LH/FSH ratio: A better marker of secondary amenorrhea in patients from eastern Nepal

Madhab Lamsal,[1] Subarna Pokhrel,[2] Ram Vinod Mahato,[3] Nirmal Baral,[1] Dhruba Upreti[4] and Magadh Gopal Sridhar[5]

[1,4]Departments of Biochemistry and Gynecology, B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal ; [2]Enzyme and Environmental Biotechnology Lab, Seoul National University, Seoul, South Korea; [3] Department of Pathology, Shree Birendra Hospital Chhauni, Kathmandu, Nepal; [5]Department of Biochemistry , Jawaharlal Nehru Institute of Post Graduate Medical Education and Research (JIPMER), Pondicherry, India

Corresponding author: Dr. Madhab Lamsal, Additional Professor, BPKIHS, e-mail: madhablamsal@yahoo.co.uk

ABSTRACT

FSH, LH and prolactin (PRL) levels were assessed by ELISA in 50 cases with secondary amenorrhea and 52 age and sex-matched healthy controls from eastern Nepal. Cases were diagnosed by differential diagnosis, and data were analyzed using standard statistical tools. Early stage (3-6 months) and long standing (> 6 months) secondary amenorrhea had no effect ($p>0.05$) in hormonal parameters studied. Pulse, SBP, DBP, weight, height, age of menarche, cycle interval and duration of flows were homogenous ($p>0.05$) in patients and controls. Median age of menarche, median cycle interval and median duration of flows in healthy subjects were 14 years, 30 and 4 days respectively. FSH in cases (15.38±7.24 mU/ml) was significantly elevated ($p<0.01$) as compared to controls (9.38±6.34 mU/ml). LH in cases (35.44±24.35 mU/ml, median 36.5 mU/ml) was significantly ($p<0.01$) elevated by almost 5 times of its mean value and 9 times of its median value as compared to that of controls (7.58±6.604 mU/ml, median 4.2 mU/ml). LH/FSH ratio in cases (2.44±1.73, median 2.00) was significantly higher ($p<0.01$) as compared to controls (0.82±0.42, median 0.76). FSH ≥12 mU/ml, LH ≥10 mU/ml and LH/FSH ratio ≥1 cut offs were significantly associated ($p=0.000$ in each) with the cases as revealed by chi-square analysis, and LH/FSH ratio ≥1 (Sensitivity=84.0%, specificity=77.0%) was found to be a stronger marker of secondary amenorrhea. As the elevation of LH was more pronounced than that of FSH, this study hints towards possible LH receptor mutation, which is generally found in premature ovarian failure (POF). Diagnosis of cases in this region may need a new cut off level for POF, as the elevation of FSH itself was not as pronounced as reported by other workers.

Keywords: Secondary amenorrhea, hormonal parameters, LH receptor mutation, eastern Nepal.

INTRODUCTION

Amenorrhea is the absence or abnormal cessation of the menses.[1] Primary and secondary amenorrhea describes the occurrence of amenorrhea before and after menarche, respectively.[2] Secondary amenorrhea, more common than primary amenorrhea, is the absence of menses for three months in women with previously normal menstruation and for nine months in women with previous oligomenorrhea.[3-5] The prevalence of secondary amenorrhea depends on the age group studied. A group of women aged 13-18 years with 3 months of secondary amenorrhea was found to have a prevalence of 8.5% in one study, whereas another study reported a prevalence of 7.6% in a 15-24 years age group and 3.0% and 3.7% in women aged 25-34 and 35-44 years age group respectively.[6] The majority of the causes of primary and secondary amenorrhea are similar, which breaks the normal menstrual cycle at three critical levels: hypothalamus, pituitary, and the ovary by disrupting the complex feedback interactions that maintain the normal menstrual cycle.[7-9] Physiologic processes (medication effect, pregnancy), interruption or failure of the hypothalamic-pituitary axis (hypothalamic: anorexia nervosa, chronic illness, histiocytosis, lymphoma, medication effect, overtraining, starvation, stress, pituitary: amenorrhea-galactorrhea syndrome, empty sella syndrome, hyperprolactinemia, pituitary adenoma, Sheehan's syndrome), ovarian failure (autoimmune ovarian failure, radiotherapy, chemotherapy), and structural disease of gynecologic organs (uterine disease: Asherman's syndrome) are the four most common causes of secondary amenorrhea.[10] The World Health Organization (WHO) has categorized the causes into three groups: Group I-no evidence of endogenous estrogen production, normal or low FSH levels, normal prolactin levels, and no evidence of a lesion in the hypothalamic- pituitary region; group II-associated with evidence of estrogen production and normal levels of prolactin and FSH; and group III-involving elevated serum FSH levels indicating gonadal failure.[11]

Deficiency of ovarian hormone production as a result of ovarian failure accounts for many cases of secondary amenorrhea. Ovarian failure can be temporary or permanent; it most often occurs before age 40; and is characterized in general by anovulation, the depletion of primordial ovarian follicles, and an elevation in the level of follicle-stimulating hormone (FSH) of more than 25 IU/dL.[12] Premature ovarian failure resulting from the early depletion of ovarian follicles is defined as primary or secondary amenorrhea associated with elevated gonadotropin levels and infertility in women younger than 40 years of age. It accounts for 10.0-28.0% of

primary amenorrhea and 4.0-18.0% of secondary amenorrhea.[13] Although intermittent ovulation may occur, many women with this condition require oocyte donation to conceive. Ovarian failure has various causes, some of which are associated with other important medical conditions that must be addressed and treated appropriately before and during pregnancy. Temporary ovarian failure can be idiopathic and often responds to a progestin challenge test. Polycystic ovarian syndrome (PCOS) characterized by the presence of cysts on the ovaries affects 5.0-10.0% of premenopausal women. The typical presentation is chronic anovulatory bleeding with androgen excess. Women with PCOS can indeed experience secondary amenorrhea or other changes in the menstrual cycle or in reproductive fecundity. For example, infertility occurs in 74.0% of women with PCOS, in 51.0% with menstrual irregularity, and in 69.0% with signs of androgen excess. The menstrual irregularities seen in women with PCOS likely relate to the associated finding of insulin resistance and hyperinsulinemia. Hyperinsulinemia is possibly involved to initiate abnormal ovarian secretions and that this, in turn, causes gonadotropin hormone deficiencies and oligomenorrhea.[14]

The feedback interactions in the menstrual cycle have been the object of intense study, and the evaluation of both the primary and secondary amenorrhea has benefited from the precise measurement of hormonal levels including gonadotroprins (FSH and LH) in the blood using readily available modern commercial assays to distinguish into distinct etiologic categories.[15] The ovary produces the most important ovarian steroid, estradiol, which is critical for the normal functioning of the uterus. As estradiol is ineffective if the uterus is unresponsive or absent, the uterus as an end organ must also be considered in the evaluation of secondary amenorrhea.[9] To further evaluate the cases presenting the clinical signs and sympotoms of secondary amenorrhea, this prospective study has been designed to see the variation in gonadotropins in the cases and healthy subjects covering the sub tropical region of eastern Nepal.

SUBJECTS AND METHODS

This was a collaborative study done in the departments of biochemistry and gynecology, BPKIHS, Dharan, Nepal from July 2002 to June 2003. Fifty non-pregnant subjects with secondary amenorrhea taking no contraceptives for at least previous six months were enrolled as cases. Patients qualified for analysis if they had menarche previously and experienced at least 3 months of amenorrhea or absence of periods for a length of time equivalent to a total of at least three previous cycle intervals. Cases with history of tuberculosis, repeated dilatation and curettage, lactational amenorrhea, presenting depression and psychosis, or on medication with antidepressant, opiate drugs, pain killers which is known to affect prolactin release, post menopause, taking lipid lowering agents, any thyroid illnesses, absence of uterus or vagina and hirsutism were excluded from further analyses. 52 non-pregnant age and sex matched healthy subjects belonging to similar socioeconomic and demographic settings like the patients were included as controls. Control subjects on contraceptives within six months were excluded. Informed consent was obtained from all the participants of this study and detailed history was recorded in a well structured proforma. Ethical clearance was obtained from Institute's Ethical Review Board, BPKIHS, Dharan, Nepal.

Laboratory Methods: All the subjects were suggested to be cool and calm during blood sample drawing, not to cause any elevation in prolactin level by stress. Blood samples were drawn from median cubital vein in antecubital fossa by venipuncture after overnight fasting (12-14 h). Serum was separated within half an hour by centrifugation, and stored at -20^0C until assayed. Follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) were assayed using standard and sensitive commercial ELISA kits (Premier Medical Corporation, marketed by Ranbaxy Laboratories Diagnostic Division, India), employing the solid phase immobilization and horse radish peroxidase (HRP) conjugated mouse monoclonal anti-hormone antibody. The test sample was allowed to react simultaneously with the antibodies resulting in hormone molecules being sandwiched between the solid phase and enzyme linked antibodies. After 45 min incubation at room temperature, wells were washed and tetramethyl benzidine (TMB) reagent was added, incubated at dark for 20 min for blue color development. Reaction was stopped with addition of 1N HCl and yellow color thus formed was read at 450 nm.

Differential Diagnosis: Diagnosis of secondary amenorrhea was made in the cases of 3 months without menses or absence of periods for a length of time equivalent to a total of at least 3 of the previous cycle intervals, or 6 months of amenorrhea.[16] After ruling out pregnancy, thyroid disease, and hyperprolactinemia as potential diagnoses, the remaining causes of secondary amenorrhea were classified as normogonadotropic amenorrhea, hypogonadotropic hypogonadism, and hypergonadotropic hypogonadism; each associated with specific etiologies.[5,17,18] None of the patients had any signs of androgen excess (Hirsutism), alopecia and hyperpigmentation (Acanthosis nigricans). The cases enrolled were with end-organ dysfunction,[19] either with

uterine pathology or ovarian pathology. As we excluded the cases with repeated dilatation and curettage, uterine pathology involvement was also ruled out. So, the cases considered for the present study were with hypergonadotropic hypogonadism or ovarian failure with no thyroid dysfunction.

Statistical Analysis: Patients were ratified into different groups; amenorrhea of < 6 months and > 6 months, married and unmarried, and data were analyzed for any difference in their hormonal profile and anthropometric parameters using Skewness test, Komogorov-Smirnov's test, t-test, Pearson's correlation and chi-square and risk analyses in SPSS version 15.00.

RESULTS

The characteristics of cases and controls are displayed in Tables 1-5. The distribution of all the parameters in cases and controls was assessed by Skewness and Komolgorov-Smirnov's test. Skewness statistic value divided by standard error (Std. error) of -2 to +2 was assumed to be satisfactory for further data analysis for t-test and f-test for each parameter on both the case and control sides. The data were assumed to be very good in case of Komolgorov-Smirnov's test significance of 0.2, and as analyzable when the Komolgorov-Smirnov's statistic value is greater than its significance (Sig.) value (Tables-1,3). Age distribution (Years) among patients (27.56±5.33) and controls (27.88±6.396) was similar ($p>0.05$). Secondary amenorrhea of 3-6 months duration was the presenting menstrual pattern in 24 women (48.0%), and >6 months in 26 women (52.0%). Early stage (3-6 months) and long standing (> 6 months) secondary amenorrhea showed no change ($p>0.05$) in hormonal parameters sought (data not shown). Mean values of various parameters are shown in Table-2. Pulse (82.92±8.02 vs. 80.92±8.168 min^{-1}), SBP (113.76±10.14 vs. 108.35±12.36 mmHg), DBP (78.80±10.38 vs. 73.65±8.212 mmHg), weight (53.12±11.04 vs. 52.58±8.14 Kg), height (151.84±6.79 vs. 153.69±9.09 cm), age of menarche (13.98±1.25 vs. 13.43±0.86 years), cycle interval (34±10 vs. 30.32±3.038 days) and duration of flows (4.65±1.92 vs. 4.08±1.1 days) were not different ($p>0.05$) in patients and controls. Median age of menarche, median cycle interval and median duration of flows in healthy Nepalese community was found to be 14 years, 30 and 4 days respectively.

On analyzing hormonal parameters (Table-1), prolactin (PRL) was normally distributed among controls but showed skewed distribution in cases (Skewness: Statistic 1.54, Std. error 0.464, Komolgorov-Smirnov's test value: Statistic 0.145, Sig. 0.187), where as FSH and LH were normally distributed in both the cases and the controls. As the hyperprolactinemic cases were excluded from the patients group, PRL was not found to be significantly different ($p>0.1$) in cases (11.18±7.47 ng/ml) as compared to controls (13.18±4.79 ng/ml). FSH in cases (15.38±7.24 mU/ml, median 15 mU/ml) was significantly elevated ($p<0.01$) as compared to that of controls (9.38±6.34 mU/ml, median 8.55 mU/ml). FSH level in follicular phase (F-phase) controls was tested against FSH level in luteal phase (L-phase) controls, but was not different ($p>0.05$). LH in cases (35.44±24.35 mU/ml , median 36.5 mU/ml) was significantly ($p<0.01$) elevated by almost 5 times of its mean value and 9 times of its median value as compared to that of controls (7.58±6.604 mU/ml, median 4.2 mU/ml). F-phase LH (7.58±6.60 mU/ml, median 4.2 mU/ml) was also slightly but significantly ($p<0.05$) higher as compared to L-phase (6.49±5.77 mU/ml, median 3.85 mU/ml). Skewness test showed LH data in F-and L-phase as analyzable, but was not so good in Komolgorov-Smirnov's test (F-phase: Statistic 0.234, Sig. 0.001 and L-phase: Statistic 0.297, Sig. 0.002). LH/FSH ratio in cases (2.44±1.73, median 2.00) was significantly higher ($p<0.01$) as compared to controls (0.82±0.42, median 0.76), but was not significantly higher ($p>0.05$) in F-phase controls (0.82±0.42, median 0.76) as compared to L-phase controls (0.84±0.41, median 0.80).

FSH ≥12 mU/ml ($p=0.000$, Pearson $\chi^2=13.117$, Odds ratio=8.036), LH ≥10 mU/ml ($p=0.000$, Pearson $\chi^2=16.769$, Odds ratio=14.25) and LH/FSH ratio ≥1 ($p=0.000$, Pearson $\chi^2=18.988$, Odds ratio=17.5) cut offs are significantly associated with secondary amenorrhea as revealed by chi-square and risk analysis (Table-5). When sensitivity and specificity of these cut offs (Table-5) were calculated in diagnosis of diseased persons and ruling out of healthy subjects, LH/FSH ratio ≥1 was stronger (Sensitivity=84.0%, specificity=77.0%) as compared to FSH (Sensitivity=76.0%, specificity=69.2%) and LH (Sensitivity=84.0%, specificity=73.0%).

DISCUSSION

Secondary amenorrhea may be the manifestation of a number of medical conditions, ranging from pregnancy to a pituitary tumor. After ruling out pregnancy, thyroid disease, and hyperprolactinemia as potential diagnoses, the remaining causes of secondary amenorrhea classified as normogonadotropic amenorrhea, hypogonadotropic hypogonadism, and hypergonadotropic hypogonadism; each associated with specific etiologies.[5,17,18] Hypothalamus and pituitary involvement can be traced by measuring serum gonadotropins and prolactin. In case on normo-prolactinomic and elevated gonadotropins in serum, as in this study, the hypothalamus and

pituitary involvement is ruled out, and the diagnosis forwards towards end organ involvement. Incidently, all the cases presented no signs and symptoms of androgen excess, so the diagnosis goes towards ovarian failure (Hypergonadotropic hypogonadism).

Median age of menarche in controls (14 years) was similar to those in cases (Table-2). There was not significant ($p>0.05$) difference in the mean age of menarche among the cases (13.98±1.25 years) and controls (13.43±.863 years). However, there was a wider range of age of menarche in cases (12-17 years) than that of controls (12-15 years). The median age of menarche in USA, according to the National Health Examination Survey, is 12.77 years, with African-American females reaching menarche a few months before Caucasian females. Although almost 90.0% of females have achieved menarche by the time they reach Tanner stage for breast and pubic hair development, there is a mean of slightly more than 2 years (range, 0.5 to 5.75 years) between the onset of breast development and menarche.[20,21] Variability in the menstrual start date, depending on the length of the month, or the possibility of having menses twice during the same calendar month makes the individual difficult in answering the exact cycle interval. We found, through questionnaire, previous cycle interval in cases (Mean±SD:34±10 days, median 31 days, range 16-60 days) and regular cycle interval in controls (Mean±SD:30.32±3.038, median 30 days, range 24-38 days) also presented wider range as compared to Americans (Range 21-35 days). Americans have less than 0.5% of women having cycles shorter than 21 days and less than 1.0% having cycles lasting more than 35 days.[20] Women of this region (Eastern Nepal) bleed for up to 10 days in cases (Mean±SD: 4.65±1.92 days, median 4 days, range 2-10 days) and 8 days in controls (Mean±SD: 4.08±1.1 days, median 4 days, range 3-8 days). US women bleed for 3 to 7 days and experience approximately 30 to 40 mL of blood loss. Cycles lasting 8 to 10 days and/or having more than 80 mL of blood loss are considered abnormal.[22]

Both primary and secondary forms of ovarian failure are biochemically characterized by high gonadotropins (LH and FSH) (hypergonadotropic amenorrhea) and low levels of gonadal hormones (estrogens and inhibins) and the elevation of FSH is usually more marked than that of LH and an FSH value >30 U/L is indicative of ovarian failure.[21,22] We observed increased level ($p<0.01$) of FSH in cases (15.38±7.24 mU/ml, median 15 mU/ml) as compared to that of controls (9.38±6.34 mU/ml, median 8.55 mU/ml). In premature ovarian failure (POF), amenorrhea, persistent estrogen deficiency, and elevated FSH levels occur prior to the age of 40, and this condition affects 1.0-5.0% of women.[23,24] Ovarian function may fluctuate, with increasingly irregular menstrual cycles before the final depletion of oocytes and permanent ovarian failure. The resulting fluctuation in gonadotropin levels accounts for the lack of accuracy associated with a single FSH value.[24] The degree of elevation in LH level in cases (35.44±24.35 mU/ml, median 36.5 mU/ml) as compared to controls (7.58±6.604 mU/ml, median 4.5 mU.ml) was more marked than the elevation of FSH. It was almost 9 times elevation of LH level in cases; where as elvation of FSH was only a two times as compared to that of controls. This is suggestive of LH receptor mutation presenting secondary amenorrhea, which is generally found in premature ovarian failure (POF). Defects in LH receptor are typically associated with a serum LH elevation (> 10 U/L) more pronounced than that of serum FSH[21]. But the elevation of FSH was not as pronounced as was recorded by Taylor *et al*[12] and Paolo *et al*[21] (FSH>25 and 30 mU/ml respectively) in POF. It means that the cases, to be diagnosed in this region seek a new cut off level for premature ovarian failure and suspect the cases might be associated with LH receptor mutation. As we observed increased LH:FSH ratio (Table-2) in cases (2.44±1.73, median 2) as compared to controls (0.82±0.42, median 0.76), and sensitivity (84.0%) and specificity (77.0%) of LH:FSH≥1 were also higher as compared to that of FSH≥12mU/ml (Sensitivity 76.0%, specificity 69.2%) and LH≥10mU/ml (Sensitivity 84.0%, specificity 73.0%), we suggest LH:FSH ratio≥1 as a better marker of the cases with secondary amenorrhea in the study group of this region.

ACKNOWLEDGEMENTS

Authors acknowledge the infrastructural and research support from BPKIHS, Dharan while undertaking this study.

REFERENCES

1. Stedman's Medical Dictionary. 27th ed. Philadelphia: Lippincott Williams and Wilkins, 2000:56.
2. Herman-Giddens ME, Slora EJ, Wasserman RC *et al.* Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatr* 1997; 99: 505-12.
3. The Practice Committee of the American Society for Reproductive Medicine. Current evaluation of amenorrhea. *Fertil Steril* 2004; 82 (suppl 1): S33-9.

4. American College of Obstetricians and Gynecologists. Amenorrhea (ACOG Technical Bulletin 128). Washington, D.C.: ACOG, 1989.
5. Speroff L, Fritz MA. Amenorrhea. In: Clinical gynecologic endocrinology and infertility. 7th ed. Philadelphia, PA.: Lippincott Williams and Wilkins, 2005; 401-64.
6. Munster K, Helm P, Schmidt L. Secondary amenorrhea: prevalence and medical contact-a cross-sectional study from a Danish county. *Brit J Obstet Gynaecol* 1992; 99: 430-3.
7. Marshall WA, Tanner JM. Variations in patterns of pubertal changes in girls. *Arch Dis Child* 1969; 44: 291-303.
8. Speroff L, Glass RH, Kase NG. Normal and abnormal sexual development. 1999, In: Clinical gynecologic endocrinology and infertility. 6th ed. Baltimore, Md.: Lippincott Williams and Wilkins, 339-79.
9. Ferin M, Jewelewicz R, Warren MP. 1993, The Menstrual cycle, New York: Oxford University Press.
10. Rosenfeld JA. Evaluating and treating secondary amenorrhea. *Women's Health Primary Care* 2001: 4: 359-67.
11. Insler V. Gonadotophin therapy: new trends and insights. *Int'l J Fertil* 1988; 33: 85-97.
12. Taylor AE, Adams JM, Mulder JE *et al*. A randomized, controlled trial of estradiol replacement therapy in women with hypergonadotropic amenorrhea. *J Clin Endocrinol Metabol* 1996; 81: 3615-21.
13. Anasti JN. Premature ovarian failure: an update. *Fertil Steril* 1998; 70: 1-15.
14. Glueck CJ, Wang P, Fontaine R *et al*. Metformin-induced resumption of normal menses in 39 of 43 (91.0%) previously amenorrheic women with the polycystic ovary syndrome. *Metabolism* 1999; 48: 511-9.
15. Rebar RW, Connolly HV. Clinical features of young women with hypergonadotropic amenorrhea. *Fertil Steril* 1990; 53: 804-10.
16. Speroff L, Glass RH, Kase NC. 1983. Clinical gynecologic endocrinology and infertility, 3rd ed. Baltimore: Williams and Wilkins.
17. Kiningham RB, Apgar BS, Schwenk TL. Evaluation of amenorrhea. *Amer Fam Physician* 1996; 53: 1185-94.
18. Pickett CA. Diagnosis and management of pituitary tumors: recent advances. *Prim Care* 2003; 30: 765-89.
19. Michelle PW. Evaluation of Secondary Amenorrhea (Clinical Review 77). *J Clin Endocrinol Metabol* 1996; 8: 437-42.
20. Paula K Braverman, Steven J Sondheimer. Menstrual Disorders. *Pediatr Review* 1997; 18: 17-26.
21. Paolo BP, Luca P. Premature ovarian failure (Review). *Orphanet J Rare Dis* 2006; 1: 9.
22. Jones GS, De Moraes-Ruehsen M. A new syndrome of amenorrhea in association with hypergonadotropism and apparently normal ovarian follicular apparatus. *Amer J Obstet Gynecol* 1969; 104:597–600.
23. Van Campenhout J, Vauclair R, Maraghi K. Gonadotropin-resistant ovaries in primary amenorrhea. *Obstet Gynecol* 1972; 40: 6–12.
24. Conway GS, Kaltsas G, Patel A, Davies MC, Jacobs HS. Characterization of idiopathic premature ovarian failure. *Fertil Steril* 1996; 65: 337–41.

Table-1: Distribution of variables in cases and controls

	Total Cases (n=50)				**Total Control (n=52)**			
	Skewness		**Komolgorov-Smirnov(a)**		**Skewness**		**Komolgorov-Smirnov(a)**	
	Sta.	**SE**	**Sta.**	**Sig*.**	**Sta.**	**SE**	**Sta.**	**Sig*.**
Age, years	0.469	0.464	0.124	0.200*	0.511	0.456	0.201	0.009
Pulse, min^{-1}	0.498	0.464	0.122	0.200*	-0.249	0.456	0.147	0.156
SBP, mmHg	-0.469	0.464	0.171	0.058	0.475	0.456	0.178	0.034
DBP, mmHg	0.590	0.464	0.174	0.049	0.655	0.456	0.326	0.000
FSH, mU/ml	0.936	0.464	0.144	0.192	0.921	0.456	0.139	0.200*
LH, mU/ml	0.288	0.464	0.150	0.149	0.996	0.456	0.234	0.001
PRL, ng/ml	1.54	0.464	0.145	0.187	0.402	0.456	0.108	0.200*
LH/FSH	0.797	0.464	0.096	0.200*	0.824	0.456	0.139	0.200*
Weight, Kg	0.388	0.464	0.193	0.017	0.011	0.456	0.107	0.200*
Height, cm	-0.709	0.464	0.140	0.200*	-2.097	0.456	0.188	0.018
AM, years	0.712	0.448	0.198	0.008	-0.821	0.580	0.344	0.000
CI, days	1.51	0.481	0.243	0.001	0.870	0.464	0.183	0.038
DF, days	1.388	0.481	0.254	0.000	2.174	0.472	0.364	0.000

= This is a lower bound of the true significance, a = Lilliefors Significance Correction, Sta.= Statistic, SE = Standard error, Sig. = Significance, AM = Age of menarche, CI = Cycle interval, DF = Duration of flow

Table-2: Distribution of different parameters in cases and controls

	Total Cases (n=50)			Total Control (n=52)			p value
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	
Age, years	27.56±5.33	27	19-40	27.88±6.40	26.5	21-40	>0.5
Pulse, min^{-1}	82.92±8.02	82	72-100	80.92±8.17	83.50	60-100	>0.1
SBP, mmHg	113.76±10.14	112	90-130	108.35±12.36	110.00	90-140	>0.05
DBP, mmHg	78.80±10.38	78	60-100	73.65±8.21	70.00	60-90	>0.05
FSH, mU/ml	15.38±7.24	15	4-37	9.38±6.34	8.550	1-25	<0.01**
LH, mU/ml	35.44±24.35	36.5	1-80	7.58±6.604	4.20	1-22	<0.01**
PRL, ng/ml	11.18±7.47	10.5	1-35	13.18±4.793	12.50	5-24	>0.1
LH/FSH	2.44±1.73	2	0.2-6.64	0.82±0.42	0.76	0.17-1.9	<0.01**
Weight, Kg	53.12±11.04	51	32-77	52.58±8.14	53.00	35-70	<0.5*
Height, cm	151.84±6.79	152	138-161	153.69±9.09	155.00	120-165	>0.5
AM, years	13.98±1.25	14	12-17	13.43±.863	14	12-15	>0.1
CI, days	34±10	31	16-60	30.32±3.038	30	24-38	>0.1
DF, days	4.65±1.92	4	2-10	4.08±1.1	4	3-8	>0.1

p<0.01** = Significance at 1.0% level, p<0.05* = Significance at 5.0% level
AM = Age of menarche, CI = Cycle interval, DF = Duration of flow

Table-3: Distribution of variables in F- and L-phase controls

	F-phase controls (n=24)				L-phase controls (n=28)			
	Skewness		Komolgorov-Smirnov(a)		Skewness		Komolgorov-Smirnov(a)	
	Sta.	SE	Sta.	Sig*.	Sta.	SE	Sta.	Sig*.
Age, years	0.511	0.456	0.201	0.009	0.552	0.597	0.265	0.009
Pulse, min^{-1}	-0.249	0.456	0.147	0.156	-0.480	0.597	0.160	0.200*
SBP, mmHg	0.475	0.456	0.178	0.034	-0.081	0.597	0.211	0.092
DBP, mmHg	0.655	0.456	0.326	0.000	0.557	0.597	0.304	0.001
FSH, mU/ml	0.921	0.456	0.139	0.200*	0.901	0.597	0.169	0.200*
LH, mU/ml	0.996	0.456	0.234	0.001	1.292	0.597	0.297	0.002
PRL, ng/ml	0.402	0.456	0.108	0.200*	0.201	0.597	0.183	0.200*
LH/FSH	0.824	0.456	0.139	0.200*	1.008	0.597	0.158	0.200*
Weight, Kg	0.011	0.456	0.107	0.200*	-0.573	0.597	0.158	0.200*
Height, cm	-2.097	0.456	0.188	0.018	-1.624	0.597	0.199	0.136

* = This is a lower bound of the true significance, a = Lilliefors Significance Correction
Sta. = Statistic, SE = Standard error, Sig. = Significance
AM = Age of menarche, CI = Cycle interval, DF = Duration of flow

Table-4: Distribution of different parameters in F- and L-phase controls

	F-phase controls (n=24)			L-phase controls (n=28)			p value
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	
Age, years	27.88±6.4	26.5	21-40	28.07±7.01	25.5	21-40	>0.5
Pulse, min^{-1}	80.92±8.17	83.5	60-100	81.93±9.85	83.5	60-100	>0.1
SBP, mmHg	108.35±12.34	110	90-140	107.86±12.51	110	90-130	>0.5
DBP, mmHg	73.65±8.21	70	60-90	73.21±7.75	70	60-90	>0.5
FSH, mU/ml	9.38±6.34	8.55	1-25	7.23±4.07	6.3	3-16	>0.05
LH, mU/ml	7.58±6.60	4.2	1-22	6.49±5.77	3.85	2-19	<0.05*
PRL, ng/ml	13.18±4.79	12.5	5-24	12.51±3.68	12.5	5-20	>0.1
LH/FSH	0.82±0.42	0.76	0.71-1.9	0.84±0.41	0.80	0.2-1.9	>0.5
Weight, Kg	52.58±8.14	53	35-70	51.79±7.89	53.5	35-65	>0.5
Height, cm	153.69±9.09	155	120-165	152.61±11.96	155	120-165	>0.1

p<0.05* = Significance at 5.0 lavel

Table-5: Risk and sensitivity/specificity analyses of hormonal parameters

	Pearson $\chi 2$	Likelihood Ratio	p	Odds Ratio	95.0% Confidence Interval		Sensitivity for cut off	Specificity for cut off
					Lower	Upper		
FSH ≥12 mU/ml	13.117	13.581	0.000	8.036	2.465	26.192	76.0%	69.2%
LH≥10 mU/ml	16.769	17.937	0.000	14.25	3.598	56.434	84.0%	73.0%
LH/FSH≥1	18.988	20.45	0.000	17.5	4.292	71.362	84.0%	77.0%