Assessment of circulating Wnt1-inducible signaling pathway protein 1 (WISP1) in obesity and type 2 diabetes mellitus patients

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ABSTRACT

Wnt-inducible signaling pathway protein 1 (WISP1) is a recently identified adipokine that is thought to be involved in mechanisms linking obesity and type 2 diabetes mellitus (T2DM). This study is designed to investigate the correlation between WISP1 serum levels and glycemic parameters in Egyptian population subjects for the first time. Anthropometric parameters, routine biochemical markers, serum levels of WISP1, insulin, proinsulin, and high sensitivity C-reactive protein (hs-CRP) were measured by ELISA kits in 90 subjects (24 non-obese patients with T2DM and 22 obese patients with T2DM compared with 24 healthy volunteers and 20 obese volunteers without T2DM). Serum WISP1 levels were significantly higher in obese patients compared with healthy controls ($p<0.05$). WISP1 was significantly correlated to waist circumference (WC) and serum triglycerides (TG). In conclusion, WISP1 might be a pivotal biomarker linking obesity and T2DM.

Keywords: Adipokines; Obesity; Type 2 diabetes mellitus; WISP1

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1. INTRODUCTION

Diabetes mellitus (DM) is considered a global emergency on the rise evidenced by the number of diagnosed cases and even more a terrifying number of undiagnosed cases\textsuperscript{1}. Globally, there are an estimated 425 million people who are suffering from diabetes with over 8 million cases in Egypt and about 39 million cases in the Middle East and North Africa (MENA) region. This number is expected to rise to 82 million by 2045 in MENA region\textsuperscript{1,2}. It’s well established that obesity is closely connected to the increased prevalence of type 2 diabetes mellitus (T2DM). Adipokines are believed to be a key player in the endless trials to understand the connection of obesity and T2D. Adipokines might have a pathophysiological role in insulin resistance and $\beta$-cell dysfunction which highlights the etiology for T2DM\textsuperscript{3,4}. However, the mechanisms by which adipokines affect the pathogenesis of T2DM are not well elucidated. Wnt signaling pathway is reported to have a role in pancreatic development and islet function as well as low-grade
inflammation in obesity. Numerous factors contribute to its regulation and activation. Wnt1 inducible signaling pathway protein 1 (WISP1, CCN4) is another remarkable validated adipokine and a downstream target of Wnt signaling pathway. WISP1 has been extensively studied in several fields including cancer, atherosclerosis, and has recently linked to obesity. It was shown that the expression of WISP1 was induced by Wnt1 which has been linked to numerous signaling pathways involved in the metabolic disturbances. Recent studies shed the light on the prominent role of WISP1, the novel adipokine in obesity and low-grade inflammation. It has been proposed that WISP1 is associated with insulin resistance following pancreatectomy. Recently, it was suggested that WISP1 is associated with pancreatic β-cell regeneration and proliferation. However, there’s a dearth of evidence regarding human studies addressing these correlations which require further studies to elucidate the role of WISP1 in the pathogenesis of obesity and DM.

We suggest that our adipokine of interest (WISP1) might be a potential key player in the pathophysiology of obesity and T2DM. However, its circulating serum levels in obesity and T2DM have not yet been assessed and properly studied. Therefore, we aimed to study WISP1 circulating levels in obesity and T2DM in the Egyptian population. Additionally, we sought to correlate its levels with anthropometric parameters, insulin resistance, and β-cell function.

2. MATERIALS AND METHODS

2.1. Study Design and anthropometric measurements

This study was conducted in the Faculty of Pharmacy, Ain Shams University, Cairo, Egypt in association with the National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt. It was approved by the ethical committee of both institutes following the regulations and recommendations of the Declaration of Helsinki. The control group subjects were recruited from healthy volunteers while the diabetic group's patients were enrolled from the outpatient clinic of NIDE after obtaining their informed consent.

Ninety subjects (47 males and 43 post-menopausal females) were enrolled in this study (46 patients with T2DM and 44 subjects without T2DM). Patients were diagnosed as diabetic according to standards of American Diabetes Association 2016 and further classified into non-obese (body mass index (BMI)<30 kg/m²) and obese BMI>30 kg/m²). The study population was classified as Group I (control group) included 24 apparently healthy volunteers; Group II included 20 obese volunteers without T2DM; Group III included 24 non-obese with T2DM patients; Group IV included 22 obese patients with T2DM. Patients with T2DM were previously diagnosed with T2DM for duration of maximum 8 years prior to the commencement of the study and they were receiving oral hypoglycemic drugs (OHA) (sulfonylurea±metformin, Su±Met). The characterization of the studied groups is demonstrated in Table (1).

Patients with the following criteria were excluded: age less than 30 or more than 65 years old, type I DM, insulin therapy, chronic and acute inflammatory conditions, cancer, thyroid dysfunctions, hormonal therapy, myocardial infarction (MI) or heart failure.

2.2. Evaluation of biochemical parameters

All biochemical tests were carried out in the laboratories of Biochemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

The blood samples (10 mL) that were drawn after 10-12 h from fasting subjects were
Circulating WISP1 in obesity and type 2 diabetes mellitus

Centrifuged for the separation of plasma and serum aliquots which stored at -80 °C to be used in subsequent assays. Serum lipid profile [triglycerides (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C)] as well as fasting plasma glucose (FPG) analyses were carried out using Spectrum Diagnostics® commercial kit (Hannover, Germany) and measured colorimetrically using a UV visible 1650 spectrophotometer (Shimadzu, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's equation\(^{18}\). HbA\(_1c\) (%) was measured by ion exchange chromatography using the Bio-Rad D-10™ system (Bio-Rad Laboratories, USA). Serum insulin and serum high sensitivity C-reactive protein (hs-CRP) were measured using Monobind Inc.(Lake Forest, USA) ELISA kits; proinsulin (PI) was quantified using DRG International (USA) ELISA kit. The updated homeostasis model assessment of β-cell function (HOMA2-%β) and insulin resistance (HOMA2-IR) was calculated from FPG (mg/dL) and serum insulin level (pmol/L) using the HOMA calculator software version 2.2.3.

Serum WISP1 was assessed using RayBio® ELISA Kit with intra- and interassay coefficients of variation of <10% and <12% respectively. All ELISA procedures were done by Hyprep automated ELISA system (Hyperion Inc, Miami, FL) according to the manufacturer’s instructions.

2.3. Statistical analysis

All data were expressed in either mean±SEM for parametric data or median (maximum-minimum) for non-parametric. Comparison between different groups was performed using either analysis of variance (ANOVA) followed by the least significant difference (LSD) as a post-hoc test between different groups for the parametric parameters or Kruskal-Wallis for non-parametric parameters p<0.05 was considered significant. Normality testing was done by Kolmogorov-Smirnov test. A logarithmic transformation was done before simple and multiple stepwise regressions. Statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp., Chicago, IL, USA).

3. RESULTS

3.1. Anthropometric, biochemical parameters, and β-cell function assessment in the studied groups

Anthropometric, biochemical, and β-cell function of the studied groups are shown in Table (1). Concerning FPG and HbA\(_1c\)\% values were significantly higher in group III and IV compared with group I and II. Lipid profile assessment showed a significant difference across the studied groups except for HDL-C. Circulating serum insulin and HOMA2-IR showed a higher significant level in group IV as compared to groups I, II, and III. A significant decline in β-cell function (HOMA2-%β) was observed in groups III and IV as compared with group I or II. HOMA2-IR correlated positively with hs-CRP and HbA\(_1c\)% \((r=0.326, \ p<0.001)\) and \((r=0.471, \ p<0.001)\) respectively while HOMA2-%β correlated negatively with HbA\(_1c\)% \((r=-0.681, \ p<0.001)\).
Table 1. The main anthropometric and metabolic parameters of the studied groups

|                  | Group I  | Group II | Group III | Group IV |
|------------------|----------|----------|-----------|----------|
| **A. 1. Anthropometric parameters** |          |          |           |          |
| N                | 24       | 20       | 24        | 22       |
| Age (y) (Mean ± SEM) | 50.25 ± 0.59 | 49 ± 0.45 | 50.92 ± 0.88 | 50.05 ± 0.84 |
| Sex (M/F)        | 19/5     | 9/11     | 14/10     | 5/17     |
| BMI (kg/m²) (Mean ± SEM) | 23.38 ± 0.87 | 33.25 ± 0.53* | 24.88 ± 0.50** | 35.5 ±0.93***,**** |
| Waist circumference (cm) (Mean ± SEM) | 88.96 ± 2.40 | 111.35 ± 1.96* | 101.5 ± 1.32*** | 119.5 ± 1.96***,**** |
| **A. 2. Metabolic parameters** |          |          |           |          |
| FPG (mg/dL)      |          |          |           |          |
| Median (Maximum-Minimum) | 84 (107-72) | 96 (123-78) | 146 (205-123)** | 149 (231-112)** |
| HbA₁c (%)        |          |          |           |          |
| Median (Maximum-Minimum) | 5.3 (5.8-4.7) | 5.6 (6.2-4.4) | 7.4 (9.8-6.6)** | 7.4 (12.4-6.6)** |
| TG (mg/dl) (Mean ± SEM) | 121 (186-85) | 142 (225-96) | 156.5 (251-105)** | 163 (229-109)** |
| TC (mg/dL) (Mean ± SEM) | 149 ± 4.2 | 177 ±6.1* | 216 ± 6.2*** | 214 ± 8.7*** |
| LDL-C (mg/dL) (Mean ± SEM) | 77 ± 3.8 | 98 ±5.8* | 137 ±5.9*** | 130 ±8.5*** |
| HDL-C (mg/dL) (Mean ± Minimum) | 46 (58-40) | 49.5 (55-45) | 47 (58-38) | 48.5 (65-42) |
| Insulin (pmol/L) (Mean ± Minimum) | 35.2 (60.42-15.14) | 42.9 (86.4-18.89) | 40.7 (136.54-19.58) | 71.5 (155.36-40.35)**,**** |
| HOMA2-IR (Median (Maximum-Minimum)) | 0.645 (1.11-0.38) | 0.8 (1.68-0.39) | 0.875 (2.75-0.43) | 1.495 (3.23-0.85)**,**** |
| HOMA2-%β (Mean ± SEM) | 80.87 ± 4.67 | 73.12 ± 4.88 | 32.80 ±3.1** | 46.56 ± 4.49***,**** |
| PI (pmol/L) (Median (Maximum-Minimum)) | 0.42 (4.02-0.24) | 0.545 (7.32-0.3) | 0.665 (6.5-0.27) | 0.547 (4.77-0.3) |
| hs-CRP (µg/mL) (Median (Maximum-Minimum)) | 550 (1701-136) | 845 (4781-115) | 882 (6000-146) | 1833 (9156-104)** |
| WISP1 (pg/mL) (Median (Maximum-Minimum)) | 630 (1600-113) | 1540 (5843-193)* | 909 (4433-103) | 785 (3757-112)** |

*Significantly different from the control (group I) at p<0.05
**Significantly different from obese without T2DM patients (group II) at p<0.001
***Significantly different from non-obese with T2DM patients (group III) at p<0.05

**Note:** Group I, Control subjects; Group II, Obese without T2DM patients; Group III, Non-obese with T2DM patients on OHA (SulMet); Group IV, Obese with T2DM patients on OHA (SulMet); BMI, Body mass index; FPG, Fasting plasma glucose; Hba₁c, glycated hemoglobin; TG, triglycerides; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; HOMA2-IR, the updated homeostasis model assessment of insulin resistance; HOMA2-%β, the updated homeostasis model assessment of β-cell function; PI, Proinsulin; hs-CRP, high sensitivity C-reactive protein; WISP1, Wnt1-inducible signaling pathway protein 1.
3.3. Associations of WISP1 levels with other biochemical parameters in the studied groups

Binary logistic regression showed a significant correlation of WISP1 with obesity (p<0.05), while simple linear regression revealed that WISP1 had a significant correlation with WC, and TG levels. Moreover, simple linear regression concluded that WC was positively correlated with WISP1 and insulin levels (β = 0.301, p = 0.002) and (β = 0.38, p<0.001) respectively. In contrast, we did not find a correlation between WISP1 and any of glycemic control parameters (FPG (r = -0.041, p = 0.704), Insulin (r = -0.171, p = 0.106) or HOMA2-IR (r = -0.167, p = 0.117)). Logarithmically transformed values were used in linear regression for non-parametric data (Table 2).

Table 2. Simple linear regression analysis using WISP1 as a dependent variable

| Variable                  | WISP1† |
|---------------------------|--------|
| BMI (kg/m²)               | 0.186  |
| Waist circumference (cm)  | 0.219  |
| FPG (mg/dL)†              | -0.064 |
| HbA1c%†                   | -0.047 |
| TG (mg/dL)†               | 0.279  |
| TC (mg/dL)                | -0.034 |
| LDL-C (mg/dL)†            | -0.099 |
| HDL-C (mg/dL)†            | 0.073  |
| Insulin (pmol/L)†         | -0.13  |
| HOMA2-IR†                 | -0.131 |
| HOMA2-%β                  | -0.028 |
| PI (pmol/L)†              | 0.035  |
| hs-CRP (µg/mL)†           | -0.007 |
| WISP1 (pg/mL)†            | - -    |

4. DISCUSSION

Several factors contribute to the progression of β-cell dysfunction, but once it occurs, the decline ultimately leads to culminating manifestations of T2DM. Obesity has a major contribution through the abnormal changes in serum adipokine profile in both β-cell dysfunction and insulin resistance, which have emerged as the culprit for the progression of T2DM.

Regarding the parameters of insulin resistance and β-cell function, their assessments have been widely proposed by HOMA model. Recently, it has been reported that HOMA2 reflects more accurate IR and β-cell function than the classical model.

Regarding WISP1, the other adipokine of interest, our current study revealed a significant correlation between WISP1 in the obese group as compared with the control group proposing a correlation between WISP1 and obesity. It was further ascertained by regression analysis showing a significant correlation between WISP1 and obesity. Our findings came in accordance with other studies showing that WISP1 expression in adipose tissue was associated with established markers of obesity. Conversely, another two studies reported higher WISP1 concentration in gestational diabetes mellitus and women with polycystic ovary syndrome as compared with the control group. Taking into consideration that both studies were conducted using study populations of premenopausal women, we cannot neglect the hormonal impact in their observation for WISP1 levels and its associations. Moreover, we have shown that WISP1 is correlated with WC and TG levels. These findings are in line with other studies proposing that WISP1 expression was highly correlated with adiposity.

Circulating serum WISP1 levels did not show a significant correlation between patients with diabetes and control. It was previously shown that there was no association with FPG or any other parameter of glycemic control suggesting that WISP1 concentration didn’t portray
overweight and diabetes per se but represents a systemic marker indicating impaired adipose tissue and adipocyte function. Although we did not find any association between WISP1 and hs-CRP nor HOMA2-IR, Barchetta et al. described a tight association between WISP1 and IL-8 suggesting the role of WISP1 in insulin resistance. Hence, further studies are required in larger sample size to detect the correlation of WISP1 with markers of insulin resistance.

Unfortunately, there is still a paucity of experimental data interrogating the postulated cellular mechanisms of WISP1 in metabolic pathways despite the conflicting results observed in clinical studies. Further studies are also warranted to delineate the molecular mechanisms of WISP1 in impairing insulin signaling in target tissues and its association with β-cell function and proliferation.

5. CONCLUSION

WISP1 might be a pivotal biomarker in the vicious cycle linking the pathogenesis of obesity and T2DM.

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Disclosure

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Author contribution

Nada S.Habib: Conducts the experiment (Sample collection, Biomarkers measurement, Data analysis). Mohamed H.EL-Hefnawy: Facilitates the sample collection. Hala O.EL-Mesallamy: Supervises the whole study as well as the study design hypothesis.

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Circulating WISP1 in obesity and type 2 diabetes mellitus

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