and deposition[7,9,10,14]. The profibrotic aspect of SPARC makes it a proposed factor to play key role in the pathogenesis of type-2 diabetes mellitus.

The results of our study are comparable to a study conducted in 2011, in Chongqing, China. Wu et al compared plasma SPARC levels in subjects categorized as different glycemic states. They observed significantly higher levels of SPARC in patients of type-2 DM than controls and subjects with impaired glucose regulation under overnight fasting conditions. These results showed that SPARC levels are positively correlated with type-2 DM as well as subjects with altered glycemic states. Therefore they concluded that SPARC might play an important role in the development of type-2 DM, possibly by altering adipose tissue metabolism.

Similar results were reproduced in a study conducted in 2013, in Beijing, China. Xu et al compared serum SPARC levels in pregnant women with or without gestational diabetes (GDM). They recruited 60 women with normal glucose tolerance as controls and 120 women with GDM. Serum SPARC levels were higher in women presenting with GDM in late pregnancy. It represented as a nonaligned determinant of insulin resistance, thus indicating a potential role of SPARC in the pathophysiology of GDM. The levels of SPARC were, although comparable, yet much higher than measured in our study. A possible explanation might be the use of ELISA kit with different assay range (Invitrogen, CA, USA). SPARC was also found to be positively correlated with inflammatory markers such as C-reactive protein, postulating a potential link between GDM- induced inflammation and insulin resistance, by ECM modulation by SPARC.

However, our study, due to time and financial limitations, could not monitor serum SPARC levels in diabetes for longer duration. Also we could not determine the exact mechanism by which SPARC leads to insulin resistance. Further studies will be needed in this regard.

CONCLUSION

In conclusion, our study showed a positive correlation between serum SPARC levels and insulin resistance, which suggests that SPARC does have a role in the development of insulin resistance in type-2 diabetes mellitus.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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