RESEARCH ARTICLE

EMERGING REPRODUCTIVE HORMONES PERTURBATIONS IN HIV POSITIVE FEMALES: A NORTH INDIAN STUDY.

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Manuscript Info	*Abstract*

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Introduction:- Reproductive hormones Testosterone, Estrogen and Progesterone forms the foundation of fertility and pregnancy issues as well as menstrual abnormalities, early menopause, lower genital tract neoplasia, sexually transmitted infections, cardiovascular complications in Human Immunodeficiency Virus (HIV) seropositive females. So measuring their levels can benefit management of HIV patients in long term.

Design:- Cross sectional and observational study.

Aims And Objectives: To estimate levels of serum testosterone, estrogen and progesterone in HIV positive, Highly Active Antiretroviral Therapy naïve females and age matched controls. To correlate these hormone levels with CD4+ cell counts of cases.

Materials And Method: Forty seven HIV seropositive HAART naïve females were taken along with forty seven age matched controls. Cases were segregated on the basis of CD4+ cell counts (A-CD4+ <200, B-CD4+ 200-350 and C-CD4+ >350 cells/mm^3). Reproductive hormones were estimated in case and control group. Results were collected and any correlation with CD4+ cell counts was searched for.

Results: Testosterone level was lower in cases than controls with significant decrease in group 2. Estrogen levels were decreased non-significantly in cases. 66.7% females in Group A, 14.3% in Group B, 14.8% in Group C and 4.2% in controls had progesterone levels less than 0.21 ng/ml. Correlation was seen between Body Mass Index (BMI) and CD4, BMI and Estrogen, CD4 and Testosterone, CD4 and Estrogen.

Conclusion: Reproductive hormones deficiency should be looked for and managed in HIV seropositive females for better management at earlier stage to avoid complications and decrease morbidity.

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Introduction:-

Chronic complications of HIV infection in females unveils in form of menstrual abnormalities, early menopause, lower genital tract neoplasias, sexually transmitted infections, cardiovascular complications and osteoporosis apart from fertility and longevity issues.(1,2) HIV infection affects endocrinal glands by direct and indirect mechanisms. Data accruing suggests that HIV positive females get diseases of aging at younger age with increased severity than uninfected ones. They lose ovarian function at earlier age also bringing menopause prematurely.(3) Management of HIV positive females can be improved substantially if endocrinal derangements are detected in advance. Very few studies have been done in India to understand the profile of endocrine dysfunctions in HIV patients. The present study was undertaken to understand the endocrine involvement in HIV seropositive females, and an effort was also made to correlate these endocrine abnormalities with stages of HIV infection and CD4 cell counts.

Aim and objectives:-
❖ To estimate the hormone levels: Testosterone, Estrogen and Progesterone in 47 HIV seropositive females and age matched controls
❖ To correlate these hormone levels with CD4 cell counts.

Subjects and method:-

This observational cross sectional study was conducted with ethical clearance in the Department of Biochemistry in collaboration with Department of Microbiology; Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India. Forty seven known female clients of HIV infection coming to Integrated counselling and testing center (ICTC) for diagnosis of HIV infection and those attending ART center for CD4 monitoring, were enrolled along with forty seven age matched controls on giving an informed written consent after full explanation of the purpose and nature of all procedures used.

Three serological rapid tests based on three different principles diagnosed a case. CD4+ cell counts were done by flow cytometry. Serum estrogen, progesterone and testosterone were estimated on ADVIA Centaur CP by a competitive immunoassay using direct chemiluminescent technology.(4,5,6) Cases were divided into three groups on the basis of baseline CD4 cell counts as: • Group A-CD4 cell counts < 200/mm3 • Group B-CD4 cell counts 200-350/mm3 • Group C-CD4 cell counts > 350/mm3. Samples were taken for routine investigations and hormones including Estrogen, Progesterone and Testosterone on the second day of menstrual cycle.

Inclusion Criteria:-
1. HIV positive females were included
2. Age group 15-45 years.

Exclusion Criteria:-
1. Pregnancy
2. Patients of age <15 and >45 years.
3. Intake of ART, steroids or drugs known to affect hypothalamo-pituitary-gonadal axis.
4. New opportunistic infection within 4 weeks of study.
5. Patients with liver disease.
6. Any chronic illness like Diabetes Mellitus, Chronic renal failure, asthma etc.

Sample collection and storage:-
Fasting early morning venous blood sample was taken in a plain red capped evacuated blood collection tube under all aseptic precautions. Samples were processed within one hour of collection. Serum was separated by centrifugation at 2000 rpm X 10 minutes after clotting. Separated serum was stored at -20◦C (maximum 3 months) for evaluating hormone levels.

Statistical analysis:-
Data were presented in mean (SD), median and percentage. IBM SPSS ver. 20 was used for various statistical analyses. Comparison of data between groups was done using 't' test / Mann Whitney Test for quantitative data and Chi-square test for qualitative data. Comparison between multiple groups was done using one-way anova/ Kruskal

wallis test. Paired samples were compared by paired't' test / Wilcoxon sign test. Correlations and regression between groups were analyzed using suitable models. Charts and graphs were prepared using IBM SPSS ver. 20 and Microsoft excel programs.

Results and observations:-

Mean age in female cases was 29±7 years with a range between 17-41 years. On the basis of CD4 cell counts cases were divided in group A (<200 cells/mm3), group B (200-350 cells/mm3) and group C (>350 cells/mm3). Mean BMI was 17.71±1.71 in group A, 18.11±2.22 in group B, 19.71±2.84 in group C and 22.45±1.70 in controls. 44.7% females were asymptomatic and 21.2% females complained of menstrual irregularities. 34% females had weight loss. Females in Group A had significantly lower BMI than controls (p<0.001). Significantly higher proportion of wasting (BMI<18.5) in group A in males and group A and B in females.(Table 1) Hemoglobin was significantly lower in all three groups in female patients than controls (p<0.001). TG and VLDL were significantly raised in female cases. Mean Testosterone Level in Group A was 21.52 ±11.58, Group B was 23.64±13.89, Group C was 34.73±19.76 and controls was 40.55±15.19 ng/dL. Significant change in testosterone level was seen in Group 2 vs 4 in females. Estrogen levels were decreased non-significantly in female cases. 4/6 (66.7%) females in Group A, 2/14 (14.3%) in Group B, 4/27 (14.8%) in Group C and 2/47 (4.2%) in controls had progesterone levels less than 0.21 ng/ml. (Table 1) Correlation was seen between BMI and CD4, BMI and Estrogen, CD4 and Testosterone, CD4 and Estrogen.(Table 2)

Table 1:- Comparison of hormone levels, CD4+ cell counts and BMI in cases and controls.

S.No	Parameter	Group A	Group B	Group C	Controls
1.	Number	N=6 (12.7%)	N=14(29.8%)	N=27(57.4%)	N=47
2.	CD4+ cell/mm^3	<200	200-350	>350	
3.	BMI	17.71 ±1.71	18.11± 2.22	19.71± 2.84	22.45± 1.70
4.	Testosterone	21.52 ±11.78	23.64± 13.89	34.73± 19.76	40.55± 15.19
5.	Estrogen	23.68 ±9.97	32.38± 9.56	39.13± 14.68	40.16± 15.55
6.	Progesterone <0.21 ng/ml	4/6 (66.7%)	2/14 (14.3%)	4/27 (14.8%)	2/47 (4.2%)

Table 2:- Correlations in females.

	R value	P value
BMI & CD4	0.392	0.007
BMI & Estrogen	0.406	0.005
CD4 & Testosterone	0.463	0.001
CD4 & Estrogen	0.289	0.049

Discussion:-

This cross sectional study was conducted in 47 HIV seropositive HAART naïve females to assess the levels of Testosterone, Estrogen and Progesterone and compare with 47 healthy controls. Correlation of hormone levels with CD4+ cell counts, BMI and each other was also sought for. Diagnosis of endocrine metabolic disorders in HIV patients is important. However, reproductive hormone analysis still remain neglected in HIV management.

Synchronous with our hypothesis of endocrinal involvement in HIV positive females, we found a significant decrease in testosterone levels in cases than controls.(Table 1, Fig 1) Patients with lower CD4+ cell counts had lower testosterone levels.(Table 2) Testosterone deficiency was found in 21.2% female cases (S. testosterone level < 14ng/dl). Mean testosterone level was 21.52±11.78 ng/dl in group A, 23.64 13.89 ng/dl in group B, 34.73 19.76 ng/dl in group C and 40.55 15.19 in controls. (Table 1)
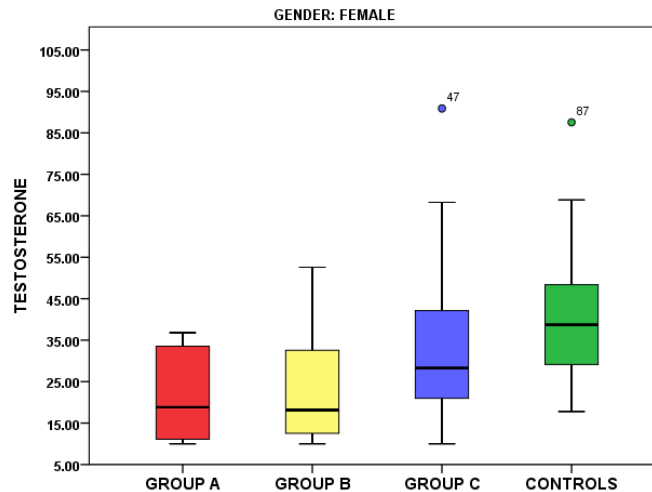
Figure 1:- Serum Testosterone levels in HIV positive females and controls.

Previous studies had also reported that significant androgen deficiency is common among women with HIV disease (7,8) occurring in 66% of cases with wasting.(9) Grinspoon et al reported that mean free testosterone, but not total testosterone levels were decreased in subjects with wasting compared to those in age matched healthy controls.(9) So women loses significant lean body and muscle mass in late stages of wasting. However, in contrast to men, women exhibit a progressive and disproportionate decrease in body fat relative to lean body mass at all stages of wasting, consistent with gender-specific effects in body composition in Acquired Immunodeficiency Syndrome wasting.(9)

Cofrancesco et al found decreased testosterone levels and increased Sex Hormone Binding Globulin (SHBG) in HIV-infected women with CD4 cell count <200cells/mm^3 (10) Another study noticed that serum total (P < 0.0001] and free testosterone levels (P < 0.0001) were significantly lower in HIV-infected women (n = 37) than in healthy women (n = 34). Testosterone levels were significantly lower in infected females even if they were menstruating normally and were independent of weight loss (11).

The Women's Interagency HIV Study (WIHS) study reported that HIV infection was associated with statistically significant differences in three measures [mean dehydroepiandrosterone sulfate (DHEAS) in HIV infected women was 73.3 µg/dl (34.0, 123) versus in uninfected women 106.0 µg/dl (65.0, 165), p<.0001)], mean testosterone in HIV infected women was 22.7 ng/dl (10.0, 36.9) versus in uninfected women 37.3 ng/dl (22.7, 53.1), p<.0001) and mean SHBG in HIV infected women was significantly higher 58.5 nmol/L (41.0, 88.0) versus in uninfected women 47.0 nmol/L (32.0, 70.0), p<.0001.(12)

Our second finding was lower serum estrogen levels in cases than controls.(Table 1, Fig 2) Correlation was found between estrogen and CD4+ cell counts and estrogen with BMI.(Table 2) In contrast, Estrogen levels were not different between HIV positive female cases and controls in study of Jain et al, and this result was not affected in a secondary analysis adjusting for Sex hormone binding globulin. But estradiol levels were lower among subjects with amenorrhea compared to eumenorrheic HIV-positive and controls and did not correlated with body composition (13).
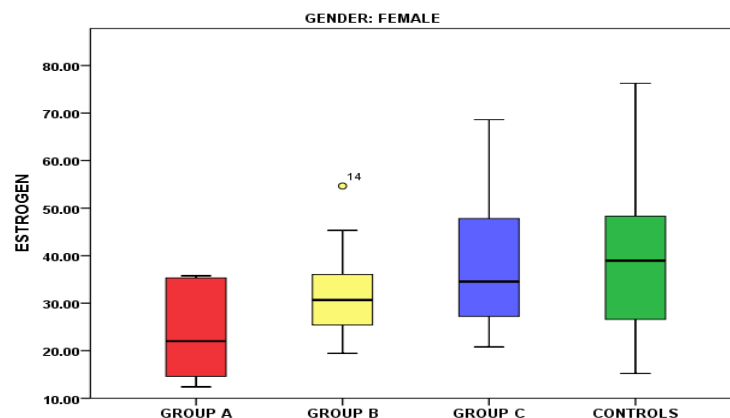
Figure 2:- Serum Estrogen levels in HIV positive females and controls.

WIHS study conducted in U.S. including HIV infected or at high risk females with regular menstrual history, who did not received sex steroids exogenously, sampled in early follicular ovulatory phase and found that the HIV infected women varied widely in their CD4 lymphocyte counts and plasma HIV RNA levels. Estradiol levels were 37.0 pg/mL (95% CI 27.0, 51.0) in the HIV infected women versus 43.5 pg/mL (31.0, 58.0) in the uninfected women, a difference that was statistically significant at the p=.001 level. (12) Santoro et al also documented Estrogen insufficiency in HIV-infected women.(14)

 Weinberg et al on the other hand studied sex steroid levels in 20 HIV infected and 20 HIV uninfected nonpregnant, not receiving exogenous sex steroids and women with regular menstrual cycles at three time points after menses and reported that the HIV infected group had CD4 lymphocyte counts ≥300cells/μL and plasma HIV RNA levels <10,000 copies/ml. They presented no significance differences in estradiol and progesterone levels between both groups. This study was limited by differences in sample size, higher CD4 cell counts and lower HIV RNA levels in the Weinberg versus the WIHS studies, indicating that HIV disease status may influence sex steroid levels.(15)

In our study, 66.7% cases in group A, 14.3% in group B, 14.8% in group C and 4.2% in controls had progesterone <0.21ng/dl.(Table 1, Fig 3) Data related to progesterone is scarce and controversial.
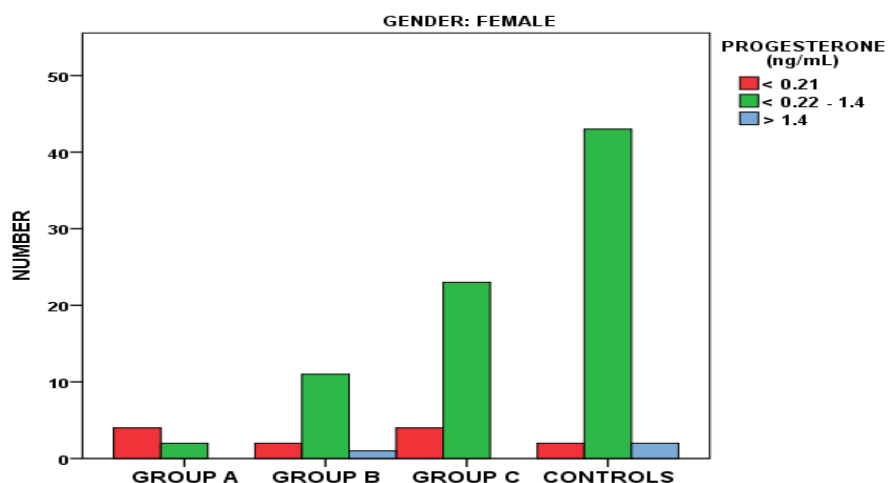

Figure 3:- Serum Progesterone levels in HIV positive females and controls.

LIMITATIONS: This cross sectional study was limited by small sample size, use of total testosterone due to constraint of resources which is less useful than free testosterone. Follicle Stimulating Hormone, Luteinizing

Hormone and SHBG measurement could have added in understanding the mechanisms behind hormonal perturbations.

Conclusion:

Endocrine involvement in HIV infection starts at an early stage and its severity amplifies with decrease in CD4+ cell counts. If these deficiencies are detected, suspected and treated in HIV management, quality of life in HIV positive patients can be improved significantly.

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