

Association between Serum Visfatin, Oxidative Stress, Inflammation and Metabolic Syndrome in Obese Premenopausal Women

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ABSTRACT

Introduction: Visfatin is an adipokine secreted by visceral fat tissue that has important effects on normal insulin secretion. An association has been found between visfatin and cardiovascular risk factors and obesity. Despite its role in the production of inflammatory cytokines, the role of visfatin in the Metabolic Syndrome (MS) is still a matter of debate.

Aim: To evaluate the relation between serum visfatin levels, oxidative stress markers, Interleukin-6 (IL-6) and (MS) in premenopausal obese women.

Materials and Methods: The study enrolled 150 premenopausal obese women and 80 age-matched healthy non-obese controls. Serum visfatin and IL-6 levels were measured by Enzyme linked Immunosorbent Assay (ELISA). Oxidative status was assessed by Total Antioxidant Capacity (TAC), Malondialdehyde (MDA), vitamins A and E and DNA damage was assessed by Comet assay.

Results: Obese MS patients had significantly higher levels of visfatin, blood pressure levels, MDA and lower levels of TAC, A and E vitamins than the control group. DNA damage was significantly higher in obese women with MS and with a history of recurrent pre-eclampsia than controls. Multiple stepwise regressions analysis showed that log visfatin was positively correlated with MS components, IL-6 and MDA, serum lipids, DNA damage and negatively correlated with TAC and vitamin A and E. The log visfatin level of 1.56 had 76% sensitivity and 71% specificity for detecting MS in obese women with the area under the curve of 0.91.

Conclusion: Significant positive correlations were observed between visfatin levels, oxidative and inflammatory markers with metabolic disturbance and pregnancy complications. These findings might emphasise the pathophysiological roles of these biomarkers in MS and pre-eclampsia and it may be useful to give treat to women in early pregnancy.

Keywords: DNA damage, Inflammatory markers, Obesity, Oxidative stress

INTRODUCTION

Visfatin is a 52 kDa multifaceted molecule, which was detected and recognised as a new adipocytokine by Fukuhara A et al., in 2005 [1]. The name “visfatin” is used to refer to this protein as it is present in visceral fat and produced by adipose tissue. It is also known as Pre-B-cell Colony-Enhancing Factor (PBEF) or Nicotinamide Phosphoribosyl Transferase (NAMPT). Visfatin is originally produced by visceral fat tissue in obese animals and humans [1,2]. It is associated with obesity and with Type 2 Diabetes Mellitus (T2DM) [3] and has an important role in pancreatic β -cell function [4] and in the production of inflammatory cytokines [5]. NAMPT is the rate-limiting enzyme in the retrieve pathway of Nicotinamide Adenine Dinucleotide (NAD) biosynthesis from nicotinamide. As visfatin is regulating the NAD metabolism so, it might control fundamental cellular processes [6]. It also activates insulin receptors and has insulin-mimetic effects [7]. Obesity is widely heterogeneous and includes >30% of the obese population are metabolically healthy [8]. The pathogenesis of MS in adults is complex. Therefore, the link between circulating level of visfatin and MS is difficult to establish. However, several studies reporting that MS patients have higher concentrations of visfatin than normal subjects [9,10]. Insulin resistance leads to MS, which is activated by inflammatory cytokines such as IL-6 [11]. Adipose tissue behaving as an endocrine organ produce adipocytokines [12]. As visfatin is secreted by visceral fats, it has been assumed that its levels are associated with the amount of visceral fats. However, no consistent results have been obtained yet [13,14].

Several different studies reported the relationship between obesity and increased serum levels of oxidative stress markers. More cell damage in obese cases was associated with oxidative stress due to the increased oxygen utilisation and consequent radical formation through mitochondrial respiration, [15]. Some authors reported that the antioxidant enzymes activity was increased in obesity [16], while others found lower antioxidant enzymes activity in obese subjects compared to control [17]. Vitamin E is a lipid-soluble vitamin with lipid antioxidant properties [18]. Serum levels of α -tocopherol <11.6 μ mol/L is defined as vitamin E deficiency [19]. It has been studied that vitamin E deficiency is associated with reduced insulin secretion and antioxidant capacity [20]. Hence, there is a possibility that vitamin E might affect the glucose metabolism independent of its antioxidant mechanism [21]. Likewise, vitamin A is involved in numerous metabolic processes in addition to its well-known role as an antioxidant [22]. Malondialdehyde (MDA) is formed during oxidation process of Polyunsaturated Fatty Acids (PUFA) by ROS [24]. Moreover, visfatin might promote premature endothelial cell death and stimulate DNA damage.

The purpose of this study was to assess the relationship between serum visfatin levels, oxidative stress markers, inflammatory marker (IL-6) and MS components in obese premenopausal women. In addition, assess the potential of the serum visfatin as a predictive marker of the MS in obese women and investigate its role in DNA damage and pregnancy complications.

MATERIALS AND METHODS

Study Population

Sample size: This study is a cross-sectional study. Sample size was calculated based on the estimated prevalence of the disorder, population size and the confidence level was 1.96, which corresponds to a 95% confidence interval.

Inclusion criteria: The study included 25-35 years old, 150 premenopausal obese women and 80 non-obese healthy controls (regardless of the marital status). The obese patients were further divided into two groups: 75 with MS and 75 without MS. Obese women were patients at the obesity clinic at the National Research Centre (NRC), Egypt. MS was defined as having three or more criteria according to the modified NCEP ATP III definition [25].

Exclusion criteria: Authors excluded the women with history of cardiovascular disease or diabetes, hypothyroidism, pregnancy or under any medication known to affect glucose levels, insulin secretion, or insulin sensitivity and smoking.

Ethical Approval

The research was approved by the Ethical Committee of NRC (No: 16361) and followed the World Medical Association's Declaration of Helsinki. Furthermore, each participant in the study signed a written consent after a full description of the study.

Anthropometric Measurements

Anthropometric parameters including calculated Body Mass Index (BMI) as weight in kilograms divided by height in meters square (kg/m^2) and Waist Circumference (WC) have been performed. Blood pressure was precisely measured as well.

Biochemical Measurements

The blood samples were collected after overnight fasting and stored at 80°C until further analysis. Enzymatic colourimetric analysis was carried out using Hitachi auto-analyser 704 (Roche Diagnostics Switzerland). Fasting plasma glucose, serum insulin concentration and serum lipids have been measured as per protocol previously described by Zaki M et al. [26].

Insulin resistance was estimated using Homeostatic Model Insulin Resistance (HOMA-IR) [27]. Enzyme linked immunosorbent assay (ELISA) kits provided by Glory Science were used to measure serum visfatin and IL-6 levels. The level of MDA, as an indicator of the lipid peroxidation, was determined by measuring Thiobarbituric Acid Reactive Species (TBARS) [28].

The TAC was measured in plasma spectrophotometrically. Serum TAC levels were determined using an automated measurement method that is based on the bleaching of the distinctive colour of 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Beckman Coulter-Fullerton, CA, USA) [29]. The results were recorded in mmol Trolox equivalents/L. Vitamins A and E analyses were carried out using high performance liquid chromatography (HPLC) [29].

Determination of DNA Damage by Comet Assay

The comet assay was performed on blood samples of MS cases and healthy controls. The assay was performed following the previous method described in Blasiak J et al., [30]. As per Zakiet M et al., protocol, the assay included three major steps of analysis include peripheral blood leucocytes preparation followed by preparation of cell microgels on slides and finally visualisation and the analysis of the comet slides [31]. The present authors aimed of this study to assess the DNA damage in obese women with MS. Methods; 30 obese women with MS aged 25-35 years and thirty age-matched healthy non-obese women were enrolled in the study. Leukocyte DNA damage was assessed by comet assay. Results Among the obese women with MS, 20% of cases met criteria for

Polycystic Ovary Syndrome (PCOS). The slides were examined at 400X magnification using a fluorescence microscope equipped with an excitation filter of 549 nm and a barrier filter of 590 nm. Evaluation of the degree of damage was done visually by scoring of the comet images. Duplicate slides were prepared for each case and one hundred cells were examined per case.

STATISTICAL ANALYSIS

The statistical analysis was done using the Statistical Package for the Social Sciences (SPSS) Version 16.0 for Windows (SPSS Inc). The normality was calculated by Kolmogorov-Smirnov test and the results were represented as Mean \pm SD. The distribution data of serum visfatin was skewed and logarithmically transformed values were used for all analyses. Mann-Whitney U-test was used in the analysis of DNA damage. In order to test the differences in baseline characteristics between the three groups (MS+, MS-, controls) one-way analysis of variance (ANOVA) has been used then, post-hoc tests were applied.

Multiple regression analysis by using stepwise linear regression with log visfatin as the dependent variable and metabolic, oxidative and inflammation parameters as the independent variables analysis was performed to assess the relationship between them in premenopausal obese women. The efficacy of the visfatin in distinguishing groups of cases with MS versus cases without was assessed by using Receiver Operating Characteristic (ROC) analysis. p-values <0.05 were considered to be statistically significant for all analyses.

RESULTS

Significant differences ($p<0.05$) in biochemical and anthropometric parameters and visfatin levels between the three groups (with MS, without MS, controls) were observed. Results revealed that MS patients had significantly higher levels of visfatin, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Fasting Blood Glucose (FBG), HOMA-IR, TC, TG, Low-Density Lipoprotein-Cholesterol (LDL-L), MDA and lower levels of TAC, vitamins A

| Characteristics | Obese With MS | Without MS | Control | p-value ^a |
|---|---------------------|-------------------|-------------------|----------------------|
| Age (years) | 31.8 \pm 4.5 | 31.3 \pm 3.5 | 32.3 \pm 4.3 | 0.67 |
| BMI (kg/m^2) | 33.24 \pm 5.5* | 28.24 \pm 4.2§ | 21.24 \pm 3.2 | <0.05 |
| WC (cm) | 96.2 \pm 8.15* | 91.7 \pm 5.2§ | 80.7 \pm 4.2 | <0.01 |
| SBP (mmHg) | 135.8 \pm 10.7* | 132.3 \pm 8.4 | 100.3 \pm 6.2 | <0.01 |
| DBP (mmHg) | 85.8 \pm 7.9* | 70.21 \pm 7.6 | 71.24 \pm 8.8 | <0.01 |
| HOMA-IR | 6.7 \pm 1.2* | 3.8 \pm 1.4 | 2.8 \pm 1.3 | <0.01 |
| FBG (mg/dL) | 103.4 \pm 14.7 | 99.0 \pm 10.9 | 97.0 \pm 8.9 | <0.01 |
| TC (mg/dL) | 259.3 \pm 16.2* | 214.6 \pm 10.01 | 199.6 \pm 9.8 | <0.01 |
| TG (mg/dL) | 225.8 \pm 23.34* | 215.2 \pm 19.32 | 115.2 \pm 16.34 | <0.01 |
| HDL-C (mg/dL) | 65.6 \pm 4.9* | 48.84 \pm 11.7 | 43.84 \pm 10.1 | <0.01 |
| LDL-C (mg/dL) | 163.89 \pm 25.88* | 153 \pm 20.88 | 119.1 \pm 18.34 | <0.01 |
| Log visfatin (ng/mL) | 1.56 \pm 0.69*# | 1.40 \pm 0.51 | 0.92 \pm 0.41 | <0.01 |
| TAC (mmol/L) | 1.62 \pm 0.25*# | 1.82 \pm 0.23 | 1.92 \pm 0.28 | <0.01 |
| MDA ($\mu\text{mol}/\text{L}$) | 7.65 \pm 1.33*# | 4.39 \pm 0.12 | 3.15 \pm 0.25 | <0.01 |
| Serum vitamin A ($\mu\text{mol}/\text{L}$) | 0.81 \pm 0.03* | 0.91 \pm 0.13 | 2.29 \pm 0.93 | <0.01 |
| Alpha-tocopherol ($\mu\text{mol}/\text{L}$) | 13.3 \pm 3.12* | 14.3 \pm 3.99 | 15.3 \pm 4.12 | <0.01 |
| IL-6 (pg/mL) | 2.95 \pm 3.96* | 2.63 \pm 1.23 | 1.63 \pm 0.99 | <0.01 |

[Table/Fig-1]: Comparison of clinical and biochemical characteristics of the study groups.

^aFor statistical analysis, one-way ANOVA test between cases with MS, cases without MS and control, * $p<0.05$ Indicates significant differences vs. control, # $p<0.05$ Indicates significant differences vs. without MS.

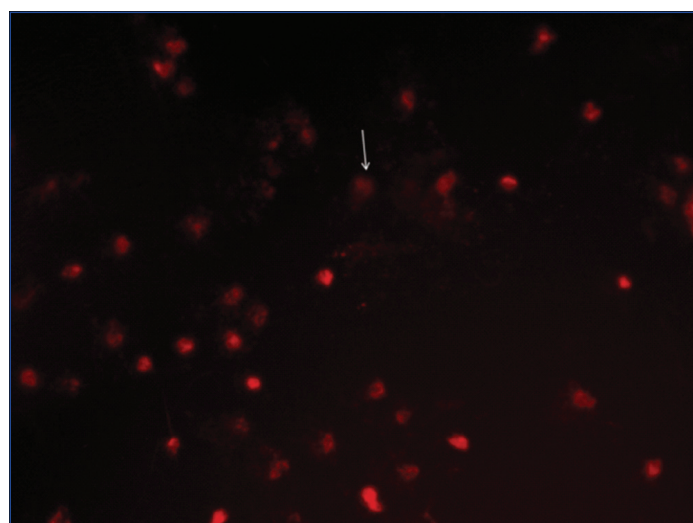
BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglyceride; FBG: Fasting blood glucose; HOMA-IR: Homeostasis model assessment of insulin resistance; TAC: Total antioxidant capacity. Values are presented as Mean \pm SD

and E compared to the control group [Table/Fig-1]. Obese MS patients also showed significantly higher levels of visfatin, MDA and significant lower TAC than those without MS. Obese cases without MS showed significantly higher BMI and WC than controls.

The results of multiple stepwise regressions show positive correlation of visfatin level with MS components, IL-6 and MDA, DNA damage, serum lipids, various measures of obesity and negative correlation with TAC and vitamins A and E [Table/Fig-2]. Comet assay was used to analyse DNA damage in combined cases of MS and pre-eclampsia. The damaged cells appeared as a fluorescent comet with tail comprising of DNA with strand breaks [Table/Fig-3].

| Variable | R | p-value |
|---------------------------|-------|---------|
| HOMA-IR | 0.44 | 0.01*** |
| FBG (mg/dL) | 0.35 | 0.02** |
| TC (mg/dL) | 0.31 | 0.01*** |
| TG (mg/dL) | 0.36 | 0.01*** |
| HDL-C (mg/dL) | -0.37 | 0.04** |
| LDL-C (mg/dL) | 0.33 | 0.05* |
| SBP (mmHg) | 0.47 | 0.03** |
| DBP (mmHg) | 0.36 | 0.04** |
| WC (cm) | 0.49 | 0.01*** |
| MDA (µmol/L) | 0.41 | 0.04** |
| TAC | -0.41 | 0.03** |
| Vitamin A (µmol/L) | -0.45 | 0.02** |
| Alpha-tocopherol (mmol/L) | -0.34 | 0.03** |
| IL-6 (pg/mL) | 0.32 | 0.02** |
| DNA damage | 0.33 | 0.02** |

[Table/Fig-2]: Multiple stepwise regression analysis with log visfatin as a dependent variable in obese women. Multiple linear regression analysis, p<0.001, **p<0.01, *p<0.05. HOMA-IR: Homeostasis model assessment of insulin resistance; FBG: Fasting blood glucose; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; WC: Waist circumference; MDA: Malondialdehyde; TAC: Total antioxidant capacity; ***IL-6: Interleukin-6



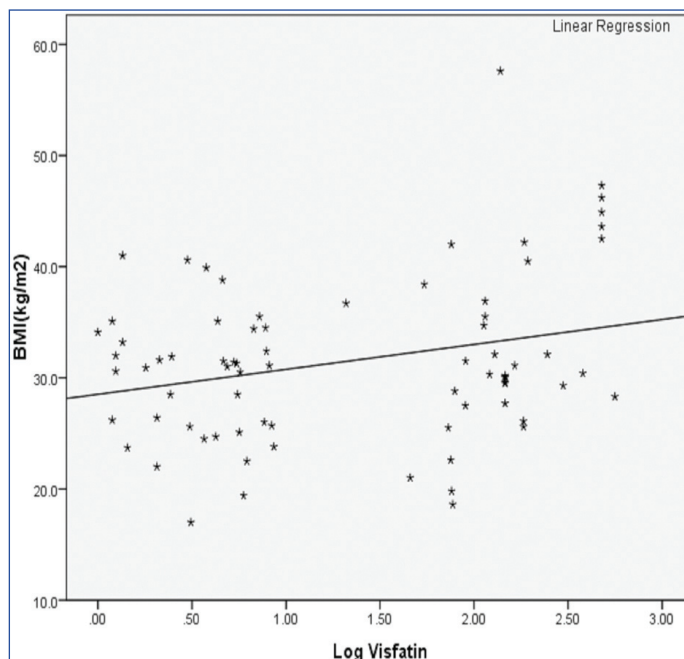
[Table/Fig-3]: DNA damage in a case with combined MS and pre-eclampsia, evaluated by the comet assay. The damaged cell is visualised as cells with the appearance of a comet (arrow) showing a brightly fluorescent head and a tail to one side formed by the DNA containing strand breaks.

The mean percent of damage was significantly higher in obese MS women with pre-eclampsia history of as well as in MS without pre-eclampsia compared to controls (p<0.002). The mean frequency of DNA damage was 33.55±3.69 in MS women with history of pre-eclampsia, 25.95±2.16 in MS women without history of pre-eclampsia and 12.81±1.94 in controls with higher levels in MS cases with pregnancy complications than those without [Table/Fig-4]. This might indicate that MS causes DNA damage that in turn leads to other problems and complications.

| Group | Damage frequency Mean±SE | Mann-Whitney U-test |
|------------------------------------|--------------------------|---------------------|
| MS with recurrent pre-eclampsia | 33.55±3.69** | 0.002 |
| MS without recurrent pre-eclampsia | 25.95±2.16* | |
| Control | 12.81±1.94 | |

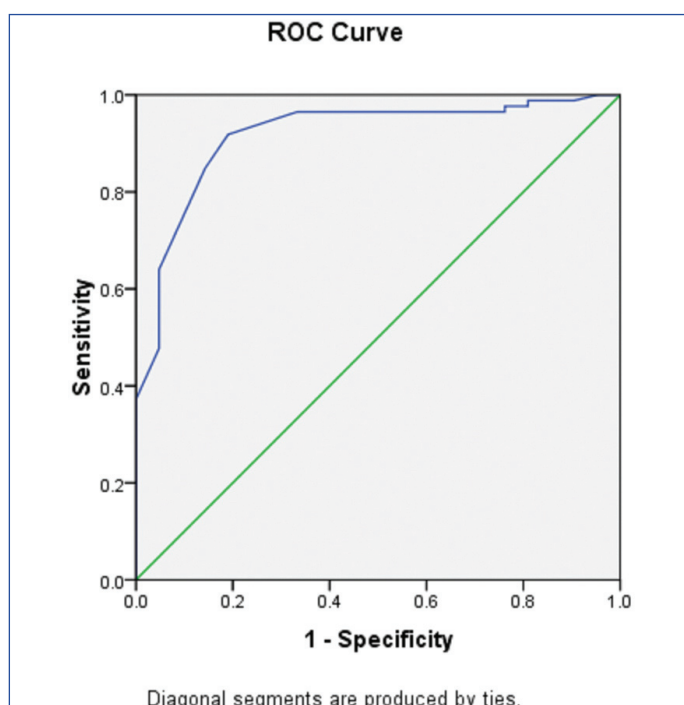
[Table/Fig-4]: Mean DNA damage in MS patients and controls. MS: Metabolic syndrome *p<0.05, **p<0.01 vs. controls

Positive correlation between log visfatin and BMI (kg/m²) in obese women with MS, indicates rise in visfatin levels along with the increase of BMI [Table/Fig-5].



[Table/Fig-5]: Positive correlation between log visfatin and BMI (kg/m²) in obese women with MS.

The cut-off of the log visfatin was 1.56 with 76% sensitivity and 71% specificity for detecting MS in obese women and the Area Under Curve (AUC) was 0.91 (p<0.001) [Table/Fig-6]. According to the results of the ROC analysis, visfatin level could be a good predictor of the MS in obese subjects.



[Table/Fig-6]: Receiver operating characteristic curve of the log visfatin in detecting the presence of MS in obese women. Area under ROC curve=0.91, p=0.001

DISCUSSION

This study is among the first to determine the optimal visfatin threshold for diagnosing the MS in Egyptian obese women. The findings demonstrate the optimal visfatin cut-off point for diagnosis of MS in obese women. The previous study on Egyptian obese children and adolescents reported that cut-off point of visfatin for obesity was 5.5 and for MS in obese with a sensitivity of 86.1% and a specificity of 86.5% and an AUC of 0.93 [32]. In the present study, the cut-off of the log visfatin was 1.56 with 76% sensitivity and 71% specificity for detecting MS in obese women and the AUC was 0.91 ($p < 0.001$). In this study, Authors also observed that women with pre-eclampsia had elevated oxidative stress and DNA damage; these findings were similar to the results obtained by Hilali N et al., [33]. Additionally, in the present study higher visfatin levels was detected in women with pre-eclampsia which is in agreement with Fasshauer M et al., 2007 and 2008 study where elevated visfatin levels have been reported in pre-eclampsia and other pregnancy complications [34,35].

Visfatin molecule has multifaceted features whose circulating levels are enhanced in metabolic disorders [5]. In the present study, significantly higher levels of visfatin have been found in obese MS cases relative to both control and obese cases without MS. The present results also indicated that serum concentration of visfatin increases along with the increasing BMI and are correlated positively with WC and lipid parameters. The results of the present study are in agreement with previous studies done by Kim JA et al., and Filippatos TA et al., that showed higher levels of visfatin in MS cases as compared to controls [36,37]. However, there have been contradictory results in the association between visfatin and obesity. Some of the studies found that serum visfatin levels are associated with obesity and visceral fat and not with subcutaneous fat when certifying sex and age-matched subjects [38]. Moreover, high visfatin levels in obese patients were found to be reduced after weight loss [12]. Conversely, Shoelson SE et al., found that visfatin levels were significantly lower in obese subjects [11]. On the other hand, Berndt J et al., in another study found a positive correlation between visfatin levels and measures of obesity as WC and BMI [2].

Oxidative Stress is associated with unbalanced levels of adipokines involved in the development of the MS [39]. It happens as a result of the high production of pro-oxidants that exceeds the ability of the antioxidant system to remove them from circulation [40]. Central adiposity may contribute to OS due to visceral fat deposition and higher free-fatty acid flux through the portal circulation. In addition, peripheral insulin resistance may be associated with mitochondrial dysfunction and overproduction of pro-oxidants that can harm or alter proteins and lipids [40-41]. It has been suggested that oxidative stress is increased due to fat accumulation in MS patients [42]. Dyslipidemia and insulin resistance, which are associated with MS, increase the production of reactive oxygen species and consequently raise the oxidation of lipid products, MDA and proteins, which lead to endothelium dysfunction, cancer and other chronic diseases [43]. The present results indicated an increase in serum concentrations of visfatin in obese MS patients, associating with rising of MDA and lowering of TAC as compared to obese without MS and controls. There are some studies looking at the changes in TAC and MDA levels during certain metabolic conditions such as MS and obesity [44,45]. Association between oxidative stress and insulin resistance has been previously reported by Evans JL [46]. The increase of the plasma visfatin level in obese women has been previously reported by Zahorska-Markiewicz B et al., he observed significant higher visfatin levels in obese women compared to normal weight women which is similar with the present findings [47]. In the present study, authors also found positive association between visfatin serum concentrations and DNA damage as previously observed by Villalobos LA et al., who reported that visfatin promotes DNA damage [48]. It has been proposed that insulin secretion is

regulated by visfatin levels and acts as an immune-modulator cytokine and involves in the inflammatory responses [49]. Elevated visfatin levels have been noticed in diabetic patients which might indicate faulty visfatin signalling or as a result of hyperglycemia or hyperinsulinemia [50]. Chronic low-grade inflammation that often accompanies the MS is a major factor in the mechanism of the MS and its consequent complications [51]. Oxidative stress markers, visfatin and IL-6 levels might yield new facts of pathways of the MS and the medical consequences of obesity such as acute coronary syndromes and atherosclerosis [52].

LIMITATION

This was a single-centred cross-sectional study, a larger multi-centric study should be done in future.

CONCLUSION

The present study found an association between visfatin levels, MS and oxidative stress. Authors suggest that serum visfatin could be used as a predictive marker for MS in obese subjects and its higher levels are associated with oxidative stress, DNA damage, IL-6 and pregnancy complications.

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