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Status of circulatory level of secreted frizzled related protein 4 in metabolic syndrome

Sushil Yadav
Yadav Sushil, Ph.D Scholar, Department of Biochemistry, Santosh Medical College, Ghaziabad, U.P, India

Vinod Kumar Singh
Singh VK, Professor, Department of Medicine, TMMC & RC, Moradabad, U.P, India

Pradeep Kumar
Professor, Department of Biochemistry, Autonomous State Medical College, Fatehpur, U.P, India

Preeti Sharma
Professor, Department of Biochemistry, Santosh Medical College, Ghaziabad, U.P, India
*Corresponding author email: prcdri2003@yahoo.co.in

Abstract---Background: Metabolic syndrome is a set of metabolic disorders and is considered as a predictor of cardiovascular risks. The expression of several specific genes and the related protein products also show their significance in pathophysiology of Metabolic Syndrome. Secreted Frizzled Related Protein 4 is a product of expression of SFRP4 genes. SFRP4 has been linked with inflammatory responses quite similar to the responses as in metabolic syndrome pathogenesis. Aim: The current study aimed to assess the circulatory level of Secreted Frizzled Related Protein 4 among metabolic syndrome individuals. Method: Estimation of SFRP4 was done by ELISA technique by using Human SFRP4 ELISA kit (Make- Bioassay Technology Laboratory, Cat no- E2327Hu, Zeijhang, China). Results: Mean concentration of serum SFRP4 in Metabolic Syndrome subjects (2.06 ± 1.39 ng/ml) was significantly higher than the mean concentration of healthy controls (1.28 ± 1.29 ng/ml) ($p< 0.05$ *). Conclusion: A significant correlation of serum SFRP4 level with Fasting blood glucose and HbA1c seen in the findings of this study puts on an extra contribution to consider this protein as a potential Biomarker for Type II Diabetes Mellitus.

Keywords--secreted frizzled related protein 4, metabolic syndrome, type II diabetes mellitus, adipocytokine.

Introduction

Metabolic syndrome is a set of metabolic disorders and is considered as a predictor of cardiovascular risks which can be seen in near future in such individuals. Specifically, metabolic syndrome is defined as a collection of 3 or more metabolic risk factors including dyslipidemia (high triglycerides & low HDL cholesterol), abdominal obesity, increased fasting plasma glucose and hypertension.[1] This syndrome acts as a culprit for a leading cause of early deaths majorly in the developed countries.[2] If left unmanaged, the syndrome is likely to land up the individual to serious complications like insulin resistance and cardiovascular diseases. Those individuals acquiring any 3 of the 5 attributes of metabolic syndrome are many folds susceptible to develop Type II Diabetes Mellitus. The release of certain adipocytokines like Interleukin-6, CRP, TNF-α, Plasminogen Activator Inhibitor-1, etc has also been reported and these pro-inflammatory mediators induces a series of events in metabolic syndrome resulting in localized inflammation initially at adipose tissues and ultimately propagating in overall systemic inflammation.[3,4,5]

Among the major contributors for metabolic syndrome, visceral adiposity, obesity and overweight are the primary triggers for most of the pathologies involved.[6] Although most of the risk factors involved in precipitating metabolic syndrome have been evaluated, the evidences show that the contributing risk factors are many more to be explored yet.[7] It has been proposed by numerous researchers indulged in this area that a concerted effort should be applied between public health solutions and clinical approaches in order to lower the burden related with metabolic syndrome. The expression of several specific genes and the related protein products are also expected to show their significance in pathophysiology of metabolic syndrome. Secreted Frizzled Related Protein 4 is a product of expression of SFRP4 genes. SFRP family has five members in humans, namely SFRP1 to SFRP5. SFRP4 protein belongs to a member of wingless- type (Wnt) signaling pathway and the researchers have explained the regulatory role of SFRP4 in Wnt pathways. SFRP4 is one of many adipocytokines with deranged expression and secretion from adipose tissues especially in the obese individuals.[8,9,10]

Secreted Frizzled Related Protein 4 has been linked with inflammatory responses quite similar to the responses seen in metabolic syndrome pathogenesis. The role of SFRP4 as an inflammatory mediator was recently found and it was proposed that the over-expression of SFRP4 and its release from the pancreatic islets tissues is stimulated by a cytokine, IL-1B, and in fact is a link between β- cell dysfunction and a chronic low- grade inflammation progressing ultimately to β-cell failure. Since, the protein SFRP4 is highly expressed by the islets tissues of pancreas, the circulatory level of the protein may be increased in Diabetes Mellitus and the studies done in this area of interest have hypothesized the elevation of SFRP4 might be several years before the diagnosis of Type II Diabetes Mellitus. It has been reported that the increase in the level of SFRP4 in the

circulation reduces glucose tolerance. The release of insulin from pancreatic β-cells is a Ca^{2+} channel mediated process and it has been explained that SFRP4 protein decreases the expression of Ca^{2+} channels in the pancreatic islets tissue and suppresses insulin exocytosis.[11] A newer approach for better management of metabolic syndrome individuals is the utmost demand as per the current scenario of exponential rise in the cases. In this approach, targeting inflammatory mediators and the use of anti-inflammatory mode of treatment will obviously be of paramount significance. Undoubtedly, targeting the release of adipocytokines and cytokines at minimal levels will suppress the course of inflammation and improvise the health risks caused by metabolic syndrome.

The current study aimed to assess the circulatory level of Secreted Frizzled Related Protein 4 among metabolic syndrome individuals which is considered as an adipocytokine and a mediator of low- grade inflammation. The available data on human SFRP4 till the date have been too limited. A relationship between the occurrence of metabolic syndrome and progression to insulin resistance and type II Diabetes Mellitus has been taken into consideration at the planning stage of this study and the study has focused to establish the relationship between SFRP4 levels in serum with attributes of metabolic syndrome if any.

Materials and Method

The study is a cross-sectional case control study and was conducted at Teerthanker Mahaveer Medical College in collaboration with Santosh Medical College after getting an ethical clearance from Institutional Ethical Committee. The subject selection was done once the written consent was taken from the enrolled patients. 110 patients of metabolic syndrome under the consultation of clinical experts of Department of Medicine between the age group of 25-55 years were included. A comparable age and sex matched 110 healthy controls were also included for comparing the test results among two groups.

Patients' Selection Criteria

The metabolic syndrome patients were selected according to *ATP III guidelines*. Those subjects meeting any 3 of the following attributes were considered as metabolic syndrome patients.

- Waist circumference: >102cm for men > 88cm for women.
- Fasting blood glucose: ≥110mg/dl
- Serum triglyceride: ≥150mg/dl
- Blood pressure: ≥130/≥85mmHg.
- HDL good cholesterol: <40mg/dl for men and<50mg/dl for women

A detailed history from all the participants was taken including their life-style, drug medications being taken, and family history as well. Those having any history of cancer, chronic inflammatory diseases like tuberculosis, arthritis, smoking and alcoholism were excluded from the study. Also, females with pregnancy and polycystic ovarian syndrome were not enrolled in this study.

Sample collection and evaluation

The anthropometric parameters (age, weight and height) and blood pressure were recorded from the subjects. A fasting blood sample was collected from the individuals for the biochemical analysis. Fluoride blood sample was used for estimating fasting plasma glucose where as a plain blood sample was stored for assessment of Secreted Frizzled Related Protein 4 and lipid parameters. After centrifugation, serum sample was separated and stored at 2-8^{0}c so that the estimation of SFRP4 could be done in a batch of 40-50 samples at a time.

Estimation of Serum Secreted Frizzled Related Protein 4

The estimation of serum Secreted Frizzled Related Protein 4 was done through enzyme- linked immune-sorbent assay (ELISA) technique by using Human SFRP4 ELISA kit (Make- Bioassay Technology Laboratory, Cat no- E2327Hu, Zeijhang, China). The kit methodology had inter-assay coefficient of variation of 10% and the intra-assay coefficient of variation of 8%. A standard curve was constructed by plotting the concentration of standards on x-axis against the average OD of the corresponding standards on y-axis. The test concentration was calculated using the standard curve.

Results

Table 1
Comparison of Baseline measures attributes of Metabolic Syndrome and SFRP4 between Metabolic Syndrome and Non-Metabolic healthy controls

S.N	Variables	Mets (n==110)	Non- Mets (n=110)	p-value
1.	Age (years)	44.42 ± 9.26	42.88 ± 8.17	> 0.05
2.	BMI (kg/m^2)	27.46 ± 2.84	24.52 ± 3.04	< 0.05
3.	Waist circumference (cm)	97.90 ± 8.27	86.88 ± 8.18	< 0.05
4.	HbA1c (%)	7.35 ± 1.42	5.0 ± 0.49	< 0.05
5.	Fasting Plasma Glucose (mg/dl)	139.98 ± 30.75	96.94 ± 4.98	< 0.05
6.	Systolic Blood Pressure (mm/Hg)	132 ± 10	118 ± 3	< 0.05
7.	Diastolic Blood Pressure (mm/Hg)	84 ± 6	80.5 ± 2.8	< 0.05
8.	HDL Cholesterol (mg/dl)	35.07 ± 8.89	76.57 ± 8.44	< 0.05
9.	Triglycerides (mg/dl)	226.12 ± 67.59	123.58 ± 9.71	< 0.05
10.	Total Cholesterol (mg/dl)	176.36 ± 52.31	87.34 ± 13.12	< 0.05
11.	SFRP4 (ng/dl)	2.06 ± 1.71	1.28 ± 1.29	< 0.05

Table 2
Comparison of parameters between Non-diabetics, Pre-diabetics and Diabetics

S.N	Variables	Non-Diabetics	Pre-Diabetics	Diabetics	Interpretation
1.	BMI (kg/m^2)	24.52 ± 3.04	26.14 ± 2.42	28.24 ± 2.79	* ND & PD * ND & D * D & PD
2.	Waist circumference (cm)	86.88 ± 8.18	97.34 ± 8.49	98.23 ± 8.18	* ND & PD * ND & D
3.	HbA1c (%)	5.0 ± 0.49	6.65 ± 0.93	7.76 ± 1.50	* ND & PD * ND & D *D & PD
4.	Fasting Plasma Glucose (mg/dl)	96.94 ± 4.98	119.12 ± 4.45	152.38 ± 32.94	* ND & PD * ND & D *D & PD
5.	HDL Cholesterol (mg/dl)	76.57 ± 8.44	36.80 ± 7.77	34.05 ± 9.39	* ND & PD * ND & D
6.	Triglycerides (mg/dl)	123.58 ± 9.71	196.93 ± 40.91	243.46 ± 74.29	* ND & PD * ND & D *D & PD
7.	Total Cholesterol (mg/dl)	87.34 ± 13.12	182.66 ± 45.05	172.62 ± 56.15	* ND & PD * ND & D
8.	SFRP4 (ng/ml)	1.28 ± 1.29	1.85 ± 1.30	2.18 ± 1.44	* ND & PD * ND & D

*: Significant, ND: Non-diabetics, PD: Pre-diabetics & D: Diabetics

Table 3
Correlation of SFRP4 with attributes of Metabolic Syndrome (n=110)

S.N	Variables	r- value	p- value
1.	Waist Circumference (cm)	0.18	< 0.05 *
2.	BMI (kg/m^2)	0.22	< 0.05 *
3.	HbA1c (%)	0.33	< 0.01 **
4.	Fasting Plasma Sugar (mg/dl)	0.54	< 0.01 **
5.	Systolic Blood Pressure (mm/Hg)	0.03	>0.05
6.	Diastolic Blood Pressure (mm/Hg)	0.09	>0.05
7.	HDL Cholesterol (mg/dl)	0.05	>0.05
8.	Triglycerides (mg/dl)	0.38	< 0.05 *
9.	Total Cholesterol (mg/dl)	0.18	< 0.05 *

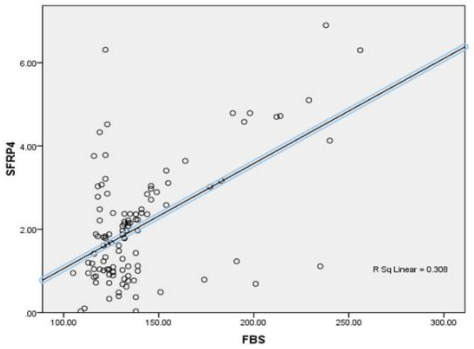

Figure 1. Scatter Diagram of correlation between SFRP4 and Fasting Blood Sugar

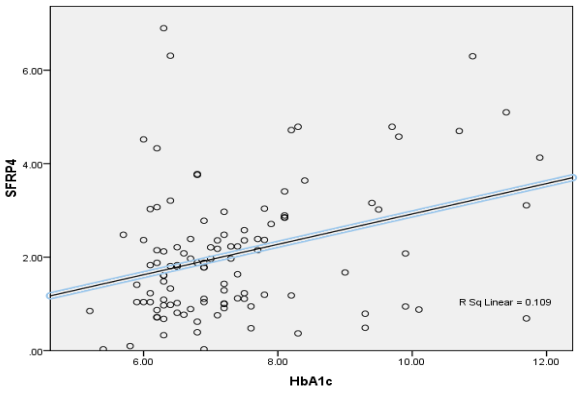

Figure 2. Scatter Diagram of correlation between SFRP4 and HbA1c

Discussion

Metabolic Syndrome, being an initial stage of multiple life- threatening diseases like Diabetes Mellitus, Cardiovascular diseases and related risks, an early diagnosis and management of the same is of utmost importance. Several molecular based researches are ongoing in the related field in order to develop the most effective clinical protocol for management and treatment of the syndrome. The implication of the biomarkers has been a prime focus so that the early diagnosis could be brought into clinical practice to halt the progression of such diseases. Secreted Frizzled Related Protein 4, being widely studied protein as a biomarker in obesity and Diabetes Mellitus, has been chosen in this study so as to find out whether this protein could be of significance in the predicting the progression of Metabolic Syndrome to an Overt Diabetes Mellitus over a period of time. Some evidences from the researches have explained that SFRP4 is an adipocytokine and its expression is increased in obese individuals. It has also been evidenced that SFRP4 might influence the secretion of adiponectin from adipocytes and is involved in adipogenesis.

In the present study, a total of 220 individuals were enrolled out of which 110 were Metabolic Syndrome subjects and 110 were healthy controls. An attempt to compare the serum levels of SFRP4 protein between Metabolic Syndrome subjects and healthy subjects was made and it was seen that the mean concentration of serum SFRP4 in Metabolic Syndrome subjects (2.06 ± 1.39 ng/ml) was significantly higher than the mean concentration of healthy controls (1.28 ± 1.29 ng/ml) ($p< 0.05$ *). The observations of serum SFRP4 level in the current study indicates that this protein is over- expressed in significant amount among the subjects of Metabolic Syndrome. Based on the findings of the present study and few other studies on SFRP4 expression in Diabetes Mellitus patients, it can be speculated that the increased expression of SFRP4 among those individuals might influence the synthesis and release of insulin. Interestingly, SFRP4 protein is evidenced to be produced by both the pancreatic α and β cells and thus some studies have explained that SFRP4 might possibly show its involvement in maintaining the Insulin/ Glucagon ratio. The mechanism involved in over-expression of SFRP4 among the subjects with deranged carbohydrate metabolism has been postulated in several studies. Mahdi T et al. in 2012 studied a group of

associated genes by collecting a global microarray expression data from islets tissues of numerous human donors with and without Type II Diabetes Mellitus. The aim of their study was to identify a molecular link in the form of SFRP4 between pancreatic islets inflammation and defective insulin secretion. The researchers stated that SFRP4 affects Wnt signaling and this in turn influences number of other genes. This influence suppresses two different voltage- gated Ca^{2+} channels and leads to reduced insulin exocytosis. More precisely, they found that the main effect of the protein was on reduction of insulin secretion by altering the calcium channels in pancreatic islets tissue whereas β cell viability and the insulin content in those individuals were unaltered.

Among the subjects of Metabolic Syndrome, 58 subjects were females and 52 were males. A gender-wise comparison of serum SFRP4 level, demographic attributes and metabolic attributes were also analyzed. No significant difference in the SFRP4 level was found gender-wise, except for HDL cholesterol and systolic blood pressure as recorded in the study. SFRP4 level was elevated in both male and female metabolic syndrome patients as compared to the counterparts in the healthy controls, although no gender-wise difference within metabolic syndrome group clarifies that there is no influence of gender on the expression of SFRP4. Meanwhile, a significant positive correlation was observed between SFRP4 levels and fasting plasma glucose among Metabolic Syndrome cases ($r= 0.54$, $p= <0.05$) as shown in Table 3. Assumably, a significant correlation between HbA1c and serum SFRP4 level was also seen on correlating these parameters. Similarly, as mentioned in the Table 3, other metabolic attributes like waist circumference, BMI, serum Triglyerides and Total Cholesterol also had significant correlation with serum SFRP4 levels ($p < 0.05$). Remaining attributes such as Systolic blood pressure, Diastolic blood pressure and HDL cholesterol had no significant correlation with the protein level.

The present study had primary objective to identify SFRP4 as an early biomarker at the stage of Metabolic Syndrome prior the progression to Type II Diabetes Mellitus. For this purpose, the enrolled study subjects were categorized into 3 different groups, namely: Non- Diabetics (Healthy controls), Pre-Diabetics (FBS < 126 mg/dl), Diabetics (FBS > 126 mg/dl). Intergroup comparison of all the measured parameters using One-Way Anova was applied in the present study as shown in the Table 2. The mean concentration of serum SFRP4 protein was found to be significantly higher among Pre-diabetic and Diabetic groups in comparison to that of Non-diabetic group. More importantly, there was no significant difference in the mean values of SFRP4 between Pre-diabetic and Diabetic group of cases since the observations in the current study showed similar values in both these groups. Based on the above mentioned findings, the present study justifies its prime aim to identify SFRP4 as an early biomarker for metabolic diseases, since SFRP4 begins to get over-expressed in the individuals several years before the diagnosis of Type II Diabetes Mellitus as evidently seen in the Pre-diabetic cases. Pre-diabetes is an early stage to Type II Diabetes Mellitus and if left uncontrolled can lead to overt Diabetes Mellitus within few years. SFRP4 circulatory concentration when assessed among the Pre-diabetic cases in this study, showed a significant rise than the healthy control and the elevated levels remained consistent among the Diabetic cases too.

The elevated blood glucose triggers β- cells of Pancreas to synthesize and release insulin for uptake of glucose by skeletal muscles and adipose tissues.[12] Metabolic stress and oxidative stress towards islets tissue initiates chronic low-grade inflammation and progresses to Type II Diabetes Mellitus. Various pro-inflammatory cytokines such as CRP, IL-6 and IL-1β might activate innate immune response and mediate the inflammatory mechanisms in pancreatic islets.[13] On the other hand, several components of Wnt signaling pathway are also linked to metabolism of lipids and glucose which aids significantly in development of metabolic syndrome progressing to Type II Diabetes Mellitus. This metabolic disorder is associated with insulin signaling and adipogenesis as a result of impairment in Wnt Signaling. Experimental evidences gathered from the researches based on experimental animals, it has been evidenced that SFRP4 is expressed highly during differentiation of pre-adipocytes to mature adipocytes, especially in obese animals as compared to lean animals.[14] The over-expression of SFRP4 causes the increase in adipogenesis and leads to the elevation in lipid accumulation.[15,16]

Similar findings have been stated in our study as we observed a positive and significant correlation of serum SFRP4 level with the lipid components like serum Triglycerides ($r= 0.38$, $p< 0.05$) and serum Total Cholesterol ($r= 0.18$, $p< 0.05$). Few researchers have revealed the role of SFRP4 protein on increasing obesity by interacting with Wnt ligands.[16] Wnt ligands including SFRP4 are produced by mature adipocytes with insulin sensitizing and anti-inflammatory properties.[17] Associated with the above mentioned findings, we also found that Body Waist circumference and BMI were also positively and significantly correlated with serum SFRP4 levels with the statistical measures of $r= 0.22$, $p< 0.05$ and $r= 0.18$, $p< 0.05$ respectively as presented in Table 3.

Conclusion

Identifying an appropriate biomarker and targeting those biomarker compounds in order to lower their expression has been the recent approach in the field of medicine to prevent a disease at an early stage and stop to progress into the full blown disease as in the case of metabolic syndrome progressing to Type II Diabetes Mellitus. The present study was planned to identify SFRP4 as an early biomarker at the stage of Metabolic Syndrome and Pre-diabetes and it was quite evident from the study that SFRP4 protein is raised in the circulation of the individuals at the stage of Pre-diabetes and Metabolic Syndrome. Also, the significant correlation of serum SFRP4 level with Fasting blood glucose and HbA1c seen in the findings of this study puts on an extra contribution to consider this protein as a potential Biomarker for Type II Diabetes Mellitus. Much more extensive studies at molecular level are suggested before considering the protein as a biomarker. The studies on inhibitor molecules targeting to inhibit the expression of SFRP4 in those individuals and following those cases to investigate the improvements in the level of blood glucose and HbA1c could be of immense significance in the field of metabolic diseases.

Limitations of the study

The present study included a small sample population which limits the generalization of the present findings. A prospective cohort study is much expected to rule out the effects of confounding factors on the results. Also, the study was limited to serum assay of the protein and no molecular mechanisms involved in the pathogenesis of Metabolic Syndrome with response to SFRP4 could be revealed which obviously can be noted by other researchers to carry on with their future studies in the area of Secreted Frizzled Related Protein 4.

Conflict of interest

There was no conflict of interest among the authors.

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