Leptin levels and menstrual function in HIV-infected women in rural India

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Introduction

- Women - nearly 50% of all people living with HIV worldwide
- Little known about alterations in reproductive endocrine function in women.
- Few studies on reproductive endocrine function specific to women with HIV infection (Grinspoon S et al, 1997; Cu-Uvin S et al, 2000).
- Our preliminary study - HIV-infected women had menstrual irregularities even before 30 years, which increased with HIV disease (Annie Phoebe.K, 2006).
- Disturbances in the pattern of menstruation are a common clinical presentation for abnormalities of the hypothalamic-pituitary-ovarian axis (Shaw RW, 2003).
- To have a better understanding of the effect of HIV/AIDS on a HIV-infected woman’s reproductive health and its sequelae, necessary to further study HIV-associated endocrine abnormalities.
What is Leptin?

• How Fat talks to the brain!!!!

• Leptin, (from leptos, meaning thin) is an adipocyte hormone, a 16 KD protein and discovered by positional cloning of the obesity gene (Zhang Y, 1994, Campfield LA, 1995).

• Signals nutritional status to the central nervous system and peripheral organs.

• ‘Anti-obesity’ hormone
Leptin- a pleiotropic molecule

Leptin and Reproduction

- Leptin—long-sought factor linking energy stores to reproduction, interacts with the HPG axis at multiple levels (Moschos, 2002; Ahima, 2004).
- Bimodal action on HPG-stimulatory effects at hypothalamus and pituitary, inhibitory actions at gonads.
- Influences menstruation, pregnancy, and lactation.
- States of leptin excess, deficiency, or resistance associated with abnormal reproductive function (Moschos, 2002).
Objective

- Low leptin levels are associated with impaired immune function and it is possible that leptin may play a role in people with weakened immune systems.
- There is no data about the role of leptin in relation to reproductive function among HIV-infected individuals. There is limited data on changes in gonadal function in HIV-infected women.
- Therefore, the objective was to study menstrual cycle disturbances as well as leptin and sex hormone levels in HIV-infected women as compared to normal controls.
Methods

- Namakkal district, Tamil Nadu, South India, at the Namakkal, Rasipuram, Thiruchengodu Govt Hospitals.
- Cross sectional study
Methods

• HIV-infected and non-infected women (18-45 years) were recruited

• Written informed consent were taken from eligible subjects who were then personally interviewed in their local language.

• Anthropometry, (Body Mass Index, Waist Hip Ratio), demographics and fasting blood samples were obtained.

• The subjects were classified in accordance with WHO staging for HIV/AIDS.
## Methods

- Patients’ self-perception of lipodystrophy was determined.
- Prevalence of short cycles (<24 days), long cycles (>6 weeks), and amenorrhea (no menstruation for 3 or more months) was assessed.
- Leptin, follicle stimulating hormone (FSH), and estradiol was measured by ELISA.
- CD4 Counts by flow cytometer.
- Statistical analysis: Chi-square, ANOVA and Pearson correlation.
Results

Study Subjects

Total No of women=161

ART-naïve=86

Asymptomatic 42 (52.5%)

Symptomatic 24 (30%)

Advanced HIV disease 14 (17.5%)

ART+ = 42

Lipodystrophic 31 (73.8%)

Non-lipodystrophic 11 (26.2%)

HIV-ve = 33
Comparison of prevalence of menstrual cycle lengths among the three groups

<table>
<thead>
<tr>
<th>Menstrual cycle length (days)</th>
<th>Proportion of women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ART-naïve n=84 (%)</td>
</tr>
<tr>
<td>Normal cycle (24-35 days)</td>
<td>63 (75)</td>
</tr>
<tr>
<td>Short cycle (&lt;24 days)</td>
<td>4 (4.8)</td>
</tr>
<tr>
<td>Long cycle (&gt;42 days)</td>
<td>11 (13.1)</td>
</tr>
<tr>
<td>Amenorrhea (&gt;90 days)</td>
<td>5 (6.0)</td>
</tr>
<tr>
<td>Irregular cycle</td>
<td>1 (1.2)</td>
</tr>
</tbody>
</table>

HIV-infected women had a higher prevalence of long cycles and amenorrhea compared to HIV-negative women (16.93% vs 6.25%, p=0.067).
ART-naïve women with Amenorrhea

- All the amenorrheic ART-naïve women were < 35 years old
- All had CD4 counts < 350/μl
All the women on ART who were amenorrheic (n=2) were lipodystrophic. (50% lipoatrophic, 50% lipohypertrophic).

1 (50%) was less than 35 years old

Also, all the women on ART with long cycles (n=3) were lipodystrophic. (75% lipoatrophic, and 25% lipohypertrophic).

2 (66.7%) were less than 35 years old

A higher proportion of HIV-infected women < 35 years had abnormal cycles compared to those >35 years old (21.51% vs 2.9%, p=0.007).
Hormone levels

HIV-positive women had lower mean leptin (p=0.00) and estradiol levels (p=0.002) compared to HIV-negative controls.
Leptin decreased with advanced HIV disease stage in ART-naïve women ($r=-.227$, $p=0.052$) and in lipoatrophic women ($r=-.326$, $p=0.035$).
Leptin and menstrual cycle length

- Increase in the length of menstrual cycle was associated with decreased leptin levels in all the groups, but not significantly.
- Women with amenorrhea had the lowest mean leptin levels (p=0.037).

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=113)</th>
<th>Short (n=4)</th>
<th>Long (n=13)</th>
<th>Amenorrhea (n=8)</th>
<th>Irregular (n=3)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>31.46±50.50</td>
<td>19.32±8.7</td>
<td>27.34±41.6</td>
<td>8.79±14.05</td>
<td>113.60±111.01</td>
<td>0.037</td>
</tr>
</tbody>
</table>
Leptin and sex hormones

- Leptin did not show significant correlations with sex hormones in HIV-infected women.

- However, leptin positively correlated with estradiol in HIV-negative women ($r=.393$, $p=0.047$).
HIV-infected women had significantly lower BMI compared to HIV-negative controls (p=0.00). Among the hormones, leptin was significantly associated with BMI (r=.455, p=0.00).
Leptin replacement therapy

• Administration of leptin to leptin-deficient obese, reproductively incompetent mice, restored their fertility (Smith GD, 2002)

• Leptin replacement improved menstrual abnormalities and low E2 levels and corrected the attenuated LH response to LHRH in a group of young women with lipodystrophy and leptin deficiency. These results add to the growing body of evidence that metabolic signals such as leptin play a role in neuroendocrine regulation. (Oral EA, 2002)

• women with hypothalamic amenorrhea when treated with recombinant human leptin appeared to have improved reproductive function (Welt, 2004)

• leptin replacement partially reversed insulin resistance, steatosis and lipid abnormalities in lipodystrophic patients. Leptin replacement restored the pituitary-gonadal axis in lipodystrophic patients (Ahima RS, 2004).
Conclusions

• A higher prevalence of abnormal cycles was seen in HIV-infected women particularly in young women <35 years.

• HIV infection per se as well as ART may have an effect on menstruation.

• The abnormal cycles and lower sex hormone levels may also be due to undernutrition.

• The relative leptin deficiency or resistance under conditions in which nutritional status is suboptimal such as advanced HIV disease and lipoatrophy may be at least partly responsible for the reproductive abnormalities that occur with these conditions.
**Acknowledgement**

- Staff at Namakkal General Hospital, PTCT Center and ART Center.
- Staff at Rasipuram and Thiruchengodu General Hospital, Namakkal District.
- All the women who took part in this study.
I take leptin in my coffee now!
sexual dimorphism of leptin

• Even after correcting for body weight and fat mass, women have higher serum leptin levels than men. This sexual dimorphism in serum leptin concentrations has been associated with or is causally related to a number of factors.
• First, the pulse amplitude, but not the pulse frequency, of leptin secretion from adipose tissue is twofold to threefold higher in females than in males.
• Second, fat mass is increased in females, and there is differential fat distribution with a higher subcutaneous/visceral fat ratio in women than men. Leptin mRNA expression is known to be higher in subcutaneous than visceral fat depots.
• Third, women have higher total serum leptin levels but lower leptin-binding protein levels than men, indicating higher free leptin levels.
• Finally, female adipose tissue may be more sensitive to hormones (i.e., insulin and glucocorticoids) or other substances that stimulate leptin production.
• It is known that sex steroids such as estrogens increase leptin levels, whereas androgens decrease leptin levels.
Clinical photographs of child B before (height = 107 cm) and 24 months after r-metHuLeptin therapy (height = 124 cm)

The Journal of Clinical Investigation.2002 ;110: 1095
References

- Ahima RS. Body fat, Leptin, and Hypothalamic Amenorrhea. 2004; 351: 959-962
Mean duration of ART = 30.46 ± 13.54 months
Lipodystrophy = 34.67 ± 10.9 months
Non-lipodystrophy = 19 ± 13.9 months
P value = 0.001

Type of ART
Sexual dimorphism of leptin
<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Testosterone</td>
<td>2-6.9 ng/ml</td>
<td>0.26-1.22 ng/ml</td>
</tr>
<tr>
<td>Estradiol</td>
<td>10-36 pg/ml</td>
<td>13-191 pg/ml</td>
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<tr>
<td>FSH</td>
<td>2-10 mIU/ml</td>
<td>2-10 mIU/ml</td>
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<tr>
<td>Leptin</td>
<td>3.84 +/- 1.79 ng/ml</td>
<td>7.36 +/- 3.73</td>
</tr>
<tr>
<td>Insulin</td>
<td>0-100 micro IU/ml</td>
<td></td>
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<tr>
<td>CD4</td>
<td>410-1590 /micro litre</td>
<td></td>
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<tr>
<td>CD8</td>
<td>190-1140/micro litre</td>
<td></td>
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<tr>
<td>TLC</td>
<td>690-2540/micro litre</td>
<td></td>
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<tr>
<td>Fasting Glucose</td>
<td>76-110 mg/dl</td>
<td></td>
</tr>
<tr>
<td>T.Chol</td>
<td>Up to 200 mg/dl</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>30-60 mg/dl</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>100-190 mg/dl</td>
<td></td>
</tr>
<tr>
<td>TGL</td>
<td>Up to 150 mg/dl</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>12-16 mg/dl</td>
<td>14-18 mg/dl</td>
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