SHORT REPORT

Normoglycemia and type 2 diabetes: exploring secreted frizzled-4, insulin resistance, and waist-height ratio

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Abstract

The current study was planned to compare serum levels of secreted frizzled related protein-4, insulin resistance and waist-to-height ratio in individuals with and without a diabetic background, and to assess the correlation of these markers with family history of diabetes. The cross-sectional comparative study comprised 80 subjects with confirmed normal glucose tolerance values. Parameters assessed included secreted frizzled related protein-4, fasting glucose, random glucose, fasting insulin, homeostasis model of assessment of insulin resistance and waist-to-height ratio values. Those without a diabetic background had significantly higher frizzled related protein-4 levels (p=0.02). Although subjects with family history of diabetes showed higher mean fasting glucose, waist circumference and waist-to-height ratio, these differences were not statistically significant (p>0.05). However, there was a strong positive correlation with waist circumference, waist-to-height ratio, fasting insulin and homeostasis model of assessment of insulin resistance (p=0.0001). There was no significant correlation of diabetic background with frizzled related protein-4 SFRP-4, homeostasis model of assessment of insulin resistance and waist-to-height ratio (p>0.05).

Keywords: Wnt signalling pathway, Frizzled related protein 4, Diabetes mellitus, Type 2, Insulin resistance, Waist-height ratio, Blood glucose, Insulin, Waist circumference, Mass screening, Cross-sectional studies, Family, NAFLD, Metabolic syndrome.

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Introduction

Secreted frizzled related protein 4 (SFRP-4), an antagonist of the wingless-related integration site (Wnt) signalling pathway, has shown promise due to its impact on glucose metabolism, insulin secretion and its association with obesity. The current study was planned to compare serum levels of SFRP-4, insulin resistance (IR) and waist-to-height ratio (WHtR) in individuals with and without a diabetic background, and to assess the correlation of these markers with family history of diabetes.

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Methods and Results

The cross-sectional, comparative study was conducted from December 22, 2020, to December 22, 2022 after approval form the ethics review board of the University of Health Sciences, Lahore, Pakistan, The sample was raised using convenience sampling technique after the sample size was calculated using standard formula in the light of literature.[1] The calculated sample size was inflated by about 50% to increase the power of the study. A diabetes screening camp was held in the outpatient department (OPD) of District Headquarters (DHQ) Hospital, Gujranwala. Normal, glucose-tolerant individuals with family history of diabetes were placed in group A, and those without a family history of diabetes were placed in group B. Family history meant either or both parents having T2DM. Normal glucose tolerance was confirmed using oral glucose tolerance test (OGTT). Those who had acute illnesses at the time of the study, known cases of all types of diabetes, cardiovascular, cerebrovascular, and peripheral arterial diseases, hypertension, chronic renal illness, malignancy, bone disease and pregnancy were excluded. Family history was taken, anthropometric measurements were noted, and blood samples were collected. Waist circumferences (WC) and height were divided to determine WHtR.[2] For adults with central obesity, the cut-off value was set at 0.5. The glucose oxidase method was used to gauge blood glucose concentration. Fasting plasma insulin (FPI) levels were tested using an automated engagement intensity analyser (EIA) Enzyme-Linked Immunosorbent Assay kit manufactured by Micorwell Diagnostic Syxtem BIOS Lab. Sandwich ELISA was operated as the test underlying methodology, with insulin in the test sera conjugated with both enzyme and solid-phase antibodies. The homeostasis model of assessment of insulin resistance (HOMA-IR) was worked out using an equation mentioned in literature.[3]

Serum SFRP-4 was measured by Human C1q and Tumour Necrosis Factor Related Protein 9 (C1QTNF9) Enzyme-Linked Immunosorbent Assay (ELISA) kit manufactured by Elabscience. The ELISA kit was able to quantify the levels of

Table-1: Anthropometric and biochemical parameters in the study groups.

Parameters	Group A		Group B		*p*-value
	Mean±SD	Median(IQR)	Mean±SD	Median(IQR)	
Age(years)	32±8.4	32(20-50)	33±9.7	30(20-50)	0.01
WC(cm)	95±14	89 (69-130)	91±21	89 (50-152)	0.52
Height(cm)	162±12	166(132-183)	167±10	170(145-185)	0.0001*
WHtR	0.6±0.12	0.56(0.4-0.9)	0.5±0.12	0.54(0.3-0.9)	0.21
FPG(mg/dl)	78±8.2	78(63-99)	76±7.4	75(63-99)	0.01*
2HRPP(mg/dl)	104±12	100(87-145)	107±13	105(89-140)	0.04*
Fasting Insulin(Mu/L)	15±12	10(1.31-54)	17±14	11(2.61-75)	0.004*
HOMA-IR	52±43	35(1.1-26.5)	59±59	38(9.16-332)	0.007*
SFRP-4(ng/ml)	5±4.5	4.2(1.1-26.5)	6.7±4.3	5.5(0.75-20)	0.28

Group A: Normal glucose tolerant with diabetic family history, Group B: Normal glucose tolerant without diabetic family history, SD: Standard deviation, IQR: Interquartile range, WC: Waist circumference, WHtR: Waist-to-height ratio, FPG: Fasting plasma glucose, 2HRPP: 2-hour post-prandial glucose, HOMA-IR: Homeostasis model of assessment of insulin resistance, sFRP-4: Secreted frizzled related protein-4.

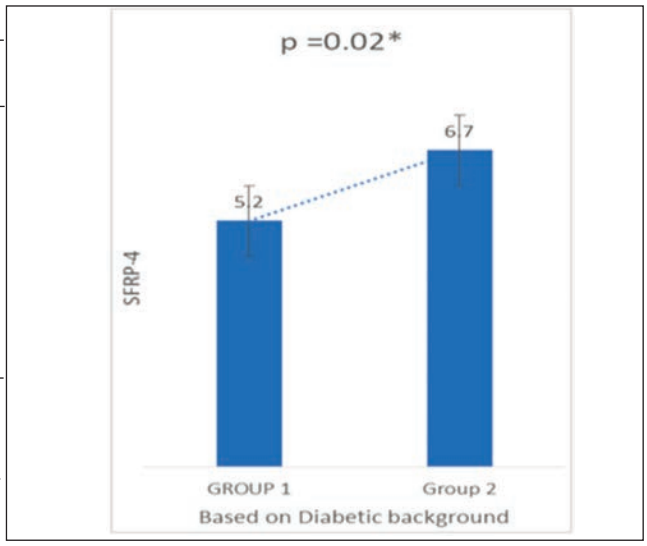

Figure-1: Intergroup comparison of secreted frizzled related protein-4 (SFRP-4) level.

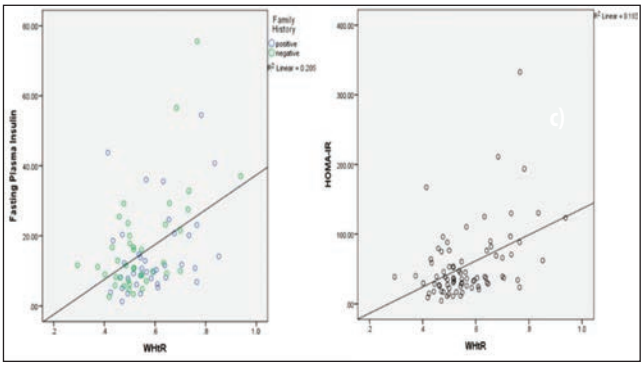

Figure-2: Significant Correlation between FP1, HOMA-IR &WHtR.

SFRP-4 in serum, plasma, and other biological fluids with a sensitivity of 0.1ng/mL. The kit was able to detect SFRP-4 in concentrations ranging from 0.16ng/mL to 10ng/mL. The coefficient of variation was claimed to be less than 10%.

Data was analyzed using SPSS version 25 and Graph Pad Prism 11. Data normality was assessed using Shapiro-Wilk's test. Parametric and non-parametric tests were applied based on data distribution. Mean±standard deviation, median with interquartile range (IQR), and frequencies and percentages were used to express the data, as appropriate. Spearman correlation analysis was used to explore association involving WHtR, IR and SFRP-4 levels. P≤0.05 was considered significant.

Of the 80 subjects, 40(50%) were in each of the 2 groups. All the participants had normal glucose tolerance confirmed through an OGTT. Height, fasting plasma glucose (FPG), random blood glucose, FPI, HOMA-IR values were significantly different between the groups (Table 1).

Table-2: Spearman's correlation of anthropometric and biochemical parameters.

		WC	Height	WHtR	FPG	2hrpp	HOMA-IR	FPI	SFRP-4	FH
Age (years)	*r*-value	**0.46****	0.02	**0.41****	0.16	0.014	**0.296****	**0.306****	-0.06	0.04
	p-value	**0.001**	0.89	**0.001**	0.201	0.902	**0.008**	**0.001**	0.599	0.66
WC	*r*-value		-0.04	**0.94****	0.06	0.121	**0.355****	**0.366****	0.12	-0.08
	p-value		0.73	**0.001**	0.6	0.28	0.001	**0.001**	0.26	0.45
Height	*r*-value			**-0.34**	-0.19	-0.04	-0.16	-0.15	-0.01	**0.23***
	p-value			**0.002****	0.09	0.67	**0.15**	**0.186**	0.87	**0.04**
WHtR	*r*-value				.07	0.08	**0.364****	**0.372****	0.13	-0.14
	p-value				.5	0.46	**0.001**	**0.001**	0.22	0.2
FPG	*r*-value					0.64**	**0.229***	0.09	-0.02	**-0.37****
	p-value					0.001	**0.04**	0.41	0.82	**0.001**
2hrpp	*r*-value						0.12	0.01	0.12	-0.15
	p-value						0.28	0.889	0.27	0.17
HOMA-IR	*r*-value							**0.98****	0.14	**-0.28***
	p-value							**0.001**	0.21	**0.01**
FPI	*r*-value								0.19	**-0.24***
	p-value								0.09	**0.03**
SFRP-4	*r*-value									0.15
	p-value									0.17

** Significant at p=0.01 level; * Significant at p=0.05 level; WC: Waist circumference; WtHR: Waist-to-height ratio; FPG: Fasting plasma glucose; 2HRPP: 2-hour post-prandial glucose; HOMA-IR: Homeostasis model of assessment of insulin resistance; FPI: Fasting plasma insulin, SFRP-4: Secreted frizzled related protein-4, FH: Family history.

The SFRP-4 level was higher in group B (Figure 1), but it was not significantly related to family history of diabetes. HOMA-IR correlated positively and significantly with age, gender, WC and FPI (Table 2).

No significant correlations were found between SFRP-4 and anthropometric or biochemical parameters in either group (p>0.05). WHtR correlated significantly with age, WC, FPI and height (p<0.05). A strong positive

correlation was observed between HOMA-IR and WHtR (p<0.0001) (Figure 2).

Discussion

The current study included individuals with and without a family history of T2DM, but unexpected findings, such as higher SFRP-4 levels in those without a family history, raise questions. One possibility is the small sample size and the underpowered nature of the study. The recruitment of subjects relied on convenient sampling, which may have led to inaccurate representation due to a variety of factors, such as eating habits, lifestyle and genetic makeup. Conducting similar research on a larger scale could help overcome these limitations. Additionally, the presence of outliers in group B may have affected the results, as indicated by the IQR values of SFRP-4 in that group (0.75-156.8ng/ml). Another factor to consider is the proportional increase or decrease of SFRP-4 levels with adipose tissue. Previous studies have shown raised SFRP-4 levels in visceral adipose tissue in obese individuals, with complex expressions of SFRP-4 during adipocyte differentiation and variations among different adipose tissue sites.[4] In the current study, a higher IQR of WC in group B indicated a higher abdominal fat percentage, which could have implications for the results related to SFRP-4. However, SFRP-4 may serve as a link between islet cell inflammation and impaired insulin secretion.

Contrary to the current study, a case-control study in India conducted between 2016 and 2018 showed higher levels of SFRP-4 in patients with T2DM compared to normal glucose-tolerant individuals. However, the study did not consider first-degree relatives (FDRs) as controls.[1] Another study revealed significantly higher SFRP-4 levels in T2DM patients compared to those with impaired glucose tolerance (IGT) (p=0.001) and normal glucose tolerance (p=0.004) groups.[5] These discrepancies suggest the possibility of genetic or ethnic variations that could influence SFRP-4 serum levels, as observed in the current study in which the inclusion of FDRs who were normal glucose-tolerant individuals was important due to their higher risk associated with genetic factors.

Another study aimed at assessing SFRP-4 levels in pregnancies with or without gestational diabetes mellitus (GDM) and found a positive association between SFRP-4 and GDM, indicating its potential as a novel biomarker for GDM.[6,7] However, in the current study, a significant correlation was found between HOMA-IR and WtHR which were positively correlated with each other, along with FPI. The mean values of SFRP-4 were significantly higher in group B than in group A, with a higher number of females in group B. Moreover, the IQR of WC in group B indicated a higher abdominal fat percentage, which could have influenced the results related to IR and SFRP-4. It is worth noting that gender did not impact WtHR in FDRs of T2DM in the current study, unlike a previous research that reported family history (FH) of T2DM as a significant predictor of abdominal adipocyte hypertrophy in females.[8]

Previous studies have proposed that SFRP-4 may serve as an early indicator of IR, T2DM, metabolic syndrome (MetS), coronary heart disease, polycystic ovarian disease, and hypertension. Furthermore, SFRP-4 may also be a predictive early marker of atherosclerosis in individuals with T2DM with or without coronary heart disease.[1,9]

One important conclusion from the current study is the positive correlation observed between HOMA-IR and WtHR along with FPI. Similar results were obtained in a study conducted in Europe in 2020, which demonstrated a positive correlation among FPI, HOMA-IR and WtHR.[10]

Non-alcoholic fatty liver disease (NAFLD) is frequently seen as a liver-related aspect of MetS, but it can be viewed as both a contributing factor and a result.[11] Also, SFRP-4 may offer insights into metabolic dysregulation associated with liver disease. Additionally, in the abdominal fat tissue of individuals with obesity and T2DM, higher levels of micro-ribonucleic acid-24 (miR-24), miR-30d and miR-146a were found. These levels were also linked to higher levels of SFRP-4.[12] Whether or not SFRP-4 may address any existing lacunae, like fibroscan test or gamma-glutamyl transferase (GGT) test in NAFLD is still an area to be explored. Interestingly, molecular docking experiments revealed that 6 phytochemicals like hesperetin, curcumin, isorhamnetin, embelin, epicatechin and methyl eugenol interacted strongly with SFRP4's active site residues, considered now a 'diabesity' marker, that is extracted from plants and could serve as anti-diabesity compounds, offering a novel avenue for treating diabesity.[13]

The current study had limitations, such as a small sample size and potential bias owing to the use of convenience sampling. Large cohort studies are needed to validate the current findings.

Conclusion

This study concluded that the mean values of SFRP-4 was significantly higher in Group B than in Group A. HOMA-IR and WHtR were positively correlated with each other along with fasting plasma insulin and there was negative correlation between SFRP-4 and WHtR.

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Conflict of Interest: None.

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Author Contribution:

SS: Concept, design, abstract, introduction, discussion, conclusion.
DK: Methodology.
SF: Discussion.
FR: Proofread, conclusion.
MOA: Referencing.
AR: Data analysis, results.