Obesity risk prediction among women of Upper Egypt: The impact of serum vaspin and vaspin rs2236242 gene polymorphism

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ABSTRACT

Background: Vaspin is an adipokine that is potentially linking obesity, insulin resistance, metabolic syndrome and type-2 diabetes.

Aim: The present study aimed to investigate the impact of vaspin rs2236242 gene polymorphism on the risk of obesity, diabetes, their metabolic traits, and serum vaspin levels in a sample of Upper Egyptian women.

Subjects and methods: A total of 224 subjects, 112 obese (62 non diabetics, 50 diabetics) and 112 controls were included in this case control study. Vaspin gene rs2236242 polymorphism was performed using tetra-amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) and serum vaspin levels were estimated by ELISA.

Results: The minor (A) allele of vaspin rs2236242 gene polymorphism was significantly lower in obese (30.8%) than controls (43.7%) (P = 0.005). The protective effect was evident in dominant and recessive inheritance models (TT vs TA + AA, P = 0.004 and TT + TA vs AA, P = 0.036). After adjusting genotypes for diabetes there were no significant association between vaspin rs2236242 gene polymorphism and obesity but significant association was maintained in the obese diabetics. Vaspin serum levels were found to be lower in minor protective (AA) genotype carriers than the other two genotypes (P < 0.001). In the mean-time serum vaspin levels were significantly higher in obese diabetics and non-diabetics than controls (P < 0.001 each). There were significant positive correlations between vaspin levels and hs-CRP, cholesterol, LDL-C, fasting glucose, HOMA-IR, insulin, and ALT values (P < 0.05 each) and a negative correlation with HDL-C (P < 0.01).

Conclusion: The minor A allele of vaspin rs2236242 polymorphism plays a protective role against obesity and diabetes but this relation is largely ascribed to its effect on insulin resistance. The serum vaspin concentration was lower in minor protective allele carriers. To the best of our knowledge, this is the first study of vaspin SNP in Upper Egyptian women. The entire understanding of vaspin intimate mechanistic action might enable the development of novel etiology-based treatment strategies for obesity, the complex genetic trait.

1. Introduction

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may lead to a decreased life expectancy and/or increased health problems (Haslam and James, 2005). Obesity is often associated with metabolic complications that include diabetes mellitus, cardiovascular disease, osteoarthritis, other degenerative joint diseases, and some forms of cancer; such as endometrial, breast and colon cancer (Navia et al., 2014). In 2014, World Health Organization (WHO) released data about obesity prevalence in both genders in Arab countries. It showed an increasing prevalence compared to 2010 and it was about 22% in males and 48% in females (WHO, 2014; Alzaman and Ali, 2016). The pathophysiology of obesity is complicated; it is caused by a combination of many factors like excessive food intake, lack of physical activity as sedentary lifestyle, hormonal, environmental factors and...
multi-genetic susceptibility. Excess fat accumulation may lead to a
dysregulation of adipocytes function, that includes increased secretion
of the deleterious adipokines with decreased secretion of advantageous
ones. (Albuquerque et al., 2015; Yazdi et al., 2015).

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder
that affects > 150 millions people worldwide and is estimated to
become 439 millions worldwide in 2030. Its prevalence is expected to
increase exponentially around the world particularly in developing
countries (El-Mesallamy et al., 2011). According to the International
Diabetes Federation Statistics, (2014), the prevalence of diabetes in
Egypt was about 17% (Alzaman and Ali, 2016). Insulin resistance and
inflammation could play a major role in the development of T2DM.
However, the molecular mechanisms that lead to the development of
these diseases are far from complete elucidation (Esteghamati et al.,
2014).

Visceral adipose tissue not only acts as a fat depot, but also appears
to be an active endocrine organ that is involved in many functions. It
has the ability to synthesize and secrete enzymes, hormones, and
adipokines (Dimova and Tankova, 2015). Vaspin, a derived serine
protease inhibitor with insulin-sensitizing effect, is considered as one of
the most important discovered adipokines. It has insulin-sensitizing
effects and some researchers suggested that it could play a role in the
development of obesity, metabolic disorders, T2DM, and could act as an
anti-inflammatory and antiapoptotic mediator (Miyatake et al., 2014).
Evidence linking vaspin to obesity and diabetes has been clouded with
contrasting results and many studies were needed to assess this
relationship (Esteghamati et al., 2014; Dimova and Tankova, 2015).

Vaspin gene locates in chromosome 14q32.13 and consists of 6
exons and 5 introns. One single nucleotide polymorphism (SNP)
(rs2236242) in intron 5 has been found and it was significantly
associated with diabetes and obesity (Wada, 2008; Kempf et al.,
2010). Its mRNA was discovered in visceral white adipose tissue (WAT)
of Otsuka Long-Evans Tokushima fatty rats, an animal model of obesity
with T2DM (Hida et al., 2005). Vaspin expression is specific to
adipocytes in visceral WAT, whereas, it is not detected in stromal
endothelial or vascular cells (Seeger et al., 2008). The link between
vaspin gene polymorphism, obesity and diabetes has been shown in
some studies (Kempf et al., 2010; Hashemia et al., 2012; Kohan et al.,
2014). To the best of our knowledge, this is the first study of vaspin
gene rs2236242 polymorphism in obesity and diabetes in Upper
Egyptian Women. The present study aimed to:

1. Investigate the impact of vaspin rs2236242 gene polymorphism
   on the risk of obesity and diabetes in Upper Egyptian women.

2. Evaluate the role of vaspin as an adipokine on the outcome of
   obesity and the relation to its genetic variants.

2. Subjects and methods

2.1. Subjects

The present study is a case control study which is an extension to
our previous work (El-Deek et al., 2017, under publication). Two
hundred and twenty-four participants were recruited between 2013
and 2014. All were Egyptians, who attended Assiut University Hospital
outpatient clinics for obesity and weight reduction programs along with
volunteers.

Medical history; including age, occupation, family history of
obesity, history of drug intake and onset of obesity; was taken. All
participants were subjected to routine physical examinations.
Measurements of blood pressure (mm Hg), body weight (kg), height
(cm), calculation of BMI (kg/m²), waist circumference (cm), hip
circumference (cm), waist/hip ratio, measurement of fat, water and
muscle percent by bioelectrical impedance scale (Korona KFW 5505
scale, Germany) were carried out by a trained nurse.

Exclusion criteria included individuals with cardiac, hepatic or
renal affections, those with endocrine disorders, pregnancy, lactation,
chronic drug intake and malignancies. 224 participants were fully
enrolled in the study. Obese individuals (112 cases) were those with a
BMI of ≥ 30 kg/m² and non-obese non-diabetic subjects (112 controls)
had a BMI between 18.5 and 24.99 kg/m² according to the definition of
obesity by WHO (Sturm, 2007). The obese subjects were subdivided
into 62 women without and 50 women with diabetes. The diagnosis of
diabetes was according to WHO criteria for fasting plasma glucose
(≥ 126 mg/dL), HbA1C (≥ 6.5%) or previously diagnosed as T2DM
(Puuilai et al., 1999). All participants signed a written informed
consent for participation in the study. The study was approved by the
Faculty of Medicine, Assiut University ethical committee in accordance
with Helsinki declaration (1975).

2.2. Biochemical analysis

Ten ml venous blood samples were obtained after an overnight fast,
and divided into 2 parts. Five milliliters were collected on EDTA for
DNA extraction, and the other 5 ml were left at room temperature for
serum separation which was preserved at −70 °C till the assay of
biochemical markers.

The levels of serum cholesterol, LDL-C, HDL-C, triglycerides, ALT
and fasting glucose were determined using enzyme colorimetric kits
supplied by Biodiagnostics, Egypt. Fasting insulin was determined by
ELISA kit supplied by DRG international instruments inc., Germany.
Homeostasis model assessment of insulin resistance (OMA-IR) was
calculated (Fasting insulin (μIU/mL) X fasting glucose (mmol/L)/22.5
(Mathews et al., 1985). Serum hs-CRP was measured by enzyme
immunoassay kit supplied by Diagnostics Biochem, Canada. Serum
vaspin was determined by enzyme immunoassay kit supplied by Ray
Biotech, USA.

2.3. Genotyping

Genomic DNA was isolated from peripheral whole blood collected
on EDTA using QIAamp DNA mini kit (Qiagen) according to the
manufacturer's instructions. Vaspin SNP rs2236242 T > A was
detected using a tetra – amplification refractory mutation system
polymerase chain reaction (T-ARMS-PCR) described previously by
Hashemia et al. (2012). A 25 μL PCR reaction mixture contained
100 ng DNA, 0.5 μL dNTP (10 mM), 0.75 μL MgCl₂ (50 mM), 1 μL
of each primers (10 pm/μL) and 0.3 U Taq DNA polymerase (5 U/μL)
was used. The primers used for detection of vaspin rs2236242 gene
polymorphism included:

Vaspin F (T allele): 5′-AACAGGGCGCCTTCTGTGAC-3′,
Vaspin R (A allele): 5′-CACAGGAGCCGATAAATTGTG-3′,
F0: 5′-GGAGGAGCAGGCGAGTACTAGAAA-3′ and
R0: 5′ ACCATCTCTCTGGCCTTGAC-3′.

The PCR conditions comprised an initial denaturation cycle at 95 °C
for 5 min, followed by 30 cycles denaturing at 95 °C for 30 s, annealing
at 58 °C for 30 s and extension at 72 °C for 30 s and a final extension
at 72 °C for 10 min. PCR products were electrophoresed on a 1.0% agarose
gel contained 0.5 μg/mL ethidium bromide viewed on BIODOC-it gel
documentation system The PCR product sizes of vaspin rs2236242
polymorphism were 174 bp for T allele, 248 bp for A allele and 378 bp
for control Fig. 1). To verify genotyping quality, the genotyping call was
carried out by two independent personnel, 20 random samples were re-
genotyped, and both showed 100% concordance. Further, 5 samples
were confirmed by DNA-sequencing of which a heterozygous genotype
sample was included with each PCR run as a positive control.

2.4. Statistical analysis

SPSS version 19 was used for data analysis. The results were
expressed as mean ± SD for continuous data or frequencies and
percent for qualitative data. Chi-square and independent student-t-test were used for comparison between the two studied groups. Genotypes and allele distributions were compared between obese and non-obese subjects using Chi-square (X²) test, odds ratio and confidence intervals were also estimated. ANOVA test and Post-hoc test (LSD) was used for comparison between different carriers. Hardy-Weinberg calculation was used to determine whether the observed genotype frequencies for the studied SNP are consistent or not with Hardy-Weinberg equilibrium. Pearson's correlation was used to evaluate the association between different parameters. P < 0.05 is considered significant.

3. Results

3.1. Baseline characteristic

Demographic, anthropometric and biochemical characteristics of 224 participants of the present study are provided in Table 1. There were 112 obese subjects and 112 controls with matched age. Relevant family history and the non-working subjects represented 75.9% and 69.6% of obese subjects respectively. Among obese women about 63.4% were obese since childhood and 44.6% were diabetics. Both systolic and diastolic blood pressure were higher in obese diabetics compared with controls (P < 0.01 for each).

Weight, BMI, waist and hip circumference, W/H ratio and fat % were significantly higher in obese than controls (P < 0.001 each), whereas height, water and muscle % were significantly lower (P < 0.001 each). Concerning biochemical parameters, serum total cholesterol, LDL-C, TG, fasting glucose, HOMA-IR value, ALT, hs-CRP and vaspin were significantly higher in obese than controls (P < 0.01 each). On the other hand, HDL was significantly lower (P < 0.001). Obese diabetics showed significantly higher values of cholesterol, fasting glucose, insulin, HOMA-IR values than obese non diabetic (P < 0.05, P < 0.001 and P < 0.05 respectively) (Table 1).

3.2. Genetic analysis

Vaspin gene rs2236242 variants were analyzed and the results were presented in Table 2. The distribution of the studied SNP genotypes followed the Hardy-Weinberg equilibrium for both the obese subjects and controls. The distribution of TA and AA genotype variants of vaspin gene was 49.1% and 6.3% among obese and 57.1% and 15.2% respectively among controls. As shown in Table 2, the A allele of vaspin rs2236242 was protective against obesity in codominant inheritance model (TT vs TA, OR = 0.494, CI = 0.278–0.877, P = 0.016 and TT vs AA, OR = 0.245, CI = 0.092–0.658, P = 0.005). Moreover this SNP was protective in additive, dominant and recessive inheritance models (TT vs TA vs AA, OR = 0.210, CI = 0.075–0.590, P = 0.004; TT vs TA + AA, OR = 0.442, CI = 0.253–0.770, P = 0.004 and TT-T-T + TA vs AA, OR = 0.373, CI = 0.148–0.938, P = 0.036).

The minor A allele of vaspin rs2236242 gene polymorphism was significantly lower in obese (30.8%) than controls (43.7%) (OR = 0.572, CI = 0.389–0.841, P = 0.005). After adjusting genotypes for diabetes there were no significant association between vaspin rs2236242 gene polymorphism and obesity except in dominant inheritance model (OR = 0.496, CI = 0.259–0.951, P = 0.039) (Table 2).

The genotype and allele frequencies among obese diabetics and controls are shown in Table 3. The minor A allele was lower in obese diabetics (27%) than control (43%) (OR = 0.475, CI = 0.284–0.795, P = 0.005). The distribution of TA and AA were 46% and 4% among obese diabetic subjects. The codominant inheritance models, (TT vs TA, OR = 0.445, CI = 0.219–0.906, P = 0.025 and TT vs AA, OR = 0.145, CI = 0.031–0.692, P = 0.015) and additive, dominant and recessive tested inheritance models (TT vs TA vs AA, OR = 0.146, CI = 0.031–0.692, P = 0.013; TT vs TA + AA, OR = 0.382, CI = 0.192–0.764, P = 0.007 and TT + TA vs AA, OR = 0.232, CI = 0.052–0.959, P = 0.053) showed significant association with diabetes. Vaspin gene polymorphism plays a protective role in obesity but this relation is largely ascribed to its effect on insulin resistance.

The genotype and allele frequencies among obese non diabetics and controls are shown in Table 4. The minor A allele was lower in obese non diabetics (33.9%) than control (43.7%) (OR = 0.572, CI = 0.389–0.841, P = 0.005). The distribution of VT-A and AA were 46% and 4% among obese non diabetic subjects. The codominant, additive, dominant and recessive tested inheritance models showed non-significant association with obese non diabetics.

3.3. Association of vaspin gene polymorphism and obesity indices

The relation between the different genotype variants of vaspin, anthropometric and biochemical parameters among obese subjects were shown in Table 5. Comparing the carriers of A allele in both heterozygous (TA) and homozygous (AA) form with TT carriers, they
showed difference as regards all the studied parameters which statistically significant for hip circumference, fat, water and muscle % (P < 0.05 each). Regarding the serum levels of hs-CRP and vaspin, both TA and AA carriers showed significant association with these biochemical parameters compared to TT carriers (P < 0.01 each). In addition, AA carriers showed lower vaspin levels compared with TA carriers (P < 0.001). The strongest associations were observed with serum vaspin and hs-CRP under additive and recessive models. In

### Table 1
Demographic, anthropometric and biochemical characteristics of controls, obese subjects and obese subgroups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n = 112)</th>
<th>Obese patients (n = 112)</th>
<th>Obese non-diabetics (n = 62)</th>
<th>Obese diabetics (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.80 ± 9.60</td>
<td>39.52 ± 9.43</td>
<td>38.95 ± 8.63</td>
<td>43.94 ± 4.38</td>
</tr>
<tr>
<td>Family history of obesity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19 (16.9%)</td>
<td>85 (75.9%)*</td>
<td>43 (69.4%)*</td>
<td>42 (84%)*</td>
</tr>
<tr>
<td>No</td>
<td>93 (83.1%)</td>
<td>27 (24.1%)*</td>
<td>19 (30.6%)*</td>
<td>8 (16%)*</td>
</tr>
<tr>
<td>Onset of obesity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Since childhood</td>
<td>71 (63.4%)</td>
<td>34 (54.8%)</td>
<td>37 (74%)</td>
<td>13 (26%)</td>
</tr>
<tr>
<td>Within adulthood</td>
<td>41 (36.6%)</td>
<td>28(45.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Systolic</td>
<td>118.93 ± 11.65</td>
<td>123.08 ± 17.90</td>
<td>118.15 ± 11.70*</td>
<td>129.20 ± 19.36*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75.31 ± 8.55</td>
<td>79.10 ± 17.90</td>
<td>77.26 ± 11.70*</td>
<td>76.77 ± 10.96</td>
</tr>
<tr>
<td><strong>Anthropometric parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>61.96 ± 11.51</td>
<td>91.08 ± 14.30*</td>
<td>93.20 ± 13.98*</td>
<td>88.45 ± 14.39*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.56 ± 9.44</td>
<td>158.74 ± 7.62*</td>
<td>158.98 ± 5.82*</td>
<td>158.44 ± 9.45*</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>22.23 ± 2.55</td>
<td>36.50 ± 5.72*</td>
<td>37.33 ± 5.74*</td>
<td>35.47 ± 5.57*</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>65.36 ± 7.19</td>
<td>101.74 ± 12.66*</td>
<td>102.26 ± 12.62*</td>
<td>101.08 ± 12.81*</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>82.13 ± 6.58</td>
<td>117.51 ± 13.16*</td>
<td>116.66 ± 11.76*</td>
<td>118.56 ± 14.76*</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>0.80 ± 0.11</td>
<td>0.87 ± 0.07*</td>
<td>0.88 ± 0.07*</td>
<td>0.86 ± 0.08*</td>
</tr>
<tr>
<td><strong>Differential body weight</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fat %</td>
<td>24.52 ± 4.50</td>
<td>43.03 ± 8.04*</td>
<td>43.53 ± 7.54*</td>
<td>42.42 ± 8.65*</td>
</tr>
<tr>
<td>Water %</td>
<td>49.51 ± 9.90</td>
<td>37.23 ± 3.60*</td>
<td>36.91 ± 3.21*</td>
<td>37.63 ± 4.03*</td>
</tr>
<tr>
<td>Muscle %</td>
<td>31.12 ± 6.43</td>
<td>23.40 ± 2.29*</td>
<td>23.42 ± 2.13*</td>
<td>23.62 ± 2.47*</td>
</tr>
<tr>
<td>Serum hs-CRP (ng/ml)</td>
<td>1.93 ± 1.61</td>
<td>4.08 ± 1.4*</td>
<td>3.95 ± 1.66*</td>
<td>4.35 ± 0.77*</td>
</tr>
<tr>
<td>Serum Vaspin (pg/ml)</td>
<td>406.65 ± 254.89</td>
<td>1304.63 ± 476.01*</td>
<td>1254.49 ± 516.27*</td>
<td>1359.19 ± 428.94*</td>
</tr>
<tr>
<td><strong>Lipid profile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>153.04 ± 37.74</td>
<td>144.20 ± 51.88*</td>
<td>173.32 ± 53.09*</td>
<td>197.59 ± 47.45*#</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>93.01 ± 40.77</td>
<td>140.60 ± 52.41*</td>
<td>140.91 ± 51.07*</td>
<td>140.23 ± 54.57*</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>46.43 ± 16.37</td>
<td>37.35 ± 15.25*</td>
<td>39.19 ± 8.75*</td>
<td>35.07 ± 8.37*</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>111.49 ± 35.93</td>
<td>156.28 ± 36.61*</td>
<td>147.44 ± 37.69*</td>
<td>167.23 ± 40.33*</td>
</tr>
<tr>
<td><strong>Insulin sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>96.63 ± 17.60</td>
<td>140.19 ± 65.26*</td>
<td>98.79 ± 15.58</td>
<td>191.53 ± 66.99*#</td>
</tr>
<tr>
<td>Serum insulin (µU/ml)</td>
<td>9.46 ± 5.51</td>
<td>11.71 ± 6.28</td>
<td>10.37 ± 5.72</td>
<td>14.48 ± 6.63*#</td>
</tr>
<tr>
<td>HOMA-IR values</td>
<td>2.93 ± 1.01</td>
<td>4.29 ± 3.64*</td>
<td>3.64 ± 1.46</td>
<td>7.69 ± 4.41*#</td>
</tr>
<tr>
<td>ALT (U/ml)</td>
<td>44.62 ± 9.44</td>
<td>51.77 ± 12.39*</td>
<td>50.57 ± 12.54*</td>
<td>53.28 ± 12.15*</td>
</tr>
</tbody>
</table>

Data represented as mean ± SD.  
P value < 0.05 is considered statistically significant.  
* Significant difference versus controls at P < 0.05.  
# Significant difference of obese with DM versus obese without DM at P < 0.05.

### Table 2
Genotyping and alleles frequencies distribution of vaspin rs2236242 gene polymorphism in controls and obese patients.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n = 112)</th>
<th>Obese (n = 112)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>50 (44.6%)</td>
<td>90 (81.8%)</td>
<td>0.494 (0.278-0.877)</td>
<td>0.016*</td>
<td>0.538 (0.274-1.056)</td>
<td>0.072</td>
</tr>
<tr>
<td>TA</td>
<td>55 (49.1%)</td>
<td>62 (55.4%)</td>
<td>0.245 (0.092-0.658)</td>
<td>0.005*</td>
<td>0.338 (0.110-0.938)</td>
<td>0.058</td>
</tr>
<tr>
<td>AA</td>
<td>7 (6.3%)</td>
<td>7 (6.3%)</td>
<td>0.210 (0.075-0.590)</td>
<td>0.004*</td>
<td>0.270 (0.081-0.901)</td>
<td>0.055</td>
</tr>
</tbody>
</table>

Data is represented as number and %.  
OR: odds ratio, CI: confidence interval.  
* Adjusted for diabetes.  
** P < 0.05 is considered statistically significant.  

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adipogenic cytokines that are involved in obesity process, T2DM and insulin resistance (Zhuang et al., 2009). The visceral adipose tissue-derived serine protease inhibitor (vaspin) is a member of adipocytokines with an insulin-sensitizing activity but the evidence linking it to obesity and metabolic syndrome has been clouded with contrasting results (Brunetti et al., 2011; Esteghamati et al., 2014).

The results of the present study showed elevated serum levels of vaspin in diabetic and non-diabetic subjects compared to the controls. These results are in agreement with Ye et al. (2009); Li et al. (2011); Han et al. (2013); Feng et al. (2014); Karbek et al. (2014); Kameshima et al. (2016). They reported significant increase in serum vaspin levels in obese diabetic and non-diabetics that are positively correlated with the class of obesity, insulin resistance and total body fat percentage.

In addition, Klöting et al. (2006) found that vaspin mRNA expression was not detected in lean subjects (BMI<25) and detected in patients with T2DM and it was significantly correlated with BMI, % body fat and plasma glucose. Moreover, Esteghamati et al. (2014); Kameshima et al. (2016) found that subjects with metabolic syndrome had higher concentrations of vaspin which significantly predicted the raised fasting plasma glucose, triglycerides, waist circumference and the reduced HDL-C. Dimova and Tankova. (2015) suggested that vaspin could be regarded as a new link between obesity and glucose intolerance which might enable the development of treatment strategies targeting metabolic and glucose tolerance disorders.

Genetic predisposition is implicated in the etiology of obesity and diabetes. Kempf et al. (2010), investigated the vaspin gene and found one SNP (rs2236242) in intron 5, having significant association with diabetes and obesity. Intronic SNPs probably represent a large mutational target that have a multiplicity of functional elements including splicing efficiency, the stability of the transcribed mRNA, enhancers and silencers activity (Hashemia et al., 2012). Although the link between vaspin gene polymorphism, obesity and diabetes has been shown in some studies (Kempf et al., 2010; Hashemia et al., 2012; Kohan et al., 2014), the precise mechanisms through which vaspin rs2236242 polymorphism exerts its effect on vaspin gene expression, vaspin secretion, and development of obesity and diabetes remain unclear.

In the present study, we evaluated the impact of vaspin rs2236242 gene polymorphism on the risk of obesity and diabetes in a sample of Upper Egyptian women. The data of the present study revealed that the minor (A) allele of vaspin rs2236242 gene polymorphism was significantly lower in obese women than controls. It was protective against obesity in codominant, additive, dominant and recessive inheritance models in obese and obese diabetic subjects. After adjusting genotypes for diabetes there were no significant association between vaspin rs2236242 gene polymorphism and obesity indicating that this gene polymorphism plays a protective role in obesity but this relation is largely ascribed to its effect on insulin resistance.

3.4. Association of vaspin gene polymorphism and serum vaspin levels

The serum vaspin levels were found to be lower in minor protective allele carriers (AA) than homozygous (TT) and heterozygous (TA) allele carriers (P < 0.001 each). Also vaspin levels were lower in subjects with TA carriers than TT carriers (P < 0.01). When we use the dominant model (TT versus TA + AA), the serum levels of vaspin were still lower in protective minor allele carriers (TA + AA) than in homozygous major allele carriers (TT) (P < 0.05) (Figs. 2 and 3).

Significant positive correlations were found between serum vaspin and each of BMI, waist, hip circumference, and fat % (P < 0.001 each). Also there were significant negative correlations between vaspin and each of water and muscle % (P < 0.001 each). Regarding the metabolic parameters, there was significant positive correlations between vaspin levels and each of hs-CRP, cholesterol, LDL-C, fasting glucose, HOMA-IR, insulin, and ALT (P < 0.001, P < 0.05, P < 0.05, P < 0.01, P < 0.001, P < 0.01 and P < 0.01 respectively) and a negative correlation with HDL-C (P < 0.01) in obese women (Figs. 4 and 5).

4. Discussion

Adipose tissue is an active endocrine organ which secretes several adipocytokines that are involved in metabolic and glucose tolerance disorders.

Table 3

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n = 112)</th>
<th>Obese diabetics (n = 56)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>31 (27.7%)</td>
<td>25 (50.0%)</td>
<td>0.445 (0.219–0.906)</td>
<td>0.025*</td>
</tr>
<tr>
<td>TA</td>
<td>64 (57.1%)</td>
<td>23 (46.9%)</td>
<td>0.145 (0.031–0.692)</td>
<td>0.015*</td>
</tr>
<tr>
<td>AA</td>
<td>17 (15.2%)</td>
<td>2 (4.0%)</td>
<td>0.146 (0.031–0.692)</td>
<td>0.013*</td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>recessive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT + TA</td>
<td>95 (84.8%)</td>
<td>48 (96.0%)</td>
<td>0.232 (0.052–0.959)</td>
<td>0.053*</td>
</tr>
<tr>
<td>TA</td>
<td>17 (15.2%)</td>
<td>2 (4.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>31 (27.7%)</td>
<td>25 (50.0%)</td>
<td>0.382 (0.192–0.764)</td>
<td>0.007*</td>
</tr>
<tr>
<td>TA + AA</td>
<td>81 (72.3%)</td>
<td>27 (50.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>126 (56.3%)</td>
<td>13 (27.0%)</td>
<td>0.475 (0.284–0.795)</td>
<td>0.005*</td>
</tr>
<tr>
<td>A</td>
<td>98 (43.7%)</td>
<td>42 (33.9%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data is represented as number and %. OR: odds ratio, CI: confidence interval.
* P < 0.05 is considered statistically significant.

Table 4

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n = 112)</th>
<th>Obese non diabetics (n = 62)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>31 (27.7%)</td>
<td>25 (40.3%)</td>
<td>0.620</td>
<td>0.166</td>
</tr>
<tr>
<td>TA</td>
<td>64 (57.1%)</td>
<td>32 (51.6%)</td>
<td>0.365</td>
<td>0.079</td>
</tr>
<tr>
<td>AA</td>
<td>17 (15.2%)</td>
<td>5 (8.1%)</td>
<td>0.270</td>
<td>0.58</td>
</tr>
<tr>
<td>Additive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>recessive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT + TA</td>
<td>95 (84.8%)</td>
<td>57 (91.9%)</td>
<td>0.490</td>
<td>0.183</td>
</tr>
<tr>
<td>AA</td>
<td>17 (15.2%)</td>
<td>5 (8.1%)</td>
<td>0.172–1.401</td>
<td>0.813</td>
</tr>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>31 (27.7%)</td>
<td>25 (40.3%)</td>
<td>0.566</td>
<td>0.088</td>
</tr>
<tr>
<td>TA + AA</td>
<td>81 (72.3%)</td>
<td>37 (59.7%)</td>
<td>0.294–1.090</td>
<td>0.043</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>126 (56.3%)</td>
<td>82 (66.1%)</td>
<td>0.659</td>
<td>0.072</td>
</tr>
<tr>
<td>A</td>
<td>98 (43.7%)</td>
<td>42 (33.9%)</td>
<td>0.417–1.039</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Data is represented as number and %. P < 0.05 is considered statistically significant.
OR: odds ratio, CI: confidence interval.
the other hand, Kempf et al. (2010) found a significant association of vaspin SNP rs2236242 T/A with T2DM but in contrary to our study they found that the AA genotype carriers had an increased diabetic risk compared with the TT genotype carriers. They reported the vaspin rs2236242 polymorphism in the 5′ to 3′ direction of the vaspin gene (reverse gene).

The vaspin serum levels were found to be lower in minor protective (AA) than homozygous (TT) and heterozygous (TA) genotype carriers. Alnory et al. (2014) investigated the association of vaspin rs2236242 polymorphism and its serum levels with the risk of developing metabolic syndrome. They concluded that, serum vaspin can be used as a diagnostic marker for metabolic syndrome while the different genotypes were not associated with different serum vaspin levels. In addition, Kohan et al. (2014) reported a significant association between vaspin rs2236242 SNP and polycystic ovary syndrome but this relation was affected by obesity status and BMI.

In contrast, Breitfeld et al. (2013) showed that the variability in serum vaspin concentration might be explained by its genetic variants. They studied another vaspin SNP variant (rs11160190) that showed a strongest association with circulating vaspin. It was in the 5′upstream of vaspin gene that may be involved in the transcriptional regulation of the gene which may explain the causality of this state. Also, Teshigawara et al. (2012) found that vaspin polymorphism could genetically define a distinct group with high levels of serum vaspin.

Table 5
The relationship between different genotype variants of vaspin rs2236242 gene and the anthropometric and biochemical data in obese subjects.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Carriers of TT (n = 52)</th>
<th>Carriers of TA (n = 53)</th>
<th>Carriers of AA (n = 7)</th>
<th>Additive P-value</th>
<th>Recessive P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>93.94 ± 16.10</td>
<td>88.16 ± 12.40</td>
<td>92.47 ± 9.31</td>
<td>0.229</td>
<td>0.556</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.52 ± 7.67</td>
<td>158.28 ± 7.80</td>
<td>156.17 ± 5.42</td>
<td>0.563</td>
<td>0.368</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>37.22 ± 6.41</td>
<td>35.61 ± 4.91</td>
<td>38.29 ± 5.67</td>
<td>0.335</td>
<td>0.362</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>103.16 ± 13.71</td>
<td>101.06 ± 11.93</td>
<td>95.50 ± 7.64</td>
<td>0.416</td>
<td>0.301</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>121.02 ± 12.48</td>
<td>115.26 ± 13.28*</td>
<td>107.33 ± 8.87*</td>
<td>0.006$</td>
<td>0.039§</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>0.85 ± 0.07</td>
<td>0.88 ± 0.07*</td>
<td>0.89 ± 0.07</td>
<td>0.064</td>
<td>0.506</td>
</tr>
<tr>
<td><strong>Differential body weight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat %</td>
<td>37.31 ± 11.35</td>
<td>31.87 ± 10.47*</td>
<td>30.79 ± 12.73*</td>
<td>0.017$</td>
<td>0.107</td>
</tr>
<tr>
<td>Water %</td>
<td>40.91 ± 8.16</td>
<td>44.50 ± 9.75*</td>
<td>46.46 ± 12.29*</td>
<td>0.012$</td>
<td>0.069</td>
</tr>
<tr>
<td>Muscle %</td>
<td>25.58 ± 5.18</td>
<td>27.99 ± 6.29*</td>
<td>29.56 ± 7.56*</td>
<td>0.012$</td>
<td>0.071</td>
</tr>
<tr>
<td>Serum hs-CRP (ng/ml)</td>
<td>5.01 ± 0.63</td>
<td>3.81 ± 1.43*</td>
<td>2.19 ± 1.65*</td>
<td>0.000$</td>
<td>0.017§</td>
</tr>
<tr>
<td>Serum Vaspin (pg/ml)</td>
<td>1627.30 ± 306.05</td>
<td>1192.78 ± 337.13*</td>
<td>343.68 ± 188.99* #</td>
<td>0.000$</td>
<td>0.000§</td>
</tr>
<tr>
<td><strong>Lipid profile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>186.94 ± 52.96</td>
<td>183.31 ± 49.52</td>
<td>168.60 ± 69.05</td>
<td>0.776</td>
<td>0.502</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>135.62 ± 52.99</td>
<td>148.11 ± 49.48</td>
<td>116.15 ± 69.29</td>
<td>0.119</td>
<td>0.134</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>33.30 ± 9.27</td>
<td>40.66 ± 13.48</td>
<td>42.72 ± 19.95</td>
<td>0.238</td>
<td>0.791</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>156.56 ± 53.51</td>
<td>155.51 ± 43.93</td>
<td>106.55 ± 23.98</td>
<td>0.456</td>
<td>0.355</td>
</tr>
<tr>
<td><strong>Insulin sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>143.33 ± 62.78</td>
<td>138.86 ± 69.91</td>
<td>126.13 ± 43.88</td>
<td>0.520</td>
<td>0.698</td>
</tr>
<tr>
<td>Serum insulin (μU/ml)</td>
<td>12.36 ± 5.71</td>
<td>11.46 ± 6.86</td>
<td>10.60 ± 3.22</td>
<td>0.746</td>
<td>0.783</td>
</tr>
<tr>
<td>HOMA-IR values</td>
<td>4.65 ± 2.74</td>
<td>3.86 ± 3.19</td>
<td>2.56 ± 0.79</td>
<td>0.409</td>
<td>0.319</td>
</tr>
<tr>
<td>ALT (u/ml)</td>
<td>52.55 ± 12.02</td>
<td>51.03 ± 12.87</td>
<td>51.78 ± 12.88</td>
<td>0.651</td>
<td>0.766</td>
</tr>
</tbody>
</table>

Data is represented as mean ± SD.

* Indicates significant differences of TA and AA vs. TT carriers at P < 0.05.
$ Indicates significant differences of AA vs. TA carriers at P < 0.05.
§ Indicates significant differences between TT vs. TA vs. AA at P < 0.05.

Fig. 2. The mean values of serum vaspin levels according to the vaspin rs2236242 genotypes.
Japanese population. Consequently, the results of different researches are contradictory. This could be due to the interactions between the vaspin rs2236242 gene polymorphism with other polymorphisms of the gene, the different ethnicities of the studied groups, the sample size of the population, diet or physical activity which can modify genetic effects (Alnory et al., 2014).

The results of the present study showed significant association between different variants of vaspin gene and hip circumference, fat%, serum hs-CRP and vaspin. Both TA and AA carriers showed significant lower levels of these parameters compared to TT carriers. Also, there were a significant positive correlations between serum vaspin and each of BMI, waist, hip circumference, fat %, hs-CRP, cholesterol, LDL-C, fasting glucose, HOMA-IR values, insulin, and ALT in obese subjects. On the other hand, there were also significant negative correlations with water %, muscle % and HDL-C in the same group.

These results are in accordance with Gulcelik et al. (2009); Handisurya et al. (2010); Li et al. (2011); Han et al. (2013) who showed increased circulating vaspin levels in obese and diabetic subjects which were positively correlated with insulin resistance. They suggested that, vaspin could increase insulin sensitivity and improve glucose tolerance in obese and diabetic patients. Han et al. (2013) in a cohort study of young Korean, found significant positive correlations of serum vaspin with the grades of obesity and insulin resistance. Collectively, it is suggested that vaspin may be one of the beneficial adipocytokines, that in addition to its compensatory mechanism for improving insulin sensitivity, had protective roles against type II diabetes. Our results could support the results of Tan et al. (2008); Karbek et al. (2014) who reported that obesity itself or obesity-related insulin resistance may increase the serum vaspin concentrations, which are found to be positively correlated with insulin resistance and can be decreased by loss of body weight and high fitness. On the other hand, Seeger et al. (2008) could not detect significant correlations between vaspin-mRNA expression and percentage of body fat, BMI and glucose in lean subjects with BMI < 25.

These results reflect the compensatory action of the body to...
decrease the insulin resistance of the fatty tissue but these trials have failed in diabetics because of the limited ability of β cells to produce the appropriate amount or the normal forms of insulin, as a result, the high concentrations of insulin can’t give the desired response and subsequently glucose is increased (Saad et al., 2013).

However, Von-Loeffelholz et al. (2010); Briana et al. (2011) showed that there is no association between serum vaspin, insulin levels and HOMA-IR in non-diabetic human. Also, Auguet et al. (2011) reported that serum vaspin levels did not correlate with BMI, markers of glucose or lipid metabolism in normal persons.

The current investigation showed that hs-cRP was higher in the serum of obese compared to non-obese subjects. These finding were in agreement with Faucher et al. (2012); Fronczyk et al. (2014); Patel et al. (2015) who found that, obese subjects were presented with higher hs-cRP levels than overweight and normal subjects. The increased CRP levels suggest that obesity may be a marker of chronic immune system stimulation and this could explain how obesity potentiated the risk for several diseases including diabetes and cancer (Power et al., 2007). On the contrary, Karadag et al. (2016) found no correlation of vaspin with hs-cRP in patients with diabetic nephropathy. In addition, the significant negative correlations between serum hs-cRP and HDL-C may indicate the role of obesity – subclinical inflammation in the development of atherosclerosis and CVD. However, vaspin could protect endothelial cells via an inhibitory effect on NF-κB (Liu et al., 2014).

Interestingly, the current study reported the significant positive correlation between serum hs-cRP, the low grade inflammatory marker and vaspin level indicating that obesity is a consequent process of a low grade inflammatory state which stimulates some anti-inflammatory legends like vaspin to combat the existence of the pro-inflammatory mediators and adipocytokines dysregulation. It could give another indication that serum vaspin is a good adipocytokine that has anti-inflammatory effects as it might suppress the production of the pro-inflammatory markers as TNFα, leptin and resistin (Karbek et al., 2014; Dimova and Tonkova, 2015).

Future similar studies should use a larger number of patients to improve the statistical power and obtain more confident results. Long term follow up studies of vaspin rs2236242 T/A SNP in obese and diabetic patients are important to improve its predictive ability for these diseases and hence clinical management. This will provide a basis that will assist prevention or developing novel therapeutic agents for obesity future therapy.

5. Conclusion

To the best of our knowledge, this is the first study of vaspin SNP in Upper Egyptian women. This study revealed the association between vaspin rs2236242 polymorphism, obesity and type 2 Diabetes mellitus, where the minor A allele of vaspin rs2236242 SNP play a protective role against obesity and diabetes. It is strongly associated with several measures of adiposity such as BMI, weight, hip and waist circumference but this relation is largely ascribed to its effect on insulin resistance. The serum vaspin concentration was found to be lower in minor protective (A) allele carriers. The entire understanding of vaspin intimate mechanism of action might enable the development of novel etiology-based treatment strategies for obesity which is a complex genetic trait.

Conflict of interest

The authors report no conflict of interest.

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