Research article
Exploring the relationship between adiponectin and reproductive transition among diabetic and non-diabetic women

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ABSTRACT

Introduction and Aim: The reproductive aging and the menopausal transition is a period of life in females which is well associated with several changes in the adipose tissue and the hormones. Little is known about the circulating levels of adiponectin and the status of the reproductive age. The aim of the study was to understand whether these transition changes affect the levels of adiponectin in the blood and the BMI.

Materials and Methods: This was a cross sectional study with 73 diabetic females matched with 106 nondiabetic females. The subjects were between the age of 30-60 years. Serum adiponectin levels were estimated using precoated ELISA technique and BMI was calculated.

Results: The study subjects showed age matched distribution. BMI showed significant difference between normal and diabetic subjects but failed to hold the significant difference when grouped based on their reproductive transition. Adiponectin failed to show any significant difference between diabetic and normal subjects, but adiponectin levels differed well with the reproductive group in diabetics only (p=0.008) and in normal subjects the difference was not statistically significant (p=0.07).

Conclusion: This study does not hold up changes in BMI because of a transition in reproductive age and this causes a change in levels of adiponectin. However, the effect of diabetes on adiponectin level seems to be valid. The variation in the level of adiponectin is because of diabetes in the menopause state. Further the adiponectin and BMI did not show any significant difference between the age groups which shows that age is not a contributing factor for changes in BMI and levels of adiponectin.

Keywords: Adiponectin; BMI; perimenopause; menopause; post menopause.

INTRODUCTION

More number of women across the continent experience menopausal and postmenopausal symptoms. These may be experienced both frequently and infrequently. The symptoms considerably reduce both physical and mental quality-of-life. These female-specific conditions and its associated burden substantially increase total health expenses across the lifespan. Also, several implications are associated with transition of menopause. The spectrum of predictors of implications remains to be elucidated.

Transition to menopause is associated with a higher rate of body weight (1,2). Subsequently aging is commonly considered as one of the reasons for the increase in both adipose tissue mass and weight in middle-aged women (3), but studies have also shown no statistically significant relationships between age and BMI (4). Similarly, post menopause state has an impact on adiponectin concentrations (5) wherein raised adiponectin levels in late postmenopausal women, has been associated with favorable lipid profiles (6, 7). Though in women even after five years of menopause increased levels of adiponectin (8) but decreased estrogen levels result in insulin resistance and dyslipidemia with increased adiponectin levels seems to be contradictory. But the scientific evidence is lacking to support or refute claims that are commonly observed.

To better understand the impact of menopause on body mass index, age, and adiponectin, we studied the associations between them among the women with reproductive transition.

MATERIALS AND METHODS

A cross-sectional study was conducted in Dakshina Kannada district of Karnataka, India. The subjects recruited for the study were between the age of 31-60 years, The inclusion criteria for the subjects were – without diabetics or known case of type 2 diabetic, on oral hypoglycemic drugs, free from any of the vascular diseases, non-pregnant females, and females free from usage of any hormones or oral contraceptives.

Among the diabetic subjects, 73 females had details of their reproductive stage and out of normal healthy
individuals, 111 females, 5 females had surgical or medical procedures (hysterectomy) and hence were not included and only 106 subjects had their reproductive stage information and were enrolled for the study.

Depending on pelvic ultrasound scans, symptoms, and visible signs, the obstetrician classified the subjects into four groups: Normal, Perimenopause, Menopause, and post menopause.

Normal subjects are those females who have no signs of any mood swings, hot flush, and regular periods. Perimenopause is the first stage of menopause, during which the periods become irregular and unpleasant symptoms, such as hot flashes, difficulty sleeping/insomnia, and perimenopause anxiety. Subjects in the menopause group were those who had no menstrual bleeding for at least 10-12 months. Post menopause is the phase, after 12 months of no menstrual bleeding to the end of their life.

This led to four groups created based on the subjects' reproductive stages: Group 0- Normal (n=48), Group 1- Perimenopause (n=28), Group 2- Menopause (n=9), Group 3- post menopause (n=21), and in diabetes subjects: Group 0 - Normal (n=18), Group 1- Perimenopause (n=26), Group 2-Menopause (n=17), Group 3- postmenopausal (n=12).

The ethics committee of Yenepoya University approved the study protocol (004-2009). The selected subjects provided informed consent for participation in the study. The blood sample was collected and the serum from fasting blood samples was used for the assessment of adiponectin using precoated ELISA kits from Ray biotech.

Height and weight were measured by investigators as per the standard protocol. Measurements of the weight close to 0.1 kg by a weighing machine and height (without shoes) to the nearest 0.1 cm by an anthropometer rod were done. Body mass index (BMI) was calculated as weight in kg divided by height in m².

Statistical analysis
Statistical analysis was performed with SPSS 15.0 for Windows (Chicago, IL), and values were expressed as median, means and SD. BMI and Adiponectin values were not normally distributed. To determine if there were significant differences in BMI and adiponectin levels between the four reproductive stages, a non-parametric Kruskal-Wallis test was performed followed by Post hoc analysis with Mann Whitney U tests was used to identify the specific differences between groups. A p-value of less than 0.05 was considered statistically significant.

RESULTS
The Kolmogorov-Smirnov and Shapiro-Wilk tests of normality indicated that the values were not normally distributed among the reproductive groups in both diabetic and normal subjects. Therefore, we opted for the Kruskal-Wallis test.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (Median)</th>
<th>Diabetics (Median)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>45 (54.5 - 38)</td>
<td>45 (55 - 38.75)</td>
<td>0.95 NS</td>
</tr>
<tr>
<td>BMI</td>
<td>27.32 (29.93 - 24.75)</td>
<td>30.85 (33.1 - 27.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>15.8 (20.5 - 11.75)</td>
<td>16 (19 - 13)</td>
<td>0.80258 NS</td>
</tr>
</tbody>
</table>

NS: Not significant

Table 1 shows the subjects in study population who were of similar age in years hence no statistical difference was observed among the diabetic and normal subjects. Diabetic subjects showed slightly higher BMI 30.85 (33.1 - 27.35) compared with the normal subjects 27.73 (29.925 - 24.75) and the difference was statistically significant. This observed difference may be because of their glycemic state. The levels of adiponectin between the groups were not statistically significant, which says that adiponectin levels are independent of BMI and diabetic state (p=0.80).

<table>
<thead>
<tr>
<th>Age</th>
<th>N (%)</th>
<th>DM (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-40</td>
<td>33(31)</td>
<td>24(33)</td>
<td>X² =1.36</td>
</tr>
<tr>
<td>41-50</td>
<td>36(34)</td>
<td>19(26)</td>
<td>P= 0.50NS</td>
</tr>
<tr>
<td>51-60</td>
<td>37(35)</td>
<td>30(41)</td>
<td></td>
</tr>
</tbody>
</table>

NS: Not significant

Table 2 shows the age distribution of subjects among the group in the study population. When the chi-square test was applied, the result showed that chi-square statistics was 1.369 with a p value 0.50. Stating that the subjects were showing age match distribution among the group of diabetics and normal of the study population the subjects were arranged based on their reproductive state. A Kruskal-Wallis test one-way ANOVA was conducted to determine whether there were significant differences in BMI, adiponectin, and age values among all the reproductive groups in the study.

The impact of the reproductive transition on the BMI of diabetic subjects does not hold up, as there is no significant increase in BMI (p= 0.76) that can be considered substantial to the changes observed in other factors. Furthermore, there is no correlation between BMI and reproductive groups in the diabetic study population. In the normal study population, there is no substantial increase in BMI during the reproductive transition, suggesting that this transition does not show any significant effect on an individual's BMI. Moreover, there is no clear connection between BMI (p=0.06) and reproductive groups, indicating that the reproductive transition does not play a significant role in BMI changes. Hence the Kruskal-Wallis test.
showed no statistically significant difference in BMI among all the reproductive groups in the study, $x^2 (3) = 1.15$ and $7.36$, $P > 0.05$ (data not shown). Hence, there is not enough evidence to establish a relationship between BMI and all the reproductive groups in the study.

However, the Kruskal-Wallis test showed a statistically significant difference in adiponectin values (Table 3) among all the reproductive groups of the diabetic study population, $x^2 (3) = 11.70$, $P = 0.008$. During the transition towards menopause, there is a reduction in adiponectin levels, and there is a correlation between adiponectin and all reproductive categories in the diabetic study population. Therefore, a relationship exists between adiponectin and all the reproductive groups of the diabetic study population. Post hoc analysis with Mann Whitney U tests was conducted resulting in a significance level set at $P < 0.05$. Median (IQR) score of age for group 0, 1, 2 & 3 for diabetics and normal study population.

To substantiate if age factor can contribute to difference in the levels of the parameter, the diabetic and normal study group was classified based on the age (Table 5 and Table 6). On applying Kruskal–Wallis test a statistically significant difference in age between all the groups of the diabetics and normal study population, $x^2 (2) = 63.18$, $P = 0.00001$ and $x^2 (2) = 93.26$, $P = 0.00001$. Hence a relationship exists between age groups among the diabetics and the normal study population.

Further Kruskal–Wallis test failed to show statistically significant difference in BMI and adiponectin, when grouped based on their age among all the groups of the diabetics and normal study population (not shown in Table).

There was no association between adiponectin and reproductive categories in the normal population, despite the observed reduction in adiponectin levels during the transition towards menopause (Table 4). Furthermore, the Kruskal-Wallis test showed a statistically significant difference in age among all the reproductive groups of the diabetic and normal study population, $x^2 (3) = 40.51$, $P = 0.00001$ and $x^2 (3) = 75.74$, $P = 0.00001$, respectively (not shown in table). Therefore, a relationship exists between age and all the reproductive groups of the diabetic and normal study population. Post hoc analysis with Mann Whitney U tests was conducted resulting in a significance level set at $P < 0.05$. Median (IQR) score of age for group 0, 1, 2 & 3 for diabetics and normal study population.

### Table 3: Data summary of diabetic study population with respect to adiponectin

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
<th>IQR</th>
<th>Test Statistics</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>18.83</td>
<td>6.25</td>
<td>9</td>
<td>32</td>
<td>15.25</td>
<td>17</td>
<td>24</td>
<td>8.75</td>
<td>11.70</td>
<td>0.008</td>
</tr>
<tr>
<td>Perimenopause</td>
<td>17.84</td>
<td>5.45</td>
<td>6</td>
<td>28</td>
<td>14</td>
<td>16</td>
<td>21.25</td>
<td>7.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>14.76</td>
<td>3.42</td>
<td>9</td>
<td>22</td>
<td>12</td>
<td>14</td>
<td>17</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post menopause</td>
<td>13.5</td>
<td>5.61</td>
<td>8</td>
<td>25</td>
<td>9</td>
<td>12</td>
<td>15</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Data summary of normal study population with respect to adiponectin

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
<th>IQR</th>
<th>Test Statistics</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>18.78</td>
<td>10.25</td>
<td>3.8</td>
<td>39</td>
<td>11</td>
<td>15.5</td>
<td>27.15</td>
<td>16.15</td>
<td>6.91</td>
<td>0.07NS</td>
</tr>
<tr>
<td>Perimenopause</td>
<td>19.92</td>
<td>9.20</td>
<td>9</td>
<td>42</td>
<td>12.47</td>
<td>18</td>
<td>23.25</td>
<td>10.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>14.52</td>
<td>7.91</td>
<td>4.6</td>
<td>28.3</td>
<td>9.8</td>
<td>14</td>
<td>17.6</td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post menopause</td>
<td>13.00</td>
<td>5.65</td>
<td>3.2</td>
<td>19.8</td>
<td>7.8</td>
<td>14</td>
<td>18</td>
<td>10.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: Not Significant.

### Table 5: Data summary of normal study population with respect to age

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
<th>IQR</th>
<th>Test Statistics</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-40</td>
<td>35.51</td>
<td>2.86</td>
<td>31</td>
<td>40</td>
<td>33</td>
<td>35</td>
<td>38</td>
<td>5</td>
<td>93.261</td>
<td>0.00001</td>
</tr>
<tr>
<td>41-50</td>
<td>44.54</td>
<td>3.41</td>
<td>41</td>
<td>50</td>
<td>42</td>
<td>44</td>
<td>47</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>56.37</td>
<td>2.97</td>
<td>51</td>
<td>60</td>
<td>55</td>
<td>57</td>
<td>59</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 6: Data summary of diabetic study population with respect to age

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
<th>IQR</th>
<th>Test Statistics</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-40</td>
<td>35.54</td>
<td>2.72</td>
<td>31</td>
<td>40</td>
<td>33</td>
<td>35</td>
<td>38</td>
<td>5</td>
<td>63.18</td>
<td>0.00001</td>
</tr>
<tr>
<td>41-50</td>
<td>44.21</td>
<td>2.85</td>
<td>41</td>
<td>50</td>
<td>41</td>
<td>44</td>
<td>45</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>55.83</td>
<td>2.96</td>
<td>51</td>
<td>60</td>
<td>53</td>
<td>55.5</td>
<td>58</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The transition towards menopause is associated with changes in adipose tissue (9-11) due to ovarian inactivity. Adipose tissue is a significant source of estrogenic and androgenic steroids in postmenopausal women (12), while the ovaries play this role in premenopausal women. These changes in adipose tissue are reflected in the increase in body weight that often accompanies the transition. However, there is no clear evidence in the literature to support the notion that this change in body weight is a definitive indicator of the menopause transition.

BMI reflects body weight transformation, and in the present study, we found that as females progress towards menopause, there is little change in their body mass index. This is consistent with findings from the Massachusetts Women's Health Study, a longitudinal study that showed the increase in weight experienced by middle-aged women is not primarily due to menopause (13) Cross-sectional and longitudinal studies (10,14,15) have found that changes during the menopausal transition are mostly linked to an increase in abdominal adiposity, which is independent of age. Abdominal obesity is associated with insulin resistance and a higher risk of type 2 diabetes, regardless of an individual's overall body fat content (16).

The shared mechanistic influence of adipokines originating in adipose tissue during the menopausal transition is of great interest. In this study, we observed a decrease in adiponectin values across both diabetic and non-diabetic subjects with similar body composition. Additionally, we found that plasma adiponectin median levels were lower in postmenopausal women than in those of reproductive age. A study conducted by Kuo et al., showed that healthy individuals had stable adiponectin levels compared to obese subjects, suggesting that changes in adipokine levels in obesity may be a consequence of associated metabolic disorders (17,18). The participants in this study were free from any vascular complications. The significant decrease in adiponectin levels observed among the diabetic and normal groups may be attributed to the diabetic state. Epidemiological studies have reported lower plasma adiponectin levels associated with metabolic syndrome, type 2 diabetes, and cardiovascular diseases (19, 20).

A study on healthy centenarians was conducted to investigate the effect of age on adiponectin levels. Results from this study showed that there were no significant differences in adiponectin levels between the group of healthy centenarians and younger elderly controls (21). This suggests that age may not play a significant role in the levels of adiponectin. These findings are consistent with the results of the present study, which also did not show a significant difference in adiponectin levels between the age groups (Data not shown).

According to previous reports suggesting that certain medications in individuals with diabetes may increase adiponectin levels (22, 23) the serum adiponectin levels were evaluated in this study in subjects taking oral hypoglycemic drugs and those without any medication. The results showed that there were no significant differences in adiponectin levels between the two groups, indicating that oral antidiabetic drugs did not influence altering adiponectin levels in diabetics. Although adiponectin levels have been shown to change during diabetic states, this study found no significant changes in adiponectin levels among the subjects. This could possibly be attributed to the fact that the study participants were free from any vascular complication.

Considering the notion that some medications used in individuals in diabetes may contribute to an increase in adiponectin levels (22,23), it was investigated whether the use of oral hypoglycemic drugs has any effect on altering adiponectin levels in diabetic individuals. Interestingly, serum adiponectin levels were found to be comparable between diabetic subjects using oral hypoglycemic drugs and those without any medication. Although adiponectin levels are known to fluctuate in the presence of diabetes, no significant changes in the adiponectin levels of the subjects were observed in this study. This observation could potentially be attributed to the absence of any vascular complications among the study participants. Several mechanisms have been proposed to explain the changes in adiponectin levels in individuals with diabetes, but the present study found no marked alterations in the levels of this adipokine.

The literature pertaining to the relationship between the transition towards menopause and the development of insulin resistance with concomitant changes in blood glucose levels is still inconclusive. There is ongoing debate as to whether insulin resistance is primarily attributable to aging or to the hormonal changes associated with menopause, resulting in central obesity and weight gain. Despite the limited available data, there is still a lack of clear demarcation on this issue. Consequently, it remains to be demonstrated whether the site of steroid production plays a significant role in the size and distribution of fat cells associated with menopause. Further research is required to elucidate the complex interplay between aging, hormonal changes, insulin resistance, and adiposity in postmenopausal women.

CONCLUSION

Despite finding a statistically significant difference in BMI between diabetic and normal study populations, our results did not observe any significant variation in BMI among reproductive groups, regardless of their diabetic status. Therefore, our study does not support
the notion that changes in BMI occur because of a transition in reproductive age, which subsequently causes alterations in adiponectin levels. However, it does confirm the previously established effect of diabetes on adiponectin levels in menopausal women.

Additionally, our study found no significant difference in age between diabetics and normal subjects, and there was no significant difference in adiponectin or BMI between age groups. This suggests that age is not a significant contributing factor to changes in BMI or adiponectin levels. Furthermore, our findings indicate that the transition towards menopause and age are inherently linked.

Overall, our study demonstrates that there is no association between BMI and adiponectin levels when classified by age group, further supporting the notion that age is not a significant contributing factor to changes in BMI or adiponectin levels.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest.

REFERENCES