Original Article

Elevated serum malondialdehyde (MDA), insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH), and reduced antioxidant vitamins in polycystic ovarian syndrome patients

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Abstract
Elevated oxidative stress and hormonal imbalance have been suggested associate with polycystic ovarian syndromes (PCOS), a causal factor for unsuccessful pregnancy outcomes and other associated complications in women. The aim of this study was to compare the oxidative stress markers and different relevant hormone between pregnant women with and without PCOS. The levels of malondialdehyde (MDA), insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), vitamin A and vitamin C were measured in 80 pregnant women with PCOS and 80 healthy pregnancies. The mean MDA and insulin levels were significantly elevated in pregnant with PCOS compared to healthy controls (1.98±0.07 vs. 1.06±0.02 nmol/mL and 11.15±0.25 vs. 6.67±0.25 mIU/L, respectively with p<0.001 for both). Compared to healthy controls, the mean concentrations of FSH (3.65±0.16 vs. 1.75±0.10 IU/L) and LH (15.67±0.63 vs. 3.65±0.16 IU/L) were significantly higher in pregnant women with PCOS, p<0.001 for both comparisons. Similarly, the concentration of serum TSH was also higher in PCOS cases compared to controls (2.79±0.22 vs. 2.34±0.06, p=0.048). In contrast, the levels of vitamin A and C were lower in PCOS cases compared to healthy pregnancy group, 0.45±0.01 vs. 1.05±0.01 and 0.26±0.01 vs. 0.53±0.02, respectively with p-values <0.001 for both comparations. In conclusion, in PCOS, serum MDA, insulin, FSH, LH and TSH levels elevated while the level of antioxidant vitamins lower compared to healthy pregnant women. Unusual hormonal imbalance and increase of oxidative stress markers during the pregnancy might important to establish the PCOS diagnosis.

Keywords: PCOS, pregnancy, oxidative stress, MDA, hormone
Introduction

Poly cystic ovarian syndrome (PCOS) is characterized as an endocrine disorder that occurs during reproductive age and occurs due to oligo-anovulation and hyperandrogenism [1,2]. PCOS is now considered as a metabolic disorder where the patient suffers from complexity like insulin resistance, impairment in glucose tolerance, dyslipidemia, and increase in various cardiovascular risk factors [3]. The studies found that insulin resistance with compensatory hyperinsulinemia is closely associated with PCOS development [4-7]. In addition, women with PCOS possess higher body mass [8-11]. PCOS’s initial hypothesis described the elevated level of intrauterine androgen is a hallmark of the disease. Oxidative stress in the follicular environmental fluid may cause pathological condition such as inadequate oocyte development, impaired embryo development, and overall consequences of the pregnancy period [12]. PCOS can be explained as a genetic predisposition and environmental influence characterized by the excessive yield of androgens [13].

The mechanism of how oxidative stress have role on PCOS not clearly understood, but studies suggest that insulin resistance plays a crucial role in the PCOS pathogenesis, accelerating oxidative stress [14]. Studies found a very close association between oxidative stress and PCOS [15,16]. MDA is a significant aldehyde formed by the metabolism of lipid hydroperoxides, which turns out to be a vital biomarker to determine lipid peroxidation level [17]. Malondialdehyde (MDA) a critical biomarker for determining oxidative stress under any clinical circumstances [19]. It is a very reactive and potentially toxic compound and reacts with thiobarbituric acid that has been explicitly used as a potential biological marker for the peroxidation of lipid of omega-3 fatty acids [18,19]. MDA is a significant indication of lipid peroxidation, which may generate due to the oxidation of lipid [20]. A previous study proved the role of circulating oxidative stress markers in inducing PCOS and the levels of the markers were elevated in PCOS patients compared to healthy individuals [21] but never observed in pregnant women with PCOS.

Hormone imbalances are associated with PCOS. In healthy women, follicle-stimulating hormone (FSH) detects ovarian follicles and stimulates the growth of small follicles (2-5 mm) and in the late follicular stage (6-8 mm) develop aromatase activity and enhance inhibin-B action which in turn reduce FSH levels [22]. In PCOS patients however these circles are disturbed leading to elevated luteinizing hormone (LH) and FSH leading to accumulating antral follicles that distinguish fast and undergo premature growth halt of follicle [23]. As sex hormones, female gonadotropins are interrelated, but their association in the procession of oxidative stress and lipid peroxidation has never studied in pregnant women with PCOS. We aimed to determine any possible alteration in oxidative stress biomarkers along with female reproductive hormones and their role in the progression of PCOS, in comparison to normal healthy pregnant women.

Methods

Study design and blood sample collection
A case-control study was conducted at Department of Gynecology and Obstetrics, Shaheed Suhrawardy Medical College, Dhaka between January and July 2019. The pregnancy women with PCOS and healthy pregnant women as control groups were recruited. A control was age and sex matched with the case. Both groups were well distributed from each trimester of pregnancy. Each PCOS case have diagnosed as PCOS before pregnancy and was further confirmed by measuring ovarian volume and ultrasound. All subjects did consume any antioxidants or antioxidant supplementation. Women with diabetes, hypertension, hypotension, weight loss, cancer, respiratory disease, thyroid disorder and those taking any medication were excluded from the study. Prior to enrolment and blood withdrawal, all PCOS patients and controls provided written informed consent. Subjects from both groups filled up a set of questionnaires collected some demographic information, biographical information (height, weight, age).
Five 5 ml of venous blood were collected from each case and control after fasting overnight. The blood sample were preserved at room temperature unsterilized for about 1 hour to clot and then centrifuged at 3000 rpm 15 minutes for serum extraction. The serum samples were then stored at -80°C till used.

**Determination of serum MDA level**
The serum MDA was quantified according to the procedure described by Kei (1978) [24] with slight modification where thiobarbituric acid (Merck, Germany) was used. Briefly, 0.5 ml of serum was mixed with 2.5 ml of 20% trichloroacetic acid and had to endure for 10 min at room temperature. Then the mixture was centrifuged for 10 min at 3500 rpm and 2.5 ml of 0.05 M sulphuric acid was added. The supernatant’s absorbance was analyzed at 530 nm by using a spectrophotometer, and the concentration of MDA was expressed in unit nmol/mL.

**Determination of vitamin A and C**
To determine the level of serum vitamin A, a UV-Vis detector has been used at absorbance 291 nm as described previously [25]. To determine the vitamin C level, the serum was mixed with 5% trichloroacetic acid (Guangzhou HIRP Chemical Co., Ltd, Guangdong, China), then centrifuged at 3000 rpm for 10 min. The concentration of vitamin C has measured as described elsewhere [26]. The absorbance was quantified at 520 nm using phenylhydrazine-based UV-spectrophotometer (UV-1201, Shimadzu, Japan).

**Determination of serum level of insulin, FSH, LH and TSH**
To measure the insulin, the Insulin Human ELISA kit from Thermo Fisher Scientific (Catalog #KAQ1251) was used following the manufacturer’s protocol. To determine serum FSH and LH, a Human FSH ELISA kit (Catalog #EH202RB), and a human LH ELISA kit (Catalog #EHLH) both from Thermo Fisher Scientific was used and the procedure was carried out according to the manufacturer protocol. Serum TSH level was measured using a previously established procedure [27].

**Ovarian volume measurement**
In all subjects, the ovary size was measured using an ultrasound (both left and right). Calculating the highest plane of ovaries in 2D view and then positioning the vaginal probe at a 90° angle to obtain the third measurement. The ovary volume was determined using the formula [length × height × width × π/6] [28].

**Statistical analysis**
The levels of vitamin A, vitamin A, MDA, and hormone concentrations were presented as mean ± standard error of the mean (mean ± SEM) and the independent samples t-test was used to compare their levels between pregnant women with PCOS group and control group. A Pearson’s correlation was employed to assess the correlation between two variables within PCOS group and control group. SPSS ver. 20.0 (Armonk, NY: IBM Corp.) was used for all statistical analyses.

**Results**

**Characteristics of the samples**
This study recruited 80 pregnant women with PCOS and 80 healthy pregnant volunteers as control group. The characteristics patient and control group are presented in Table 1. The mean age patients and of control was 27.17 years and 27.90 years, respectively. There was no different of gestational weeks and hemoglobin concentration between PCOS and control group. There was a slightly different of body mass index (BMI) between case and control group (28.08±0.53 vs. 26.01±0.29, p<0.05). The ovarian volume was significant larger in PCOS patients compared to healthy group (14.52 cm³ vs. 8.22 cm³).

**Comparison of oxidative stress markers and hormonal**
The mean level of serum MDA was significantly higher in pregnancy women with PCOS compared to healthy pregnancy control group (1.98 vs. 1.06 nmol/mL, p<0.001). The level of
vitamin A (0.45 vs. 1.05 µmol/L), vitamin C (0.26 vs. 0.52 mg/dL), however, were significantly lower in PCOS patients in comparison to the normal healthy individuals (p<0.001 for both comparisons) (Table 1 and Figure 1).

Table 1. Sociodemographic and clinical characteristics, biochemical, and hormonal parameters of pregnant control and pregnant PCOS patient.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCOS patients (mean ± SD)</th>
<th>Healthy control (mean ± SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>80</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>Age, years</td>
<td>27.17 ± 0.87</td>
<td>27.90 ± 0.79</td>
<td>0.538</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.08 ± 0.53</td>
<td>26.01 ± 0.29</td>
<td>0.01*</td>
</tr>
<tr>
<td>Hemoglobin, mg/dL</td>
<td>10.11 ± 0.24</td>
<td>10.73 ± 0.22</td>
<td>0.067</td>
</tr>
<tr>
<td>Gestational weeks</td>
<td>28.47 ± 1.31</td>
<td>29.42 ± 1.35</td>
<td>0.617</td>
</tr>
<tr>
<td>Ovarian volume, cm³</td>
<td>14.52 ± 0.35</td>
<td>8.22 ± 0.15</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Malondialdehyde (MDA), nmol/mL</td>
<td>1.98 ± 0.07</td>
<td>1.06 ± 0.02</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Vitamin A, µmol/L</td>
<td>0.45 ± 0.01</td>
<td>1.05 ± 0.01</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Vitamin C, mg/dL</td>
<td>0.26 ± 0.01</td>
<td>0.52 ± 0.02</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Follicle-stimulating hormone (FSH), IU/L</td>
<td>13.03 ± 0.39</td>
<td>1.75 ± 0.10</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Luteinizing hormone (LH), IU/L</td>
<td>15.67 ± 0.63</td>
<td>3.95 ± 0.16</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Thyroid-stimulating hormone (TSH), mIU/L</td>
<td>2.79 ± 0.22</td>
<td>2.34 ± 0.06</td>
<td>0.048*</td>
</tr>
<tr>
<td>Insulin, mIU/L</td>
<td>11.15 ± 0.25</td>
<td>6.67 ± 0.25</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

* Statistically significant at p=0.05; ** Statistically significant at p=0.001

Figure 1. Comparison of serum oxidative stress marker, antioxidant and hormonal between pregnancy women with polycystic ovarian syndromes (PCOS) and healthy pregnancy. Compared to healthy control, in PCOS patients there is a reduction of vitamin A level (A), reduction of vitamin C level (B), elevated of MDA level (C), increased of ovarian volume (D), elevated of FSH level (E), elevated of LH level (F), increased of insulin level (G) and decreased serum of TSH level. * Statistically significant at p=0.05; *** Statistically significant at p=0.001.
Our data suggested that the level of FSH (13.03 vs. 1.75 IU/L), LH (15.67 vs. 3.65 IU/L), TSH (2.79 vs. 2.34 mIU/L) and insulin (11.15 vs. 6.67 mIU/L) were statistically significant higher in pregnancy women with PCOS compared to healthy pregnancy control group and all with p<0.05 (Table 1 and Figure 1).

**Correlation analysis**

To understand the inter-parameter relationship, a Pearson correlation test was performed within PCOS patient and control group. The results of the correlation analyses are presented in Table 2. In PCOS group, there was a statistically significant positive correlation between the ovarian volume and the level of FSH (r=0.796, p<0.001). As expected, there was a negative correlation between the level of MDA and vitamin C in PCOS group but not in control group. There was no other significant correlation between parameters within PCOS group (Table 2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy pregnant control</th>
<th>PCOS patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian volume and MDA</td>
<td>r: 0.026 p-value: 0.875</td>
<td>r: -0.139 p-value: 0.392</td>
</tr>
<tr>
<td>Ovarian volume and Vitamin A</td>
<td>r: 0.195 p-value: 0.228</td>
<td>r: -0.109 p-value: 0.505</td>
</tr>
<tr>
<td>Ovarian volume and Vitamin C</td>
<td>r: 0.126 p-value: 0.439</td>
<td>r: 0.099 p-value: 0.954</td>
</tr>
<tr>
<td>Ovarian volume and FSH</td>
<td>r: -0.214 p-value: 0.185</td>
<td>r: 0.796 p-value: &lt;0.001***</td>
</tr>
<tr>
<td>Ovarian volume and LH</td>
<td>r: -0.066 p-value: 0.688</td>
<td>r: 0.309 p-value: 0.052</td>
</tr>
<tr>
<td>Ovarian volume and TSH</td>
<td>r: -0.220 p-value: 0.172</td>
<td>r: 0.004 p-value: 0.982</td>
</tr>
<tr>
<td>Ovarian volume and Insulin</td>
<td>r: 0.199 p-value: 0.219</td>
<td>r: 0.301 p-value: 0.059</td>
</tr>
<tr>
<td>MDA and Vitamin A</td>
<td>r: 0.197 p-value: 0.114</td>
<td>r: 0.485 p-value: &lt;0.001***</td>
</tr>
<tr>
<td>MDA and Vitamin C</td>
<td>r: -0.322 p-value: 0.043*</td>
<td>r: 0.023 p-value: 0.889</td>
</tr>
<tr>
<td>MDA and FSH</td>
<td>r: 0.132 p-value: 0.418</td>
<td>r: -0.248 p-value: 0.122</td>
</tr>
<tr>
<td>MDA and LH</td>
<td>r: -0.077 p-value: 0.638</td>
<td>r: -0.060 p-value: 0.711</td>
</tr>
<tr>
<td>MDA and Insulin</td>
<td>r: -0.018 p-value: 0.912</td>
<td>r: 0.049 p-value: 0.762</td>
</tr>
<tr>
<td>Vitamin A and Vitamin C</td>
<td>r: 0.086 p-value: 0.597</td>
<td>r: 0.079 p-value: 0.626</td>
</tr>
<tr>
<td>Vitamin A and FSH</td>
<td>r: 0.017 p-value: 0.917</td>
<td>r: 0.040 p-value: 0.805</td>
</tr>
<tr>
<td>Vitamin A and LH</td>
<td>r: -0.194 p-value: 0.231</td>
<td>r: -0.111 p-value: 0.497</td>
</tr>
<tr>
<td>Vitamin A and TSH</td>
<td>r: -0.134 p-value: 0.411</td>
<td>r: -0.066 p-value: 0.684</td>
</tr>
<tr>
<td>Vitamin A and Insulin</td>
<td>r: 0.022 p-value: 0.893</td>
<td>r: -0.170 p-value: 0.293</td>
</tr>
<tr>
<td>Vitamin C and FSH</td>
<td>r: 0.122 p-value: 0.452</td>
<td>r: -0.126 p-value: 0.439</td>
</tr>
<tr>
<td>Vitamin C and TSH</td>
<td>r: 0.253 p-value: 0.115</td>
<td>r: 0.052 p-value: 0.749</td>
</tr>
<tr>
<td>Vitamin C and LH</td>
<td>r: -0.278 p-value: 0.083</td>
<td>r: 0.275 p-value: 0.086</td>
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<tr>
<td>Vitamin C and Insulin</td>
<td>r: -0.060 p-value: 0.715</td>
<td>r: 0.080 p-value: 0.624</td>
</tr>
<tr>
<td>FSH and LH</td>
<td>r: -0.193 p-value: 0.234</td>
<td>r: 0.138 p-value: 0.396</td>
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<tr>
<td>FSH and TSH</td>
<td>r: -0.269 p-value: 0.093</td>
<td>r: 0.001 p-value: 0.995</td>
</tr>
<tr>
<td>FSH and Insulin</td>
<td>r: -0.150 p-value: 0.256</td>
<td>r: -0.187 p-value: 0.248</td>
</tr>
<tr>
<td>LH and TSH</td>
<td>r: -0.108 p-value: 0.506</td>
<td>r: -0.006 p-value: 0.668</td>
</tr>
<tr>
<td>LH and Insulin</td>
<td>r: 0.112 p-value: 0.491</td>
<td>r: -0.088 p-value: 0.591</td>
</tr>
<tr>
<td>TSH and Insulin</td>
<td>r: 0.293 p-value: 0.066</td>
<td>r: 0.090 p-value: 0.582</td>
</tr>
</tbody>
</table>

r: correlation co-efficient
* Statistically significant at p<0.05 (two-tailed)

**Discussion**

PCOS is responsible for anovulatory infertility [29] where oxidative stress plays a negative impact on women’s fertility by preventing implantation, embryo development, fertilization, and ovulation [30]. Studies have found that oxidative stress is more predominant in PCOS women than normal or control cases [31,32]. Our present study suggested elevated levels of MDA and low levels of vitamin A and vitamin C in pregnant PCOS patients compared to normal subjects. Our study also found the elevated level of essential hormones in PCOS patients such as LH, FSH, and insulin in comparison to control individuals. The development of PCOS is very complex and PCOS is found to be responsible for early pregnancy loss (EPL) comparing to normal pregnant women [33]; even more pregnant PCOS women are at a high risk of premature delivery, gestational diabetes mellitus, and pregnancy related hypertensive disorders [34].
MDA, the most common metabolic end-product that is generated at the time of lipid peroxidation, plays a vital role in oxidative stress. Despite being harmful, ROS sometimes can provide a beneficial effect on the physiological process [35]. For instance, releases of pro-inflammatory cytokines and cellular damage is triggered due to oxidative stress [36]. Further, when the MDA level rises then it may help to stimulate the phospholipase A2 and interrupt cellular membrane integrity. In our study, we have observed that the MDA level was elevated in PCOS patients in comparison to controls. Similar results have been reported where increased MDA level also associated with hyperglycemia and insulin resistance [37]. Our results also similar with previous studies [38–40]. However, a study has reported no significant difference [41]. In our observation, we also found elevated of insulin level, suggesting that PCOS patients at risk of developing type-2 diabetes and insulin resistance [42].

Vitamin C plays a significant role in human physiological process such as inhibiting lipolysis, modulating lipid accumulation, interfering with adipocyte-macrophage crosstalk, and finally scavenging reactive oxygen species [43]. Both peritoneal fluid and endometrial tissue of PCOS patients contain a low level of vitamin C in comparison to normal pregnant women indicating, PCOS patients are more prone to oxidative stress [44]. It has been found that oxidative stress is responsible for more than 50-60% of recurrent pregnancy loss [45]. Our current study demonstrated that the serum vitamin C levels in PCOS were significantly lower than that of the control group, which is similar to that of the previous results [46-48]. A statistically substantial negative correlation has been observed between MDA and vitamin C, which indicates that when MDA level rises, then vitamin C level depletes, and our data in line with the previous studies [16,49,50].

Retinaldehyde is the initial metabolite of vitamin A that consists of the heme and rhodopsin [51]. The other metabolite is retinoic acid, a lipid-soluble biomolecule that helps in gene expression with a receptor-mediated process. During pregnancy, the recommended dietary vitamin A boost is 770 mcg per day. Our present study found a significant lower of serum vitamin A levels in PCOS patients compared to normal pregnant which may be due to the oxidative stress in the pregnant PCOS patients. Previous studies found no relationship between low vitamin A level and intrauterine growth retardation [52–54]. However, a British study has observed a correlation between anthropometric indexes and birth weight [55]. Therefore, there might be a critical association with vitamin A levels with PCOS’s progression and etiology during pregnancy requiring further investigation.

Generally, plasma FSH levels are minimized during pregnancy for elevated levels of estrogen and progesterone are observed but abnormally elevated levels of FSH are observed in the case of pregnant PCOS patients. After ovulation, there is a minor role of FSH and ovarian FSH receptor (FSHR) for a successful outcome in pregnancy, but studies suggest the role of extra ovarian FSHR on the female reproductive tract [56]. For pregnant PCOS patients, elevated FSH found to be positively correlated for the increment of ovarian volume. Our study revealed elevated FSH and LH levels in pregnant PCOS patients where they have decreased conception rate, and the increment of miscarriage were observed for PCOS women compared to normal women with standard LH levels [57]. A study also showed that women who have elevated levels of LH are more prone to miscarriage comparing to normal healthy women and women with PCOS [58]. Restored LH levels in PCOS women could increase the ovulation rates and pregnancy induction, proving the debilitating role of elevated LH for women [59]. Considering the above evidence, LH concentration is associated with healthy pregnancy outcomes and a critically important risk factor diagnosis and treatment target for pregnant PCOS women.

As increased LH surge is observed in PCOS patients, this hyperandrogenic condition in theca cells of the ovary is augmented by increased insulin production, which is a response to PCOS [60]. Women with PCOS mostly have type-2 diabetes, insulin resistance, elevated inflammation, and obesity, where all and/or either of this evidence is prevalent [61]. In our study, increased levels of serum insulin were observed, and this hyperinsulinemia condition contributes to obesity development in PCOS patients [62]. Impairment in glucose uptake resulted in blastocyst apoptosis, and this impaired glucose uptake responsible for Insulin-like growth factor 1 receptor downregulation [63].
Moreover, elevated risk of preeclampsia and gestational diabetes are closely linked with PCOS condition [64]. TSH levels are generally raised in women with PCOS condition where TSH levels above 2.5 mIU/L can significantly increase spontaneous pregnancy loss at the first trimester with complications in mother and child [65]. PCOS patients express their prevalence of metabolic problems, dyslipidemia, and insulin resistance [66–68]. Elevated TSH levels in PCOS women were also positively correlated with systolic blood pressure, fasting insulin, HDL, and total-cholesterol/HDL ratio compared to PCOS women with normal TSH values [69]. Our data support previous evidence of increased TSH levels in PCOS subjects [70] and for pregnant PCOS women [70], resulting in an increased risk of pregnancy loss, insulin resistance, and type 2 diabetes, suggesting further clinical investigation over other biochemical parameters. PCOS women with pregnancy must be under the intervention of hormonal monitoring, and also increment in MDA in pregnant PCOS patients influence the progression of PCOS where these parameters might be used as a pathological lead. Dietary supplementation with antioxidant is suggested for minimizing clinical conditions and complications of pregnant PCOS patients.

There are some limitations of this study. Our study did not correlate any metabolic diseases with PCOS patients and the role of nutrition in disease prognosis should be carried out. Moreover, we did study on a small number of population and we did not interlink racial differences with PCOS. For in-depth understanding of the disease, a large number of study-subject and samples from various races might be important.

**Conclusion**

There is an increase in oxidative stress and gonadotropin hormones in pregnant PCOS patients, including some metabolic dysfunction. We can end up that increased MDA, insulin, TSH, LH, FSH, and minimized vitamin A, C levels are evident and highly associated with pregnant PCOS women.

**Ethics approval**

The research protocol study was approved by the Ethical Committee of Manarat International University (BPM-14340/2019). The principles of the Helsinki declaration were strictly followed in this study.

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**Conflict of interest**

The authors declare that they have no competing interests.

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**Underlying data**

Derived data supporting the findings of this study are available from the corresponding authors on request.
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