INTRODUCTION:

Autonomic system is vital to the maintenance of internal homeostasis and achieves this by mechanism that regulates blood pressure, fluid electrolyte balance and body temperature. It is directly involved in tonic reflexes and adaptive control of autonomic functions and integrates autonomic with hormonal immunomodulatory and pain controlling responses to internal and external environmental challenge.

Menstrual cyclicity is one of the physiological challenges in a normal individual and is an indicator of endocrine function and a surrogate for underlying reproductive health. Characteristics of menstrual cycle like cycle length, bleeding duration etc may reflect a woman's underlying hormonal milieu and potentially offer an immediate non-invasive measure of reproductive health. Different phases of menstrual cycle are accompanied by variations in autonomic functions up to different degrees. So any factor which causes disruption of the pattern of menstrual cyclicity in an individual shall also be reflected in her autonomic activity.
Number of host and environmental factors influence menstrual patterns like strenuous physical exercise, psychosocial stress, low body fat, endocrine disturbances and lifestyle factors such as increase fat intake smoking etc. All these factors may perturb menstruation and affect woman's reproductive health culminating into risk of infertility. Body fat plays a significant role in reproduction and both the extremes of the weight distribution have deleterious effects on menstrual cycle and infertility.

As the population of underweight girls in India is substantial, these girls are more prone for cycle irregularities, infertility problems. The present study is carried out to explore variations in autonomic responses relating to different phases of menstrual cycle in relation to body mass index.

AIM & OBJECTIVES

Aim: To evaluate autonomic responses at different phases of menstrual cycle with reference to body mass index.

Objective: To assess and compare the autonomic responses of young healthy females of normal and low BMI during menstrual, follicular and luteal phases of menstrual cycle and to find out correlation, if any, between the same.

MATERIALS & METHODS

Study design: Cross-sectional study.

Subjects selection: Sixty (60) randomly selected Female subjects of age group 18-25 years were chosen from 1st year MBBS students of SAMC & PGI, Indore.

Inclusion criteria: On the basis of International Classification of underweight, overweight and obesity in adults according to Body Mass Index (BMI), the study subjects were classified into two groups:

Group I- Subjects with BMI between 18.5 to 24.99 kg/m² (n=30, Normal BMI)

Group II- Subjects with BMI < 18.5 kg/m² (n =30, Low BMI)

Exclusion criteria:

Subjects with known history of diabetes, hypertension, heart disease or any other medical complications and taking medications which might influence their autonomic functions and BMI were excluded.

Ethical considerations

- Prior to the commencement of the study ethical clearance was obtained from the Institutional Ethical Committee.
- The participants were informed well in advance about protocol of the study and were asked for their willingness to participate.

Parameters studied

- Anthropometric data
- Body mass Index (BMI)
- Menstrual History
- Relevant clinical examination

The following Autonomic Function Tests (AFT) were performed:

A battery of five autonomic function tests was carried out on each subject under normal resting conditions.
Tests for assessing Parasympathetic system activity:

i) Deep Breathing test
ii) Valsalva maneuver
iii) Heart rate response (30:15 ratio)

Tests for assessing Sympathetic system activity:

iv) Handgrip test
v) Cold Pressor test
vi) Lying to standing test

Equipments Used for study:

1. Physiograph machine (RMS POLYRITE D)
2. Valsalva apparatus
3. Hand grip dynamometer
4. Sphygmomanometer and Stethoscope
5. Thermometer
6. Weighing machine and Height measuring scale

METHODOLOGY:

The Subjects were asked to report to the Physiology Research Laboratory on specific date between 2-4 pm hours of the study. All the subjects were evaluated for the parameters in one sitting. General examination including anthropometric measurements was done and detailed menstrual history was noted. Menstrual cycle characteristics were self-reported and usual cycles were defined as Short (≤ 25 days), Normal (26-34 days), or Long (≥ 35 days). Cycles were defined as irregular if there were < 15 days between the longest and shortest cycle in the past 12 months. Age at menarche was obtained through recall method.

In subjects having variations in the duration of menstrual cycle the phases were calculated by considering 14 days duration fixed for luteal phase.

Autonomic function tests (AFT) were carried thrice on each subject under normal resting condition and in various phases of the menstrual cycle to ensure AFT recordings at times of low (Menstrual) and high (Follicular and Luteal) hormonal influence. The time durations for different phases of menstrual cycle is as follow:

1. Menstrual phase: 1st to 5th day of bleeding.
2. Follicular phase: 6th day to 14th day of menstrual cycle.
3. Luteal phase: 15th day to 28th day or the next menstrual bleeding.

Subjects were asked to remove clothes except for light personal clothing. Shoes were also removed. Height and weight were recorded to the nearest 0.1 cm and 0.1 kg respectively. Height was recorded after the subject was standing erect with the head held in horizontal Frankfurt's plane. BMI was computed from height and weight using standard formula (Weight in kg / height in meters²). (Table1)

Autonomic function tests (AFT) were carried thrice on each subject under normal resting condition and in various phases of the menstrual cycle to ensure AFT recordings at times of low (Menstrual) and high (Follicular and Luteal) hormonal influence. The time durations for different phases of menstrual cycle is as follow:

Different phases of menstrual cycle is as follow:

1. Menstrual phase: 1st to 5th day of bleeding.
2. Follicular phase: 6th day to 14th day of menstrual cycle.
3. Luteal phase: 15th day to 28th day or the next menstrual bleeding.

In subjects having variations in the duration of menstrual cycle the phases were calculated by considering 14 days duration fixed for luteal phase.

A. Tests for assessing Parasympathetic system activity:

1. Deep Breathing Test: The subject was made to lie down comfortably in supine position with head elevated at 30°. ECG electrodes for recording lead ECG were connected. The subject was made to
breathe deeply and steadily at 6 breaths/min for 2 minutes. The expiratory/inspiratory (E/I) ratio was calculated taking the R-R intervals of each respiratory phase into consideration.

2. Valsalva Maneuver: Subject was made to sit comfortably in chair. Proper instructions were given to the subject regarding how to exhale forcefully into the manometer so as to raise the mercury column up to 40 mm of Hg and to maintain this for 10-15 sec. The subjects were asked to repeat the same. In the end, the subject was asked to release the pressure and continue normal breathing. The maximum/minimum R-R ratio was derived from the results obtained.

3. Heart Rate Response (30:15 Ratio): ECG was recorded continuously in the lying down position (about 30 beats), while standing and after standing for about 60 beats. From this HR variation, 30:15 ratio was calculated which is the ratio of the longest R-R interval around 30th beat divided by the shortest R-R interval at around 15th beat after standing.

B. Tests for assessing Sympathetic system activity:

1. Handgrip test: Subject was made to sit comfortably. Basal blood pressure was recorded. Continuous recording of heart rate and blood pressure was done. Maximum voluntary activity in pressing the hand grip dynamometer was recorded. Subject was asked to press the hand grip dynamometer for four minute (30% of maximal voluntary effort) and the change in BP was noted at the first, second and fourth minutes.

2. Cold Pressor Test: Basal blood pressure was recorded. The subject was asked to immerse her both feet in cold water of 10°C for 1 minute and changes in BP were noted at the first and second minute after the test.

4. Lying to Standing Test: Subject was made to lie down for at least 5-10 mins, then her basal BP was recorded. Then she was asked to stand actively taking 2-5 seconds and BP was measured immediately and then at time intervals of 1 min, 2.5 min, 5 min, 7.5 min and 10 min.

Statistical analysis:

Data was collected in Microsoft Excel; mean, standard deviations of all variables were calculated. Unpaired t-test was performed in both the groups and also for the intra- and inter-phasal comparison of autonomic functions in both the groups. ANOVA test was applied to compare the sympathetic/parasympathetic dominance between the two groups.

RESULTS:

Table-1: Distribution of values of anthropometric parameters in Normal and Low BMI

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal BMI (Mean ± SD)</th>
<th>Low BMI (Mean ± SD)</th>
<th>Unpaired T-test</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18.73±.86</td>
<td>18.23±.77</td>
<td></td>
<td>2.37</td>
<td>0.02</td>
</tr>
<tr>
<td>Weight (kgs)</td>
<td>51.8±4.8</td>
<td>40.3±4.7</td>
<td></td>
<td>9.37</td>
<td>0.0001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158±6±5.3</td>
<td>157.08±5.37</td>
<td></td>
<td>0.95</td>
<td>0.34</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.6±1.51</td>
<td>16.43±1.66</td>
<td></td>
<td>10.17</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>13.4±1.77</td>
<td>13.63±1.06</td>
<td></td>
<td>0.61</td>
<td>0.54</td>
</tr>
</tbody>
</table>

It is apparent from table 1 that there is statistical difference in weight and BMI in low BMI group as
compared to normal BMI.

Table 2: Comparison of E/I ratio between Normal and Low BMI groups in different phases

<table>
<thead>
<tr>
<th>Deep Breathing</th>
<th>Normal BMI (Mean ± SD)</th>
<th>Low BMI (Mean ± SD)</th>
<th>Unpaired T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>t-value p-value</td>
</tr>
<tr>
<td>Menstrual</td>
<td>1.38±0.17</td>
<td>1.34±0.13</td>
<td>0.5 0.6</td>
</tr>
<tr>
<td>Follicular</td>
<td>1.31±0.15</td>
<td>1.21±0.12</td>
<td>1.77 0.08</td>
</tr>
<tr>
<td>Luteal</td>
<td>1.30±0.15</td>
<td>1.45±0.11</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 2 shows highly statistical significant difference in E/I ratio in the luteal phase of menstrual cycle between the groups. It was lower in the normal BMI group as compared to the low BMI group.

Table 3: Comparison of Valsalva ratio between Normal and Low BMI groups in different phases

<table>
<thead>
<tr>
<th>Valsalva</th>
<th>Normal BMI (Mean ± SD)</th>
<th>Low BMI (Mean ± SD)</th>
<th>Unpaired T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>t-value p-value</td>
</tr>
<tr>
<td>Menstrual</td>
<td>1.15±0.15</td>
<td>1.20±0.22</td>
<td>1.02 0.3</td>
</tr>
<tr>
<td>Follicular</td>
<td>1.21±0.12</td>
<td>1.50±0.22</td>
<td>6.3 0.001</td>
</tr>
<tr>
<td>Luteal</td>
<td>1.23±0.13</td>
<td>1.86±0.27</td>
<td>11.51 0.001</td>
</tr>
</tbody>
</table>

Table 3 shows statistically highly significant difference in follicular and luteal phases of menstrual cycle showing increase in Max:Min R-R ratio in low BMI as compared to normal BMI group.

Table 4: Comparison of rise in DBP (mm Hg) in handgrip test between Normal and Low BMI

<table>
<thead>
<tr>
<th>Handgrip</th>
<th>Normal BMI (Mean ± SD)</th>
<th>Low BMI (Mean ± SD)</th>
<th>Unpaired T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>t-value p-value</td>
</tr>
<tr>
<td>Menstrual</td>
<td>13.36±5.6</td>
<td>8.96±4.69</td>
<td>0.12 0.8</td>
</tr>
<tr>
<td>Follicular</td>
<td>15.16±7.5</td>
<td>10.5±7.2</td>
<td>2.45 0.01</td>
</tr>
<tr>
<td>Luteal</td>
<td>13.06±4.56</td>
<td>12.8±7.44</td>
<td>3.32 0.001</td>
</tr>
</tbody>
</table>

Table 4 shows a statistically significant difference in DBP in follicular and luteal phases of menstrual cycle with increased DBP in normal BMI group as compared to low BMI group.

Table 5: Comparison of rise in DBP (mm Hg) in Cold Pressor test between Normal and Low BMI

<table>
<thead>
<tr>
<th>Cold Pressor</th>
<th>Normal BMI (Mean ± SD)</th>
<th>Low BMI (Mean ± SD)</th>
<th>Unpaired T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>t-value p-value</td>
</tr>
<tr>
<td>Menstrual</td>
<td>10.4±4.5</td>
<td>12.6±4.58</td>
<td>2.5 0.1</td>
</tr>
<tr>
<td>Follicular</td>
<td>11.3±6.2</td>
<td>11±5.4</td>
<td>0.1 0.08</td>
</tr>
<tr>
<td>Luteal</td>
<td>10.8±4.46</td>
<td>14.4±6.34</td>
<td>1.99 0.05</td>
</tr>
</tbody>
</table>

Table 5 shows a rise in DBP in all the phases of menstrual cycle in both normal and low BMI groups with a significant difference in DBP in luteal phase of menstrual cycle, where it was higher in low BMI group as compared to the normal BMI group.

Table 6: Comparison of change in SBP from lying to standing position in Normal and Low BMI

<table>
<thead>
<tr>
<th>Lying to standing (fall in SBP mmHg)</th>
<th>Normal BMI (Mean ± SD)</th>
<th>Low BMI (Mean ± SD)</th>
<th>Unpaired T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>t-value p-value</td>
</tr>
<tr>
<td>Menstrual</td>
<td>6.2±3.32</td>
<td>6.2±4.05</td>
<td>0.9 1.00</td>
</tr>
<tr>
<td>Follicular</td>
<td>7.0±3.29</td>
<td>5.6±4.11</td>
<td>1.42 0.15</td>
</tr>
<tr>
<td>Luteal</td>
<td>6.7±3.18</td>
<td>7.8±2.99</td>
<td>1.41 0.16</td>
</tr>
</tbody>
</table>

Table 6 shows a comparison of fall in systolic blood pressure, changes were seen but are not significant in all the phases of menstrual cycle in both the groups.

Table 7: Comparison of heart rate (30:15 ratio) between Normal and Low BMI groups in different phases

<table>
<thead>
<tr>
<th>Lying to standing (30:15 ratio)</th>
<th>Normal BMI (Mean ± SD)</th>
<th>Low BMI (Mean ± SD)</th>
<th>Unpaired T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>t-value p-value</td>
</tr>
<tr>
<td>Menstrual</td>
<td>1.12±0.09</td>
<td>1.17±0.11</td>
<td>1.92 0.05</td>
</tr>
<tr>
<td>Follicular</td>
<td>1.11±0.09</td>
<td>1.25±0.17</td>
<td>3.98 0.002</td>
</tr>
<tr>
<td>Luteal</td>
<td>1.09±0.08</td>
<td>1.19±0.16</td>
<td>3.06 0.003</td>
</tr>
</tbody>
</table>
As shown in table 7, statistically significant changes are seen across all the phases of menstrual cycle showing an increase in the 30:15 R-R ratio. Maximum change was seen in luteal phase in the low BMI group as compared to normal BMI group.

DISCUSSION:

On intraphasal comparison in all the phases of the present study, statistically significant changes were found in E/I ratio, R-R ratio and 30:15 ratio in low BMI subjects with unaltered difference in normal BMI indicating altered sympathovagal balance in underweight. (Table 2, 3, 7) BMI is a major determinant of cardiac autonomic nervous modulation causing sympathovagal imbalance. 13, 14, 15 These results were in consonance with other authors 16, 17 who have shown reduced parasympathetic and increased cardiovascular sympathetic responsiveness in luteal phase. They linked this altered response to nutritional deficiencies 18, 19, 20 High estrogen levels in the menstrual cycle are accompanied with decreased sympathetic nervous system activity and increased vagal tone i.e. better functioning of parasympathetic nervous system. 21

In the present study resting systolic blood pressure in different phases of menstrual cycle showed difference in value across the phases but that was not statistically significant. No significant differences were recorded in DBP. The above findings are in consistent with Greenberg G et al 22 who found changes in the systolic blood pressure in different phases of menstrual cycle and observed higher changes in the luteal phase. According to him this may be due to increase in progesterone levels. According to Christopher T et al 21 the hormonal fluctuations that occur during normal menstrual cycle mainly increase norepinephrine in mid luteal phase which might be responsible for higher systolic pressure in this phase. On the other hand, Victoria Hall et al 23 observed significantly higher blood pressure in ovulatory phase than in luteal phase and didn't find any difference in resting diastolic blood pressure between the phases. In the present study changes were seen in SBP but not in DBP. Hassan AK et al 24 in their study found that there was significant reduction in DBP and mean arterial pressure in the luteal phase. Makato Tamaka et al 25 found significant correlation between plasma estradiol concentration and cardiovagal baroreflex sensitivity which indicated that cardiovagal baroreflex sensitivity during follicular phase is greater than mid luteal phase indicating parasympathetic dominance in the follicular phase. Some authors in their studies 26 have shown increased levels of plasma renin, aldosterone and norepinephrine during luteal phase which might be contributing to the increased cardio-vagal baroreflex sensitivity. Physiological and psychological stress also contributes to the rise in SBP in premenstrual phase which is also called progesterone phase. Also, administration of exogenous progesterone and combined oral contraceptive pills are known to induce hypertension. 22

Thus it is possible to conclude that the changes in ovarian steroids in the normal menstrual cycle may alter autonomic nervous system activity with parasympathetic dominance in the follicular phase and sympathetic predominance in the luteal phase.

In the present study when handgrip test, cold pressor and lying to standing (sympathetic tests) were applied, there were changes in systolic and diastolic blood pressure in all the phases of menstrual cycle in normal and low BMI girls but
the changes were more pronounced in the diastolic blood pressure of low BMI girls. On intraphasal comparison it was found that there was significant change in diastolic blood pressure only in low BMI girls when follicular and luteal phase were compared. Sympathetic dominance was responsible for the increase in SBP and DBP. These results are in consistent with the results other authors\textsuperscript{23,26} where they found that baroreflex sensitivity was greater in luteal phase when both estrogen and progesterone were markedly elevated. (Table 4,5,6,)

In the present study, mean systolic blood pressure response to cold pressor test was higher in the luteal phase compared to menstrual and follicular phases in low and normal BMI subjects. The difference of systolic blood pressure between the three phases of the menstrual cycle when compared, were not statistically significant. The DBP did not show any significant increase in both the groups. Rolinda R et al\textsuperscript{26} concluded that pain perception varies across the menstrual cycle as shown by higher pain threshold and tolerance during follicular phase of menstrual cycle and so less pain is felt during earlier phases of cycle. Same were the observation of Hapidou and Catanzaro.\textsuperscript{27} However Stenting et al\textsuperscript{28} have reported no significant changes in the pain tolerance time of the cold pressor test during the menstrual cycle in sixteen students. This could be due to the fact that they have kept the cut-off limit for tolerance time as 300 seconds in which many participants had reached the cut-off limit.

They also demonstrated a significant correlation between the serum progesterone level and reduced activation time during the luteal phase suggesting progesterone to be pronociceptive. Animal studies indicates that the induction of luteinizing hormone (LH) surge leads to a diminished analgesic response to morphine resulting from desensitization of brain opiate receptors.\textsuperscript{29} Thus, one can speculate that hormonally induced opiate receptor desensitization could enhance luteal phase pain sensitivity among women during this phase.

While another study demonstrates less pain sensitivity during phases of the menstrual cycle associated with high estrogen.\textsuperscript{30} Many estrogen receptor expressing neurons are opioidergic.\textsuperscript{31} In the present study the responses obtained in the cold pressor test expressed, that pain perception varies across menstrual cycle.

**CONCLUSION :**

BMI plays a crucial role in sympathovagal imbalance as hormones are stored in body fat layer, and low body fat content may lead to hormonal imbalance leading to disruption of menstrual cyclicity which leads to anovulatory cycles culminating into risk of infertility. Proper preventive measures employed early to control appropriate weight can effectively arrest autonomic alterations before it becomes pathological.

**REFERENCES:**


3. Diaz A, Laufer MR, Breech LL.
Menstruation in girls and adolescents: using the menstrual cycle as a vital sign, Pediatrics 2006;118(5):2245-2250.


