Prader–Willi syndrome (PWS) is the commonest genetic cause of obesity. Oxidative stress and chronic low-grade inflammation play a crucial role in the pathogenesis of obesity. Alterations of vitamin D (25-OHD) levels are commonly encountered with obesity. The aim of this study was to analyze serum chemerin, oxidized low-density lipoprotein (ox-LDL), and 25-OHD values in pediatric PWS patients in comparison with obese healthy children and nonobese control groups, highlighting possible correlations with body mass index (BMI) and obesity. Twenty-six PWS Egyptian patients and 26 obese healthy individuals referred to the outpatient clinic of the Clinical Genetics Department, National Research Centre, Cairo, Egypt, and 20 control patients with matching age and sex were enrolled in the study. Patients were clinically diagnosed and confirmed by routine cytogenetic and fluorescence in-situ hybridization analysis. Anthropometric measurements were performed, and BMI was calculated by weight/height² (kg/m²), and BMI z score was also determined. Serum chemerin, ox-LDL, and vitamin D were determined by enzyme-linked immunosorbent assay. Chemerin levels, which reflected chronic inflammation, were significantly elevated as compared with obese and nonobese controls (p ≤ 0.0001). Concerning oxidative damage, children with PWS showed higher Ox-LDL levels compared with obese and nonobese controls (p < 0.0001). Vitamin D levels were significantly lower in PWS patients compared with obese and nonobese controls (p ≤ 0.0001). Our data showed that obesity in PWS is associated with oxidative stress and chronic low-grade inflammation. Ox-LDL is a good indicator of oxidative stress, and chemerin could be used as a biomarker for the chronic inflammatory state. Furthermore, vitamin D supplementation is recommended in PWS patients.
Introduction

Prader–Willi syndrome (PWS: OMIM 176270)\(^1\) is a genetic disorder with multisystem involvement. Its prevalence is \(~1/15,000\) live births\(^2\) and is reported to be more in Caucasians.\(^3\) PWS is due to loss of paternally expressed genes from a de-novo paternal 15q11-q13 deletion (65–75% of cases), maternal uniparental disomy 15 (20–30% of cases), or imprinting defect (1–3%).\(^4\)

PWS is characterized by infantile hypotonia and feeding difficulty leading to failure to thrive, but they develop hyperphagia and excessive weight gain with subsequent obesity in early childhood.\(^5\) PWS patients have increased total body fat mass and decreased resting energy expenditure, which promote more gain of weight and abnormal body composition.\(^6\) PWS is considered to be the commonest genetic cause of life-threatening obesity.\(^7\) Other features of PWS include short stature, small hands and feet, hypogonadism, developmental and intellectual disability (ID), and behavioral problems.\(^8,9\)

Patients with PWS have distinctive facial features\(^13\) and commonly encounter endocrine disturbances in the form of insulin resistance and diabetes, hypothyroidism, adrenal insufficiency,\(^12,14\) and growth hormone (GH) deficiency.\(^13,15\)

Oxidative stress is the imbalance between the production of reactive oxygen species and the antioxidant defense system with disruption of redox signaling causing damage to biomolecules, including proteins, nucleic acids, and lipids. Dyslipidemia and increased lipid oxidation were found to be associated with obesity. Oxidized low-density lipoprotein (ox-LDL) is considered as one of the major and early lipid peroxidation products and, therefore, is used as one of the oxidative stress biomarkers.\(^16\) Ox-LDL was found to be elevated in obesity.\(^17\) insulin resistance,\(^18\) metabolic syndromes,\(^19\), and cardiovascular disease.\(^20\)

The adipose tissue is a metabolically active organ, secreting various adipokines, and pro-inflammatory cytokines, which play an important role in the inflammatory and atherosclerotic processes.\(^21\) Obesity is a pro-inflammatory state, usually associated with low-grade chronic inflammation and elevated levels of pro-inflammatory adipokines that are positively correlated to the body mass index (BMI).\(^22,23\) Chemerin is an adipokine that is linked to inflammation and adipo genesis. It also has a vital role in glucose and lipid metabolism\(^24,25\) by modulating the expression of adipocyte genes involved in glucose and lipid homeostases such as glucose transporter-4, adiponectin, fatty acid synthase, and leptin.\(^24\) Consequently, chemerin is linked to obesity and inflammation in humans.\(^25\)

Obesity is associated with 25-hydroxy vitamin D (25-OHD) deficiency.\(^26\) Vitamin D (25-OHD) deficiency is also associated with insulin resistance in obese children\(^27,28\), and adults.\(^26\) PWS patients show a higher percent of body fat, more subcutaneous adipose tissue, and frequently develop insulin resistance, glycemic alterations, and metabolic syndrome as a consequence of obesity, which results in a 25-OHD deficiency.\(^29\)

The aim of the work is to assess the role of oxidative stress (ox-LDL) and chronic low-grade inflammation (chemerin) as comorbidity factors affecting obesity in PWS patients in comparison to nongenetic obesity. In addition, the study aims to compare 25-OHD levels in obese PWS patients and obese healthy children. To the best of our knowledge, this is the first study to test for ox-LDL and chemerin markers in PWS children.

Patient and Method

Patient

This case–control study was conducted on patients referred to the outpatient clinic of the Clinical Genetics Department, National Research Centre, Cairo, Egypt. Their parents gave written informed consent. The study was approved by the Ethical Committee of the National Research Centre in accordance with the Declaration of Helsinki protocols. The study included three groups: group I (26 children clinically diagnosed as PWS), group II (20 nonobese healthy controls), and group III (26 obese healthy children) with matching age and sex. All children enrolled in the study were subjected to full history taking, three-generation pedigree construction, a thorough clinical examination of all body systems to detect any system abnormality, and essential anthropometric measures. Exclusion criteria for all the participants were type 1 diabetes, presence of metabolic disease (confirmed by the use of drugs), pulmonary diseases, and those on medications. Anthropometric measurements were performed as follows: height was measured to the nearest 0.5 cm, body weight was measured to the nearest 0.1 kg, BMI was calculated as weight/height\(^2\) (kg/m\(^2\)), and Z-score BMI was calculated according to the WHO Anthro Plus program\(^10,30\).

Method

All 26 clinically diagnosed children were of the deletion subtype confirmed by routine cytogenetic and fluorescence in-situ hybridization (FISH) analysis. Diagnosis of PWS depends on the neonatal history, characteristic clinical features followed by chromosomal, and FISH analysis. FISH was performed on metaphase spread from peripheral blood according to modification of Pinkel et al\(^31\) and manufacturer’s instructions by using a locus-specific probe (LSI) Prader–Willi/Angelman chromosome region probe labeled by SinoGeneClonBiotech, Co., Ltd. Ox-LDL was determined by ELISA assay catalog No. SG–11266. Serum 25 hydroxy-vitamin D (25-OHD) was assessed by vitamin D direct ELISA kit supplied by Cytocell FISH probes.

The cytogenetic study was performed by the conventional G-banding technique.\(^32\) At least 25 metaphases were karyotyped and nomenclature, according to ISCN, 2016.

FISH studies: FISH was applied using 170 kb SNRPN Prader–Willi/Angelman chromosome region probe labeled in red and covers the whole SNRPN gene as well as the entire imprinting center. The 15qter subtelomeric specific probe (clone 154P1), labeled in green, allows identification of chromosome 15 and acts as a control probe.\(^33\)

Serum chemerin was determined by ELISA assay supplied by SinoGeneClonBiotech, Co., Ltd. Ox-LDL was determined by ELISA assay catalog No. SG–11266. Serum 25 hydroxy-vitamin D (25-OHD) was assessed by vitamin D direct ELISA kit (EIA–4696) DRG International, Inc.; NJ, USA, according to Wielders and Wijnberg.\(^34\)

Statistical Analysis

Analysis of data was performed using SPSS 23 (Statistical Package for Scientific Studies) for Windows. The description
of the quantitative variables was presented in the form of mean and standard deviation (SD). The description of qualitative variables was in the form of numbers (No.) and percent (%). Comparing means of the quantitative variable between the three groups were performed after data were explored for normality using Shapiro–Wilk tests of normality. Whenever the results of the test indicated that the data were normally distributed, the one-way analysis of variance (ANOVA) test (parametric tests) was used to carry out the comparisons, followed by the Tukey test for multiple comparisons whenever a statistical significance was detected by the ANOVA. When the data were not normally distributed, comparisons were performed using the Kruskal–Wallis H test (nonparametric tests) followed by a series of Mann–Whitney U tests for every two groups to detect the group(s) responsible for the significance of the Kruskal–Wallis H test. Chi-squared test ($\chi^2$) was used to detect the independence between groups and the qualitative variable (Gender). Binary correlations were performed by Pearson correlation. Results were expressed in the form of correlation coefficient ($R$) and $p$-values. The following points are the accepted guidelines for interpreting the correlation coefficient:

- 0 indicates no linear relationship.
- +1 indicates a perfect positive linear relationship: as one variable increases in its values, the other variable also increases in its values via an exact linear rule.
- –1 indicates a perfect negative linear relationship: as one variable increases in its values, the other variable decreases in its values via an exact linear rule.
- Values between 0 and 0.3 (0 and -0.3) indicate no or a weak positive (negative) linear relationship, respectively.
- Values between 0.3 and 0.7 (0.3 and -0.7) indicate a moderate positive (negative) linear relationship, respectively.
- Values between 0.7 and 1.0 (-0.7 and -1.0) indicate a strong positive (negative) linear relationship.

The level of significance for all tests was set at $p \leq 0.05$.

**Results**

This case–control study included three groups: group I (26 children clinically diagnosed as PWS), group II (20 nonobese healthy controls), and group III (26 obese healthy children). Group I comprised 26 patients clinically diagnosed as PWS and cytogenetically confirmed as PWS-deletion subtype using FISH analysis. Their ages ranged from 3 to 15 years of age, with a mean of 8.54 years ± 3.42 SD (Table 1). Group II comprised 20 nonobese healthy children. Their ages ranged from 3 to 15 years of age, with a mean of 7.8 years ± 3.47 SD. Group III comprised 26 healthy obese children. Their ages ranged from 4 to 15 years of age, with a mean of 9.58 years ± 3.30 SD. There were 12 males and 14 females among the PWS cohort. Gender as a variable was found to be independently and almost equally distributed among the patients and control groups, which was confirmed by the insignificant result with $p$-value $= 1.000$ (Table 2). Anthropometric measurements, including weight, height, and Z-score BMI, were done for all children enrolled in the study. As shown in Table 1, analysis of the measurements revealed obesity with elevated BMI in PWS patients as compared with the normal controls with no significant difference with the obese healthy controls.

Demographic and clinical data of patients are summarized in Table 3. Seventeen patients (65.3%) were the offspring of consanguineous marriage. Dysmorphic features were present in 20/26 (76.9%) patients, including a round face, full cheeks, broad bossing forehead, and almond eyes. Epicanthic fold was present in 12/26 (46.1%) patients, and nystagmus was present in 6/26 (23%) of cases. Hand anomalies in the form of short fingers (42.3%), syndactyly (53.8%), and Simian crease (34.6%) were also detected in PWS patients. Neurological examination revealed hypotonia (100%) in all patients and microcephaly in 23% of cases. Dermatological changes were observed in some patients in the form of skin hyperpigmentation (38.4%) and café au lait patches (19.2%). Abdominal and pelvic ultrasonography showed hepatosplenomegaly in four patients. External genitalia showed absent labia minora and/or hypoplastic clitoris in eight females. Micropenis and hypospadias were observed in 9 and 5 males, respectively, and undescended...
controls (444.69 ± 33.43 vs. 263.07 ± 9.25; p < 0.0001). Higher Ox-LDL levels were also noticed in PWS patients when compared with the obese healthy controls (444.69 ± 33.43 vs. 350.69 ± 79.83; p < 0.0001) (Table 4; Fig. 1). In addition, vitamin D levels were significantly affected in PWS patients in comparison with the normal healthy nonobese controls (16.39 ± 2.90 vs. 23.10 ± 2.22; p ≤ 0.0001). A significant difference was also observed between PWS patients and the obese healthy controls (16.39 ± 2.90 vs. 18.46 ± 1.45; p ≤ 0.0001). The results in Table 4 indicate that BMI positively correlated with the levels of chemerin and ox-LDL, while it was negatively correlated with vitamin D levels (Figs. 2–4).

**Discussion**

PWS is a genetic disorder that affects many systems in the body and is the most common obesity genetic syndrome. However, clinical diagnosis of patients is still challenging, as many features of PWS are nonspecific, and patients may present with various features depending on patients’ age. In this study, 68% of the clinically diagnosed patients were confirmed by cytogenetic analysis. Routine cytogenetic analysis is essential to identify chromosomal abnormalities that contribute to Prader–Willi like phenotypes, known as Prader–Willi like syndromes (PWLS).35 Many PWLS were reported with imbalances of chromosomes 1, 2, 3, 6, 10, 12, 14, and X.36 However, the polymerase chain reaction-based methylation test and Multiplex ligation probe dependent amplification of 15q11-q13 region are the most specific tests for confirming the diagnosis of PWS.9

In our study, positive consanguinity was present in 65.3% of cases denoting a high incidence of consanguinity in our population, which is estimated to be around 60%.38 The patients presented with dysmorphic facies, hypotonia, hypogonadism, and ID, ranging from mild to moderate. These findings were consistent with other studies.39,40 Abdominal ultrasound showed hepatosplenomegaly in four patients. This could be due to extreme obesity and liver function impairment.

Excessive weight gain and obesity, especially in early childhood, are considered major complications of this disease. Obesity was the main problem in our patients as the measures of weight and BMI, when compared with the healthy controls, were significantly above the normal values. This is in agreement

**Table 4** Levels of oxidized LDL, chemerin, and vitamin D level in the studied patients as compared with the healthy nonobese controls and healthy obese controls

| Parameter                | Group I Mean ± SD | Group II Mean ± SD | Group III Mean ± SD | p-Value |
|--------------------------|-------------------|--------------------|---------------------|---------|
| Chemerin (µg/L)          | 85.04 ± 15.13a    | 58.25 ± 1.79b      | 71.42 ± 8.02c       | < 0.0001* |
| Oxidized LDL (mg/dL)     | 444.69 ± 33.43a   | 263.07 ± 9.25b     | 350.69 ± 79.83c     | < 0.0001* |
| Vitamin D level (ng/mL)  | 16.39 ± 2.90a     | 23.10 ± 2.22b      | 18.46 ± 1.45c       | < 0.0001* |

Abbreviations: LDL, low density lipoprotein; SD, standard deviation.

*a, b, c: Different small letters indicate statistical significance between the groups.

Abbreviation: PWS, Prader–Willi syndrome.
with previous studies denoting that obesity is a prominent feature of PWS and could be severe and life-threatening.\(^\text{6,40}\)

Oxidative stress is involved in the pathogenesis of PWS\(^\text{41}\) and is linked to obesity\(^\text{42}\) with the consequent lipid peroxidation and production of ox-LDL. In our study, ox-LDL levels were significantly elevated in comparison to the healthy nonobese controls and were significantly (positively) correlated with higher BMI and obesity. These results confirm the association of an oxidative stress state in obese PWS patients. Several observations reported the association between oxidative stress and PWS.\(^\text{16,17}\) Kelly et al\(^\text{43}\) highlighted that overweight and obese children and adolescents had higher levels of ox-LDL as compared with controls. Many studies demonstrated that adipocytes could have a role in the metabolism of circulating lipoproteins, including ox-LDL.\(^\text{44,45}\) The sensitivity of biomarkers of oxidative damage is higher in obese individuals and correlate directly with BMI. Oxidative stress in obesity among children requires serious consideration. Infants may exhibit peculiar susceptibilities to the effects of oxidative stress as they are undergoing rapid tissue growth and development.\(^\text{46}\) In fact, individuals with PWS harbor a higher fat mass than non-PWS

**Fig. 1** Mean levels of chemerin, oxidized low-density lipoprotein (ox-LDL), and vitamin D in Prader–Willi syndrome patients (group I), non-obese controls (group II), and obese controls (group III).

**Table 5** Pearson correlation of Z-BMI with chemerin, oxidized LDL, and vitamin D in PWS patients

|                      | Correlation coefficient “R” | p-Value |
|----------------------|-----------------------------|---------|
| Chemerin             | 0.625                       | < 0.0001* |
| Oxidized LDL         | 0.561                       | < 0.0001* |
| Vitamin D            | -0.653                      | < 0.001*  |

Abbreviations: BMI, body mass index; LDL, low density lipoprotein; PWS, Prader–Willi syndrome.

*Statistically significant p-value ≤ 0.05.

**Fig. 2** Pearson correlation of Z-score body mass index (Z-BMI) with chemerin in Prader–Willi syndrome patients.
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Obesity is considered a pro-inflammatory process, and chronic low-grade inflammation is proposed as one of the mechanisms involved in the pathogenesis of obesity.\(^{48}\) Chemerin is a pro-inflammatory adipokine.\(^{49}\) In our study, chemerin levels were significantly elevated in PWS patients as compared with the controls (\(p < 0.0001\)). These results indicate a state of ongoing chronic inflammation in those patients. Also, chemerin levels positively correlated with higher BMI and obesity with statistical significance in PWS patients (\(p < 0.0001\)). This is in accordance with other studies that highlighted the role of chemerin as an adipokine marker of chronic inflammation and has an effect on adipogenesis and lipid metabolism.\(^{24,25}\) A study by Sell et al.\(^{50}\) reported that overweight and obese patients show higher chemerin levels than normal weight, whose plasma levels decrease after diet. A study by Perumalsamy et al.\(^{51}\) reported increased levels of chemerin in patients with obesity, diabetes, and cardiovascular diseases. Interestingly, many studies showed that chemerin gene expression and its levels positively correlated with higher BMI and obesity-related biomarkers.\(^{50,52–54}\) In addition, Helfer and Wu\(^{54}\) added that chemerin affects cell expansion and increases inflammation and angiogenesis in the adipose tissue, leading to adiposity. The association of obesity and metabolic syndrome has been reported in many studies.\(^{55,56}\) Stejskal et al.\(^{57}\) confirmed that chemerin is a marker of obesity and metabolic syndrome. Additionally, Ross et al.\(^{58}\) identified an association between chemerin and appetite with body weight regulation in the hypothalamus in genome-wide expression analysis. Another study showed that the release of chemerin from the adipose tissue in obese individuals was higher than that in the normal controls and that it was positively correlated with BMI, waist-hip ratio, and fat cell mass.\(^{52}\) Consistent with these studies, chemerin levels were lowered in obese patients who lost weight by diet regimen or bariatric surgery.\(^{50,53}\) Interestingly, weight loss by exercise leads to more decrease in chemerin levels, implicating that chemerin could be used as a predictor for insulin resistance in obese individuals.\(^{53,59,60}\) On the other hand, a study conducted by Buechler et al.\(^{61}\) detected higher chemerin levels in obese mice and humans, while its bioactivity was not concomitantly changed. Contradictory results were reported on the association of chemerin with obesity and insulin resistance with no or little effect on body composition and glucose homeostasis.\(^{62–64}\)

As previous studies demonstrated the occurrence of chronic low-grade systemic inflammation in obese individuals,\(^{57,65,66}\) it is assumed that chemerin being a pro-inflammatory adipokine marker has a superadded role in the association of chronic low-grade inflammation in obese individuals. Previous studies reported the association between increased levels of pro-inflammatory adipokines and low-grade chronic inflammation.\(^{21,22,40,48}\) Huang et al.\(^{67}\) revealed that inflammation plays a vital role in the pathogenesis of obesity and its related complications. In accordance, our results showed significantly higher chemerin levels in obese PWS patients as compared with the controls (\(p < 0.0001\)). In contrast, some studies that investigated the correlation between obesity with low-grade inflammation and insulin resistance did not detect elevated chemerin levels in all patients.\(^{57,68–70}\) An additional study by Catalán et al.\(^{71}\) linked elevated levels of Chemerin and its receptor, Chemokine-Like receptor-1, in obese patients to inflammation and reported that TNF-\(\alpha\) stimulates the production of chemerin through mRNA in visceral adipocytes in these patients. Other studies reported a positive correlation of chemerin with other inflammatory cytokines and C-reactive protein in chronic inflammatory diseases.\(^{72,73}\)

PWS patients exhibit excess body fat and more subcutaneous adipose tissue leading to increased sequestration of 25-hydroxy vitamin D (25OHD) levels.\(^{74}\) Moreover, PWS patients suffer from GH deficiency\(^{11,15}\), which contributes to 25-OHD deficiency as GH and insulin growth factor-I (IGF-I) play a role in stimulating the hydroxylation of vitamin D to its active form.
In addition, vitamin D regulates gene expression of the GH/IGF-I axis and promotes the effect of IGF-I by increasing IGF-I receptors.\textsuperscript{75,76} In the present study, the estimated 25-OHD levels were significantly lower in comparison to the healthy controls ($p < 0.0001$). The study also demonstrated a statistically significant negative correlation between BMI and 25-OHD levels ($p < 0.001$). These results indicate an inverse relationship between obesity and vitamin D levels in PWS patients. This is in agreement with previous studies showing an inverse correlation between 25-OHD levels with BMI and fat mass in different ethnic and geographical groups of obese children and adults.\textsuperscript{77–79} A growing body of evidence suggests that adipocytes from obese people with insulin-resistant might have impaired release function of 25-OHD.\textsuperscript{80,81} Only a few studies on 25-OHD levels in PWS were available. A study done by Brunetti et al\textsuperscript{82} showed a significantly lower 25-OHD serum level in PWS patients than the controls. Purtell et al\textsuperscript{83} demonstrated in their study deficiency of vitamin D in adult PWS patients correlated with obesity and GH deficiency. Interestingly, Rubin et al\textsuperscript{84} observed a deficiency of 25-OHD alongside calcium in adolescents with PWS, although 33% were taking vitamin D and calcium supplements. On the other hand, Fintini et al\textsuperscript{74} showed no difference in 25-OHD levels between PWS pediatric patients and BMI matched control subjects. They owed it to the smaller sample size and other factors determining 25-OHD serum levels such as dietary intake and sunlight exposure.

Interestingly, when comparing genetic obesity (PWS patients) and nonobese obesity (obese healthy children), we found a significant difference in the studied parameters (chemerin, ox-LDL, and vitamin D), denoting that PWS patients were more affected than obese healthy children.

In conclusion, obesity in PWS is associated with oxidative stress and chronic low-grade inflammation and ox-LDL is considered as a sensitive marker for oxidative stress. Since chemerin acts as a pro-inflammatory adipokine, it could be used as a biomarker reflecting the chronic pro-inflammatory status. Vitamin D supplementation is recommended in PWS patients.

Conflict of Interest
None declared.

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