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ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2021, 10(8): 142-147

Association of Secreted Frizzled Related Protein-4 (SFRP-4) and Adiponectin (APN) in the Early Stage Progression of Insulin Resistance in Pre-diabetic Obese Subjects

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ABSTRACT

Background: *Pre-diabetes is a condition characterized by increased plasma glucose levels that fall somewhere between normoglycemia and hyperglycemia. It can be caused by the derangement in the fasting glucose, glucose tolerance, or both. SFRP-4 (Secreted Frizzled-Related Protein-4) is extensively expressed in adipose cells and beta cells of the pancreas, and its levels are elevated many years before diabetes is diagnosed. Adiponectin (a peptide hormone) is secreted by the adipose tissue that was reported to have insulin-sensitizing properties.* ***Aim:*** *To compare the levels of serum SFRP-4 and serum adiponectin in pre-diabetic obese subjects to the levels in control subjects. We also studied the correlation of serum SFRP-4 and serum adiponectin with anthropometric parameters, IGT, fasting blood glucose, and lipid profile.* ***Materials and Methods:*** *There were 200 pre-diabetic and 100 non-diabetic participants in the present study. Serum SFRP-4, serum adiponectin, fasting blood glucose, IGT and lipid profile were all measured in blood samples. Subjects were categorized into two groups based on their BMI into control and case. The Control group was healthy individuals with a mean of BMI, 24.87 kg/m^2, and the case group was having pre-diabetes and obesity with a mean of BMI, 30.80 kg/m^2. During the screening period, anthropometric parameters were measured using standard techniques.* ***Results:*** *Mean serum SFRP-4 and serum adiponectin were statistically significant and were different in control and case groups ($p<0.001$).* ***Conclusions:*** *Present study shows a strong relationship between serum SFRP-4 and serum adiponectin in cases and controls.*

Keywords: Pre-diabetes, Obesity, Wnt signaling, Diabetes mellitus

Abbreviations: SFRP-4: Secreted Frizzled Related Protein-4, IGT: Impaired Glucose Tolerance, IFG: Impaired Fasting Glucose, BMI: Body Mass Index, OGTT: Oral Glucose Tolerance Test, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

INTRODUCTION

Obesity is a significant cause of T2DM (Type 2 Diabetes Mellitus), with 90% of those diagnosed being overweight or obese [1]. Pre-diabetes is an intermediate hyperglycemic state that exists between normoglycemia and hyperglycemia, characterized by the reduced Glucose Tolerance (IGT), Impaired Fasting Glucose (IFG), or both [2]. It is either impaired fasting glucose (plasma glucose concentration 100 mg/dl-125 mg/dl after 8 hours-12 hours of fasting) or impaired glucose tolerance (plasma glucose concentration 140 mg/dl-199 mg/dl after 2 hour OGTT) or HbA1C (5.7%-6.4%) according to the American Diabetes Association (ADA) criteria. A plasma glucose concentration of 200 mg/dl after 2 hour OGTT or a random fasting plasma glucose concentration of 200 mg/dl was used to diagnose T2DM [3]. Every year, almost 11% of individuals with pre-diabetes develop T2DM [4]. Secreted Frizzled-Related Protein (SFRP-4) is a recent pro-inflammatory cytokine that influences the secretion of insulin in the human pancreatic beta cells as an extracellular regulator of the Wnt beta-catenin pathway. SFRP-4 has been correlated to low-grade inflammation and β-cell dysfunction [5]. In obesity, adipose cell contributes by increasing SFRP-4 levels [6]. SFRP-4 is perhaps a new pro-inflammatory cytokine that connects adipose tissue inflammation to β-cell dysfunction. Therefore, SFRP-4 may be a marker for β-cell dysfunction along with a remedial target for preserving β-cell activity. A recently discovered

adipocyte secreted protein adiponectin that is the most abundant adipose tissue-derived protein [7]. Adiponectin is reduced in obese individuals, unlike other adipokines that are often elevated [8].

Our research investigated the correlations between the serum SFRP-4 and serum adiponectin in prediabetic obese individuals. Hence, we planned to determine SFRP-4 and adiponectin, which are recent, discovered early predictors of insulin resistance in prediabetic subjects with obesity.

MATERIALS AND METHODS

Study Design

This present study included 300 human subjects, 200 of whom were cases and 100 of whom were controls. The subjects or participants were chosen from the city center, Gwalior. Ethical Committee of G. R. Medical College, Gwalior (Madhya Pradesh), India (Ref. No: 269/Bio/MC/Ethical, date: 03 /03/2017) approved this present study. All the procedures followed the ethical principles of the Helsinki Declaration, and all the participants were given a written consent form and screening questionnaires before participating.

Inclusion Criteria

Subjects with a history of T2DM in their family, a BMI of <25 kg/m^2, females with Gestational Diabetes Mellitus (GDM) and Polycystic Ovary Syndrome (PCOS), physically inactive subjects, and others with insulin resistance disorders, like extreme obesity and coronary artery diseases, etc. were included. Subjects were identified by using a screening questionnaire.

Exclusion Criteria

Subjects using insulin or other drugs that can influence plasma glucose concentration, endocrine disorders, malignant diseases, less physical activity in the previous week, visual and hearing impairments, elevated triglyceride levels (400 mg/dL and above), women having BMI <35 kg/m^2 and documented pregnancies were omitted from the study. Those who refused to give consent for this study were also excluded.

Diagnostic Criteria

The pre-diabetic participants were diagnosed using the American Diabetes Association (ADA) guidelines as:

a) Impaired fasting glucose, IFG (100 mg/dL-125 mg/dL)

b) Impaired glucose tolerance, IGT (140 mg/dL-199 mg/dL) [9]

The subjects in the control group had normal blood glucose concentration (fasting plasma glucose <100 mg/dL), 2-hour plasma glucose concentration, OGTT after giving 75 g of glucose (<140 mg/dL).

Anthropometric Measurements

Standard equipment has been used for the anthropometric measurements. Before any measurements, the subject's consent was always obtained. Name, surname, sex, date of birth, date of measurement, the justification provided by a subject who did not offer permission to be measured, and records of measured weight and height were obtained using a subject's Report Form. Subjects were asked to remove their shoes, bulky clothing, and personal belongings such as wallets, keys, cell phones, hair ornaments or braids, and so on. The clothes that a person wore when being weighed are recorded in the subject Record Form. Bodyweight was calculated for the weight of the clothes worn by the subjects as they were measured during data analysis. The same anthropometric equipment was used by all subjects. Measurements were taken using a digital weighing scale and a mobile stadiometer. Bodyweight has been calculated in kilograms and reported to the closest 100 g (0.1 kg) unit. The weight has been measured in kilograms and noted to the very close to 100 g (0.1 kg) unit. The height of all subjects was taken in centimeters and the reading was taken to the very last millimeter (0.1 cm). The BMI (Body Mass Index) of all study subjects was computed by the formula: weight in kg was divided by square height in meter (m^2). After a normal expiration, the Waist Circumference (WC) was calculated in an upright position using the measuring tape at the point that lies between the lowest rib and the iliac crest. In the standing posture, the Hip Circumference (HC) was measured around the widest part of the gluteal area at the pubic tubercle. The waist circumference (cm) was divided by the hip circumference (cm) to calculate the Waist-to-Hip Ratio (WHtR).

Definition of Obesity

Subjects with a BMI ≥ of 30 Kg/m^2 are defined as obese [10].

Biochemical Measurements

After 8 hours-12 hours of overnight fasting, approximately 6 mL of blood has been collected from all individuals keeping in mind the maximum possible aseptic conditions, and dispensed into two separate tubes depending on the examination to be performed. Approximately 3 mL blood was dispensed into a clear bulb for examination of lipid parameters, serum SFRP-4, serum adiponectin, and fasting insulin, while the remaining 3 mL blood was dispensed into a fluoride bulb for estimation of fasting plasma glucose. Along with that, each subject was given glucose (approximately 75 g) orally, and 2 hr later the plasma glucose was estimated by an OGTT. To obtain serum/plasma, the collected blood was centrifuged at approximately 3000 rpm for about 10 minutes. Parameters, including fasting glucose and lipid profile, were analyzed using kits from ERBA Diagnostics on an automated analyzer (Mindray BS-400 Chemistry Analyzer). LDL-cholesterol and Very Low-Density Lipoprotein (VLDL) cholesterol were calculated by using the Friedewald equation [11]. Serum SFRP-4, adiponectin, and fasting insulin were measured using ELISA (enzyme-linked immunosorbent assay) kits (catalog no. SEF878Hu, USCN Life Science Inc.), (Catalog no. AP348G, CALBIOTECH), and (Catalogue no. IS130D, CALBIOTECH), respectively. HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) is an index of insulin resistance, which was calculated by the equation represented by Matthews DR, et al. [12].

Statistical Analysis

The statistical calculations were performed using SPSS 23. All the findings were represented by the mean and standard deviation. The student's independent sample t-test was used for two groups. The 'r' value of IGT is calculated with anthropometric indices (Age, BMI, WC, HC, and W/H ratio) fasting glucose, lipid profile, SFRP-4, adiponectin, fasting insulin, and HOMA-IR. The p-value <0.001 was considered statistically significant.

RESULTS

In the present study, 200 subjects were taken with Impaired Fasting Glucose (IFG), Impaired Glucose Tolerance (IGT), and a BMI of less than 30 kg/m^2 was taken as cases, while 100 subjects presented as controls. Table 1 represents the anthropometric and biochemical parameters in control and case groups respectively. The pre-diabetic obese group had increased levels of age, BMI, WC, HC, and WHtR than the control group. The lipid profile was significantly higher in cases than in the control. The case group had significantly lower HDL levels than the control group. The FPG (Fasting Plasma Glucose), fasting insulin, SFRP-4, adiponectin, IGT, and HOMA-IR were found to be significantly higher in the case group than the control group ($p<0.001$). The Pearson's correlations of SFRP-4, adiponectin, IGT, FPG, fasting insulin, and HOMA-IR with anthropometric parameters (age, BMI, WC, HC, and WHtR) and lipid profile tests (TC, TG, HDL, LDL, and VLDL) are shown in Table 2. All anthropometric parameters (age, BMI, WC, HC, and WHtR), FPG, fasting insulin, IGT, HOMA-IR, TC, TG, LDL, and VLDL had a positive and significant correlation with SFRP-4 but negatively correlated with HDL. Adiponectin was correlated negatively and significantly with all anthropometric parameters (age, BMI, WC, HC, and WHtR), FPG, fasting insulin, IGT, HOMA-IR, TC, TG, LDL, and VLDL but not with HDL, which was correlated positively. Furthermore, IGT, FPG, fasting insulin, and HOMA-IR were correlated positively with the anthropometric parameters and lipid profile tests but with HDL, there was a negative correlation (Table 3).

Table 1 Anthropometric indices of the control and case groups (pre-diabetic obese subjects)

Parameter	Control (n=100)	Case (Pre-diabetic obese, n=200)
Age (Yrs.)	34.19 ± 13.76	50.41 ± 9.00***
BMI (Kg/m^2)	24.87 ± 3.05	30.80 ± 1.93***
WC (cm)	82.83 ± 12.28	104.98 ± 10.95***
HC (cm)	75.66 ± 14.55	112.04 ± 12.78***
WHtR	0.91 ± 0.06	0.94 ± 0.04***
***Significant at p<0.001; Data is represented as means ± SD		

Table 2 Biochemical parameters of control and case subjects

Variables	Control (n=100)	Case (Pre-diabetic obese, n=200)
FPG (mg/dl)	85.00 ± 5.39	114.35 ± 7.88***
F. Insulin (μIU/mL)	8.80 ± 2.66	10.91 ± 8.14*
SFRP-4 (ng/mL)	103.32 ± 26.91	169.18 ± 21.82***
IGT (mg/dL)	104.27 ± 10.06	152.34 ± 8.81***
HOMA IR	1.80 ± 0.86	3.00 ± 2.28***
TC (mg/dL)	193.49 ± 18.04	203.16 ± 20.77***
TG (mg/dL)	98.33 ± 23.70	136.28 ± 28.46***
HDL (mg/dL)	47.24 ± 4.75	45.27 ± 4.74***
LDL (mg/dL)	119.92 ± 21.20	129.85 ± 21.18***
VLDL (mg/dL)	22.48 ± 6.70	29.06 ± 5.69***
*Significant at p<0.05; ***Significant at p<0.001; Data is represented as means ± SD		

Table 3 Pearson's correlations of SFRP-4, adiponectin, IGT, FPG, fasting insulin, and HOMA-IR with anthropometric and biochemical parameters

	SFRP-4	APN	IGT	FPG	HOMA-IR
Age	0.331**	-520**	0.285**	0.347**	0.449**
BMI	0.571**	-0.536**	0.385**	0.385**	0.346**
WHtR	0.371**	-0.520**	0.355**	0.415**	0.323**
TC	0.809**	-0.478**	0.866**	0.950**	0.408**
TG	0.848**	-0.493**	0.890**	0.977**	0.448**
HDL	-0.566**	0.441**	-0.474**	-0.599**	-0.324**
LDL	0.645**	-0.390**	0.689**	0.790**	0.385**
VLDL	0.848**	-493**	0.890**	0.977**	0.448**
**Significant at p<0.01(two-tailed)					

DISCUSSION

Many previous types of the research reported that central obesity can cause insulin resistance, which is identified by a faulty insulin response in peripheral tissues and results in impaired glucose uptake and utilization [13]. This condition causes high plasma glucose concentration and a compensatory rise in insulin, additionally other complications like hypertension, dyslipidemia, and hepatic steatosis. These findings show that pre-diabetes is more significantly connected to markers of central obesity than general obesity [14]. Pre-diabetes is characterized by blood glucose concentrations that are greater than normal values but not as high as in diabetes. Impaired Fasting Glucose (IFG), Impaired Glucose Tolerance (IGT), and IFG combined with IGT are all included [15]. In this study, the anthropometric indices, SFRP-4, and adiponectin, additionally lipid profile, were determined as the supporting parameters in the both case and control group and they were correlated. According to several previous studies, SFRP4 is a Wnt antagonist that inhibits the Wnt signaling pathway in adipose tissues [16,17]. Adipose tissue is a potential contributor of SFRP-4, which plays a significant role in glucose metabolism and insulin action; therefore, SFRP-4 levels were significantly increased in the case group than the control group. In our research, we observed elevated levels of SFRP-4 cases than the controls [18]. Our results regarding the serum SFRP-4 are very similar to previous researches [19,20]. A strong positive correlation was seen among BMI, W/H ratio, IGT, and HOMA-IR with SFRP-4 in the case group. Adiponectin is an adipocyte-derived peptide hormone that promotes inflammation [21]. Its concentration is reported lower in obese individuals, particularly those with visceral obesity and it was inversely associated with the progression of insulin resistance [22]. In our study, the adiponectin levels were decreased significantly in the case group than in the

control group. A strong negative correlation is seen among BMI, W/H ratio, IGT, HOMA-IR, and adiponectin in cases. Decreased levels of adiponectin were closely associated with Impaired Glucose Tolerance (IGT) [23]. Our findings are very similar to previous findings [24,25]. In the present study, we found a significant relationship between adiponectin and other indices (anthropometric parameters, SFRP-4, lipid profile, fasting insulin, and HOMA-IR). Adiponectin was correlated negatively with the anthropometric parameters, FPG, triglycerides, fasting insulin, and HOMA-IR, while HDL was positively correlated with it. Adiponectin and HDL have a positive significant relationship, as per our findings.

However, there are numerous limitations to this study, including the inability to evaluate major pro-inflammatory cytokines including interleukin-1, and the lack of serial assessments for long-term follow-up of pre-diabetic obese subjects.

CONCLUSION

From the above results, it may conclude that the case group has higher levels of SFRP-4 and adiponectin was found to be decreased. Hence, altered levels of SFRP-4 and adiponectin derange glucose metabolism, thus producing insulin resistance in the case group. Therefore, SFRP-4 and adiponectin may be used as diagnostic markers in pre-diabetic individuals with obesity but it needs further validation.

DECLARATIONS

Conflicts of Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Informed Consent

All of the participants gave their informed consent to participate in the study.

Financial Support

The authors have not received any funds for the present research.

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