Normative range of various serum hormonal parameters among Indian women of reproductive age: ICMR-PCOS task force study outcome

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Summary

Background The hormonal profile varies considerably with age, gender, ethnicity, diet or physiological state of an individual. Limited population-specific studies have studied the variations in hormonal parameters among apparently healthy women. We aimed to analyse the biological reference interval for various hormonal parameters in the reproductive-aged healthy Indian women.

Methods Out of 3877 participants that were clinically evaluated, 1441 subjects were subjected to laboratory investigations. All participants underwent a detailed clinical, biochemical and hormonal profiling. The hormone analysis was carried out at a single centre using a uniform methodology. Among the participants evaluated for biochemical and hormonal parameters, subjects that presented any abnormal profile or had incomplete investigations (n = 593) were excluded for further analysis.

Findings The mean age (±SD) of the subjects retained in the final analysis (n = 848) was 29.9 (±6.3) years. In the present study, the biological reference interval (2.5th–97.5th centile) observed were: serum T4: µg/dL (5.23–12.31), TSH: µg/mL (0.52–4.16) and serum prolactin: ng/mL (5.13–37.35), LH: mIU/mL (2.75–20.68), FSH: mIU/mL 2.59–15.12), serum total testosterone: ng/mL (0.06–0.68), fasting insulin: mIU/mL (1.92–39.72), morning cortisol: µg/dL (4.71–19.64), DHEAS:µg/dL (50.61–342.6) and SHBG: nmol/L (21.37–117.54). Unlike T4, TSH, LH, and E2, the biological reference interval for prolactin, FSH, testosterone, C-peptide insulin and DHEAS varied when the subjects were stratified by age ($p < 0.05$). The comparative analysis showed marginal differences in the normative ranges for the hormones analysed among different populations.

Interpretation Our first large composite data on hormonal measures will benefit future endeavours to define biological reference intervals in reproductive-aged Indian women.

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Research in context

Evidence before this study

Globally, limited population-specific studies have reported the variations in hormonal parameters among apparently healthy women. However, no such large countrywide study has been carried out in India.

Added value of this study

Our first large composite data on hormonal measures generated by a uniform methodology on a cohort of reproductive-age women truly representative of the reproductive age across the country would provide the biological reference range for the studies hormones.

Implications of all the available evidence

The study is likely to fill the vacuum of biological reference interval data for various hormones among women of reproductive age.

Introduction

Women's health requirements are dynamic and pulsatile not only to their growing age but to their sexual and reproductive functions as well.[1] The reproductive phase is the most critical period in a woman's life that alters the physiology of the body, and wherein any dysregulation can lead to serious health consequences. Menstruation-related illnesses and uterine or ovarian diseases are the specific physiological alterations in women throughout this stage of development. Although, the physiological changes that take place during the reproductive phase usually follow a uniform order, the pace of these changes varies depending on external factors (e.g. food habits, lifestyle, etc).[2] The clinical significance of female reproductive physiology is relevant for a variety of clinical problems, including menopause, pregnancy, infertility, and adolescent entrance into childbearing years.

A panel of blood tests including biochemical and hormonal profiles, routinely prescribed by healthcare professionals, are critical in representing the normal functioning of the body. Of late, with the recent advances in quantitative estimation approaches, hormone profiling is in a critical transition period.[3,4] While the precision in estimation has significantly increased in the recent past, the accuracy of biological reference intervals employed, not only depends upon the assay type but on other indicators as well.[5] Moreover, the hormonal parameters fluctuate significantly depending upon the individual's age, physiological state of the body, gender, ethnicity, nutritional status, lifestyle, and use of medications. Among these factors, age and gender are two pivotal drivers of variations in hormonal parameters. Besides, the biological reference intervals of ready-to-use kits provided by different vendors lack comprehensive information on such issues.[5]

The biological reference interval for hormones is critical in reproductive endocrinology, particularly in clinical assessments of the presence of any other reproductive or metabolic anomalies in women. A functional assessment of the hypothalamus-pituitary-gonadal axis requires measurements of the hormones, serum testosterone, prolactin, cortisol, estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), dehydroepiandrosterone sulphate (DHEAS) and sex hormone binding globulin (SHBG). The generation of biological reference intervals for hormonal parameters in a country is essential for enhancing the standard of care, because failure to do so could result in wasteful spending or denial of care to those in need. These reference values for hormonal parameters in healthy individuals are subjected to variations due to analytical and pre-analytical factors. For that purpose, their determination for every country, even every region, is of major importance. Health data in India is scarce and its quality is a perennial source of contention.[6] This scarcity can be attributed to India's large heterogenous population, and inaccessibility of the available data.[6] For a normative range of hormonal parameters, India also lacks such data, especially for healthy women of reproductive age. Therefore, in the current large study, we attempted to evaluate the biological reference interval for various hormones in reproductive-aged Indian women.

Methods

Study design

The detailed protocol of the study subjects is published elsewhere.[7] Briefly, this national cross-sectional study envisioned to estimate the burden, and associated risk factors of polycystic ovary syndrome (PCOS), recruited subjects from 2018 to 2022 in six zones (North India, Northeast, South India, Eastern India, Central India, and Western India) spreading across the country, involving 10 regional centres that represent a majority of ethnic groups. The screening of the subjects was done employing a uniform methodology using a pre-designed screening questionnaire, designed by the coordinating centre. The questionnaire was pilot tested and then circulated among various study sites. The study was conducted following the guidelines enshrined under the Helsinki 1975 declaration and was approved by the

institute Ethics Committees (IECs) of the respective study institutions. Informed written consent was taken from all the subjects before their enrolment. The data on PCOS cases is not reported here.

Study population

The study subjects included apparently healthy women aged 18–40 years from the urban and rural areas of the country. Inclusion criteria included those participants who lived in the area for at least a year, were non-pregnant, with regular menstrual cycles, apparently healthy, and were willing to participate. Subjects with any previously confirmed disease or disorder, or were on any medication for the same, and those who didn't want to participate in the study were excluded. We used a structured questionnaire to record their socio-demographic characteristics such as place of residence, age, marital status, level of education, occupation and socioeconomic status,[8] religion, and type of family followed by uniform clinical and laboratory assessment.

Clinical and laboratory assessment

Subjects underwent a detailed clinical history and physical examination which included measurement of parameters like body weight, height, BMI, and waist circumference measurements by using standard calibrated instruments (SECA 213, Hamburg Germany), and blood pressure measurements (Omron HEM7120). A mean of three readings was taken as the final value for these parameters. All subjects were further evaluated for biochemical and hormonal parameters. The oral glucose tolerance test (OGTT) was performed after an overnight fast of 10–12 h. A total of 5 mL of venous blood was drawn in a fasting state between day 2 and day 7 of the menstrual cycle. The samples were immediately separated in a cold centrifuge and aliquoted for hormones and other required laboratory investigations. The aliquots for hormones and other required investigations were shipped to the co-ordinating center in a cold chain (dry ice) and were stored at –80 °C until the assay. The quantification of hormones (including serum total T4, thyroid-stimulating hormone (TSH), total testosterone, 17-OHP, cortisol, prolactin, luteinizing hormone, and follicle-stimulating hormone, E2, C-peptide, insulin, and DHEAS) and SHBG (a transport carrier that binds and regulates the biological activity of oestrogen and androgens) was conducted using electro-chemiluminescent immunosorbent assay on Cobas e411 (Roche Diagnostics).

Quality control assessment

The standards (reagents with known concentrations) were run daily to validate the accuracy of the hormone analyser and was calibrated when required as per the manufacturer's instructions.

Data management and statistical analysis

Data were entered into Microsoft Excel database. The biological reference intervals were calculated using nonparametric methods. Besides the median, reference intervals were determined at 2.5th and 97.5th percentiles. Subjects were further stratified by age, BMI (WHO-Asian BMI criteria Normal: 18.5–22.9, Overweight: 23–27.5, and Obese: ≥27.5 kg/m^2) and WHO general population BMI criteria (Normal: 18–24.9, Overweight: 25–29.9, Obese: ≥30 kg/m^2), and residence (rural and urban) for the estimation of the reference intervals. Mean, median and standard deviations were computed for each of the biochemical and hormonal parameters of the study subjects. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS; Version 26, IBM). Kruskal–Wallis test was used to determine the differences in the data after stratification by age, BMI, and place of residence. All tests were considered significant with two-tailed $p < 0.05$. Moreover, a literature survey for comparative analysis was also performed for comparing the data from the present study with the already published data.

Role of funding source

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Results

The details of all the subjects recruited in the study are provided in Fig. 1. A total of 7107 apparently healthy controls were enrolled for the present study out of which 3877 were screened for detailed socio-demographic

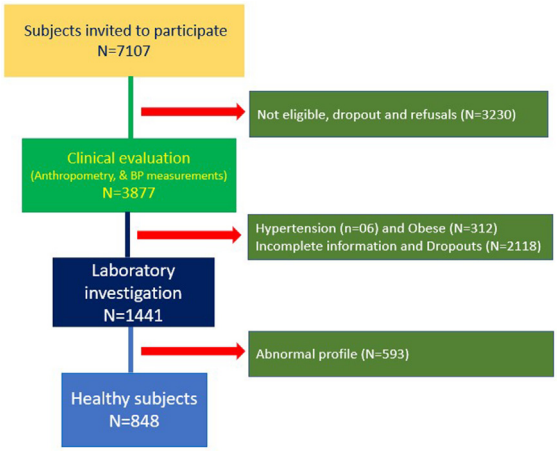

Fig. 1: **Pert chart showing the distribution of subjects enrolled in the current study.**

information. Among the screened subjects, 1441 participants were assessed for hormonal parameters. After excluding the subjects (n = 593) with any underlying hormonal imbalance or comorbidities like subclinical hypo/hyperthyroidism, hyperprolactinemia, premature ovarian failure, or exogenous Cushing syndrome, a total of 848 subjects were retained for the analysis. The majority of subjects were unemployed (64.8%) and represented the lower and middle socioeconomic class (31%) of the population. Based on the population structure of the country,[9] expectedly, most of the participants were Hindu (71%) followed by Muslim subjects (26.1%).

The mean age and BMI (±SD) of the subjects recruited in the study were 29.9 (±6.3) and 23.2 (±3.3) respectively. The mean values (±SD) of various hormones analysed are presented in Table 1 and all the centile distribution is presented in Table 2. The biological reference interval (2.5th–97.5th centiles) in our cohort were: serum T4: µg/dL (5.23–12.31), TSH: µg/mL (0.52–4.16) and serum prolactin: ng/mL (5.13–37.35), LH: mIU/mL (2.75–20.68), serum total testosterone: ng/mL (0.06–0.68), fasting insulin: mIU/mL (1.92–39.72), FSH (mIU/mL) (2.59–15.12), E2 (pmol/L) (52.03–439.91), fasting plasma insulin (mIU/mL) (2.00–15), morning cortisol (µg/dL) (4.71–19.64), DHEAS:µg/dL (50.61–342.6), and SHBG: nmol/L (21.37–117.54) (Table 3). The biological reference intervals (median and 2.5th–97.5th percentiles) of prolactin, FSH, testosterone, E2, C-peptide, fasting insulin and DHEAS varied when stratified by age ($p < 0.05$) (Table 3). The data on biological reference intervals for the studied hormones reported in the relevant published literature across different populations is presented in Table 4. The comparative analysis showed marginal differences in the normative ranges for these hormones among different populations. The biological reference intervals for prolactin, C-peptide, T4, DHEAS, fasting insulin, and SHBG varied when the subjects were stratified by BMI ($p < 0.05$) (Supplementary Tables, S1–S3). The data on anthropometrical and biochemical indicators are not reported here.

Total subjects, N = 848

Parameter	Mean ± SD
Age (years)	29.9 ± 6.3
BMI (kg/m$^{2)}$	23.2 ± 3.3
Systolic Blood Pressure (mm/Hg)	112.4 ± 8.7
Diastolic Blood Pressure (mm/Hg)	74.1 ± 6.8
Serum T4 (µg/dL)	8.09 ± 1.91
Serum TSH (µl/mL)	2.26 ± 1.00
Serum Prolactin (ng/mL)	15.46 ± 8.28
Serum Cortisol (8.00 am) (µg/dL)	10.38 ± 4.13
DHEAS (µg/dL)	161.3 ± 76.93
Serum LH (mIU/mL)	8.46 ± 4.97
Serum FSH (mIU/mL)	6.45 ± 3.14
Serum Total Testosterone (ng/mL)	0.28 ± 0.17
SHBG (nmol/L)	65.19 ± 28.17
Estradiol (E2) (pmol/L)	216.27 ± 104.8
C-peptide (Fasting) (ng/mL)	1.96 ± 1.13
Plasma Insulin (Fasting) mIU/mL	11.72 ± 9.03

BMI, Body mass index; TSH: Thyroid stimulating hormone; LH: Luteinising hormone; FSH: Follicle stimulating hormone; DHEAS, Dehydroepiandrosterone sulphate; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol.

Table 1: **Distribution of clinical and hormonal parameters.**

Discussion

The present country-wide study was aimed to frame the biological reference values of clinically important hormones among Indian women of reproductive age. To the best of our knowledge, this is the first large pan-India study to report the normative values of the studied hormones. The data on detailed clinical and biochemical and hormonal examination from a total of 1441 apparently healthy women, aged between 18 and 40 years, were analysed for the study. After the exclusion of various comorbidities, a total of 848 healthy subjects were further analysed for generating hormonal reference intervals.

Serum levels of hormones in women fluctuate significantly during different stages of life, starting from menarche to menopause.[24] Age and health status are two characteristics that have an impact on the levels of sex hormones, with age being the primary influencer in women.[25] Thyroid dysfunction (clinical or biochemical) is a common clinical condition with its presentation modulated by gender, ethnicity, genetic predisposition, and iodine status.[26] An expanding set of research outlines the negative consequences of subclinical thyroid illness in women, thereby drawing attention to early intervention of T4 therapy. This may progress over a period of time to overt hypothyroidism particularly in females, those with thyroid peroxidase antibody positivity and a TSH of more than 10 mIU/mL.[27] Thyroid function reference intervals for adults have been thoroughly established in various populations including India, where Marwaha et al. reported the TSH level of 2.2 ± 0.9 mIU/L (mean) for adult Indian women.[23] The United States National Health and Nutrition Examination Survey III (NHANES-III) similarly evaluated a disease-free population and observed significantly lower TSH and T4 values as compared to the general population.[21] The results for T4 and TSH from our study are consistent with the findings of NHANES-III report. However, our normative values for T4 and TSH vary from this earlier Indian study by Marwaha et al.[23] Unlike ours, the study by Marwaha et al.[23] was based on subjects from a single Indian city (Delhi), with a smaller sample size and the values presented are 3rd and 97th centile. On further stratification of the subjects based on age, the aggregated normative values for serum T4 and

Parameters	Centiles							
	2.5th	5th	10th	25th	50th	75th	90th	97.5th
Serum T4 (μg/dL)	5.23	5.40	5.64	6.62	7.91	9.24	10.80	12.31
Serum TSH (μl/mL)	0.52	0.75	1.01	1.50	2.18	3.00	3.73	4.16
Serum Prolactin (ng/mL)	5.13	6.12	6.77	9.41	13.20	18.78	27.66	37.35
Serum Cortisol (8.00 am) (μg/dL)	4.17	4.59	5.47	7.11	9.65	13.00	16.24	19.64
DHEAS (μg/dL)	50.61	59.31	73.98	101.50	148.50	203.20	276.20	342.6
Serum LH (mIU/mL)	2.75	3.21	3.65	4.87	7.03	10.18	16.24	20.68
Serum FSH (mIU/mL)	2.59	2.88	3.49	4.51	5.86	7.28	9.85	15.12
Serum Total Testosterone (ng/mL)	0.06	0.08	0.10	0.16	0.25	0.36	0.49	0.68
SHBG (nmol/L)	21.37	23.67	29.94	43.04	62.88	86.65	108.90	117.54
Estradiol (E2) (pmol/L)	52.03	57.22	70.70	140	200	280	377.66	439.91
C-peptide (ng/mL)	0.22	0.43	0.68	1.10	1.68	2.79	3.89	4.09
Plasma Insulin (Fasting) mIU/mL	1.92	2.20	3.37	5.51	9.25	14.82	22.97	39.72

TSH: Thyroid stimulating hormone; LH: Luteinising hormone; FSH: Follicle stimulating hormone; DHEAS: Dehydroepiandrosterone sulphate; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol.

Table 2: **Reference intervals (2.5th–97.5th percentiles) of various hormonal parameters among reproductive age Indian women.**

TSH didn't change (P > 0.05) suggesting our reference can be applied to all the studied age subgroups. Moreover, unlike a large Chinese study,[28] the median values for T4 were elevated in higher BMI groups in our study. While our study was based on female subjects of reproductive age, this earlier Chinese study did not stratify the subjects based on gender.[28]

The normative ranges for quantifying prolactin secretion show it depends upon multiple biological or clinical factors, including gender, age, BMI, other hormones, nutrition, stress, physical activity, medications and renal disease.[29] In the current study, we found our reference interval for serum prolactin levels was higher in younger women (18–25 year group) when compared with older women (≥26 years). Earlier studies have also found a decrease in prolactin levels with the increase in the age of women.[30,31] Moreover, in the current study, we did not observe any significant difference in the biological reference range of prolactin with the increase in BMI. More replicative studies are warranted to substantiate these findings.

DHEA plays a pivotal physiological role in the reproductive tissues, bone, mood, cognition, vasculature, breast, muscle, and other systems in women.[32] Nearly all androstenedione produced in the adrenal gland is synthesised from DHEA in humans.[33] Elevated DHEAS is considered a marker for hyperactive adrenal cortex production of androgens.[32] Majority of women with PCOS are reported to have increased DHEAS levels.[34] Similar to the published literature, in the current study we found the median levels of DHEAS decreased with age.[35–37]

	Total N = 848			18–25 Years N = 236			26–33 Years N = 311			34–40 Years N = 301			[a]p-value
Parameters	2.5	50	97.5	2.5	50	97.5	2.5	50	97.5	2.5	50	97.5	
Serum T4 (μg/dL)	5.23	7.91	12.31	5.29	7.89	12.30	5.17	7.80	12.74	5.26	8.12	12.16	0.062
Serum TSH (μg/mL)	0.52	2.18	4.16	0.48	2.11	4.11	0.65	2.27	4.17	0.40	2.17	4.17	0.746
Serum Prolactin (ng/mL)	5.13	13.20	37.35	6.00	14.20	38.08	4.61	12.50	35.85	5.04	12.99	37.65	0.008
Serum Cortisol (8.00 am) (μg/dL)	4.17	9.65	19.64	3.64	9.40	19.79	4.45	9.60	19.60	4.20	10.65	19.90	0.141
DHEAS (μg/dL)	50.61	148.50	342.6	53.26	169.40	337.10	45.67	145.80	343.23	49.27	134.80	352.15	<0.001
Serum LH (mIU/mL)	2.75	7.03	20.68	2.66	6.88	20	2.75	7.17	20.76	2.61	6.98	24.72	0.562
Serum FSH (mIU/mL)	2.59	5.86	15.12	2.37	5.81	13.52	2.74	5.74	13.57	2.73	6.11	18.48	0.025
Serum Total Testosterone (ng/mL)	0.06	0.25	0.68	0.07	0.27	0.68	0.06	0.25	0.67	0.06	0.24	0.77	0.037
SHBG (nmol/L)	21.37	62.88	117.54	21.78	65.21	118.10	22.12	61.53	116.20	19.57	62.40	118.25	0.051
Estradiol (E2) (pmol/L)	52.03	200	439.91	54.93	200.00	433.24	51.21	200	450.03	50.36	200	430.45	0.068
C-peptide (ng/mL)	0.22	1.68	4.09	0.42	1.78	4.09	0.21	1.56	4.10	0.20	1.85	4.11	0.008
Plasma Insulin (Fasting) (mIU/mL)	1.92	9.25	39.72	1.64	8.17	36.31	1.79	9.15	37.34	2.18	10.24	43.04	0.014

TSH: Thyroid stimulating hormone; LH: Luteinising hormone; FSH: Follicle stimulating hormone; DHEAS: Dehydroepiandrosterone sulphate; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol. [a]Kruskal Wallis test.

Table 3: **Reference intervals (2.5th, 50th, and 97.5th percentiles) of various hormonal parameters stratified by age in Indian women of reproductive age (18–40 years).**

Based on the findings of a recent meta-analysis, serum cortisol is believed to have increased circulating levels in the follicular phase of the menstrual cycle.[38] The cause–effect relationship between obesity and cortisol is poorly understood. Moreover, the data on its correlation with BMI and age is varying.[39] Some studies have reported low cortisol levels in obese than normal-weight subjects[39,40] while another study shows elevation in the cortisol levels with age.[41] In the current study, we observed a marginal rise in the median cortisol levels with age or BMI but did not attain statistical significance.

In a typical menstrual cycle, E2 levels are lowest during the early follicular phase of monthly bleeding and rise subsequently until ovulation. Just before

Parameter	Age (Years)	Reference interval	Ethnicity	Author	Method (N)
Serum Total Testosterone (ng/mL)	16–45	<0.49	North Europe	Neale et al., 2013[10]	LC-MS (90)
	18–40	0.16–0.79	North America	Pesant et al., 2012[11]	CICLA (44)
	18–54	0.11–0.43	South Europe	Fenelli et al., 2011[12]	ID-LC-MS/MS (–)
	19–49	0.12–0.55	Central Europe	Eisenhofer et al., 2017[13]	LC-MS (525)
	18–40	0.07–0.68	Indian (South Asian)	Present study	ECLIA (848)
SHBG (nmol/L)	18–19	16–212	North Europe	Sorenser et al., 2007[14]	TR-IFMA, (212)
	18–40	21–105	North Europe	Pesant et al., 2012[11]	CICLA, (44)
	18–40	21.37–117.54	Indian (South Asian)	Present study	ECLIA (848)
DHEAS (µg/dL)	16–40	13.3–210	Central Europe	Kulle et al., 2017[15]	CLIA (–)
	19–49	43.8–284.8	Central Europe	Eisenhofer et al., 2017[13]	LC-MS/MS (298)
	18–40	50.61–342.6	Indian (South Asian)	Present study	ECLIA (848)
LH (mIU/mL)	18–48	0.5–7.7	South America	Woloszynek et al., 2015[16]	IFMA, (133)
	18–40	2.75–20.68	Indian (South Asian)	Present study	ECLIA (848)
FSH (mIU/mL)	18–48	2.3–9.8	South America	Woloszynek et al., 2015[16]	IFMA, (133)
	20	2.0–8.5	Central Africa	Okunola et al., 2016[17]	ELISA (65)
	25	3.0–7.0	Central Africa	Okunola et al., 2016[17]	ELISA (65)
	35	6.5–12.5	Central Africa	Okunola et al., 2016[17]	ELISA (65)
	40	3.0–15.0	South Europe	Grisendi et al., 2014[18]	Chemiluminescent (192)
	18–40	2.59–15.12	Indian (South Asian)	Present study	ECLIA (848)
Cortisol (F) (ug/dL)	16–40	5–29.4	Central Europe	Kulle et al., 2017[15]	CLIA (–)
	18–54	5.44–29.8	South Europe	Fenelli et al., 2011[12]	ID-LC-MS (416)
	19–49	3.52–35.5	Central Europe	Eisenhofer et al., 2017[13]	LC-MS/MS (298)
	18–40	4.17–19.64	Indian (South Asian)	Present study	ECLIA
E2 (nmol/L)	18–19	0.053–0.688	Central Europe	Elimlinger et al., 2003[19]	CLIA (381)
	25–44	<0.303	Central Europe	Schuring et al., 2016[20]	Immulite (139)
	25–44	<0.355	Central Europe	Schuring et al., 2016[20]	Immulite (139)
	18–40	0.05–0.44	Indian (South Asian)	Present study	ECLIA (848)
Serum TSH (µIU/mL)	12–19	0.44–3.59	American (NHANES 1988–1994)	Hollowell et al., 2002[21]	CLIA (6182)
	20–29	0.38–3.52	American (NHANES 1988–1994)	Hollowell et al., 2002[21]	CLIA (6182)
	30–39	0.32–370	American (NHANES 1988–1994)	Hollowell et al., 2002[21]	CLIA (6182)
	40–49	0.60–3.92	American (NHANES 1988–1994)	Hollowell et al., 2002[21]	CLIA (6182)
	12–49[a]	0.33–4.69	American (NHANES 1999–2002)	Aoki et al.[22]	NA
	18–30[a]	0.70–3.80	Indian (South Asian)	Marwaha et al.[23]	ECLIA (425)
	31–40	0.80–3.90	Indian (South Asian)	Marwaha et al.[23]	ECLIA (201)
	18–40	0.52–4.16	Indian (South Asian)	Present study	ECLIA (848)
Serum T4 (µg/dL)	12–49	5.19–12.89	American (NHANES 1988–1994)	Hollowell et al., 2002[21]	ECLI A (6182)
	12–49	5.2–11.60	American (NHANES 1999–2002)	Aoki et al.[22]	–
	18–30[a]	9.63–14.67	Indian (South Asian)	Marwaha et al.[23]	ECLIA (425)
	31–40[a]	9.40–15.45	Indian (South Asian)	Marwaha et al.[23]	ECLIA (201)
	18–40	5.23–12.31	Indian (South Asian)	Present study	ECLIA (848)

TSH: Thyroid stimulating hormone; LH: Luteinising hormone; FSH: Follicle stimulating hormone; DHEAS: Dehydroepiandrosterone sulphate; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; TR-IFMA: Delfia Time-resolved immunofluorescence; CLIA: Chemiluminescence immune-assay; IFMA: Immuno-fluorimetric assay; CICLA: competitive immunochemiluminescent assay; LC/MS: Liquid chromatography/Mass spectrometry. [a]The values presented are 3rd and 97th centile. N = sample size.

Table 4: **Comparative analysis of reference intervals (2.5th–97.5th percentiles) of various hormonal parameters in different populations and the present study.**

ovulation, levels of LH and FSH spike, but progesterone (P4) levels rise following ovulation and peak at the mid-luteal phase. In our study, the median normative serum levels of LH, E2 and SHBG didn't vary across different age groups which is inconsistent with the findings of the studies carried out in different populations.[10,11,13,18] However, the biological reference interval for FSH elevated significantly with age. The increase in the FSH levels has been coupled with a decline in fertility rates typically beginning in the third decade onwards of a woman's life.[42]

In women, androgen levels begin to decline with age.[37] In line with the published literature, we also observed a decline in the levels of testosterone when the subjects were stratified by age. Moreover, unlike men, serum androgens levels positively correlate with BMI in women,[43] however, in the current study the testosterone levels did not change with the increase in BMI. The difference might be attributed to a smaller sample size in the model after stratification, and needs further validation.

C-peptide is a widely used and useful method of assessing the functioning of the pancreatic β-cells, and its measurements serve as indicators of insulin production.[37,44] C-peptide is secreted in equimolar levels and has a longer half-life than insulin. As a result, C-peptide levels provide a more accurate reflection of the long-term level of insulin than does the actual amount of insulin. High C-peptide levels are related to increased mortality risk in both men and women in the general population between the ages of 40 and 74.[45,46] As expected, our data support homeostatic insulin secretion in glucose normo-tolerant subjects following the glucose load. Moreover, similar to earlier studies, in the current study, we observed significantly elevated median levels of fasting insulin and C-peptide in the higher age and BMI groups.[47] However, Tohidi et al. reported a decrease in trend with age in Iranian subjects.[48] Unlike ours, the study by Tohidi et al. was based on a smaller sample size, thus warranting further research with a larger sample size to substantiate these findings.

Studies have persistently propounded upon the ethnic differences on the normative reference interval for various biochemical and hormonal indicators.[49] Most of the data on these reference intervals is published from developed countries. Barring the study by Marwaha et al. for T4 and TSH, no comprehensive study has been carried out in India for establishing normative reference intervals. On comparative analysis of our data with the already published literature, we observed marginal differences in the normative ranges for the studied hormones among different populations. However, these differences can be attributed to different estimation methods employed in these studies.[3,50]

Our community-based study based on a countrywide reasonably large sample size utilized a robust study design to establish a population-specific normative range of common hormonal parameters in reproductive-aged Indian women and explored its variability with age, residence, and BMI. For establishing normal hormone reference intervals, the current study has employed reliable sensitive and uniform methodology using ECLIA at a single study centre in addition to stringent exclusion criteria.

The data on single-gender within a specific age group, and a selected number of hormone parameters limits the generalisability for reference ranges. Besides a relatively smaller sample size after subsequent stratifications might be a limiting factor of the present study. However, these factors are unlikely to change the main outcome of the study.

In conclusion, this is the first large composite data from India on common hormonal parameters generated using a uniform methodology on a cohort of reproductive-age women representative of the country. Therefore, this is likely to fill the vacuum of normative data among these women in future.

Contributors

Study conception and design: MAG, SC, VS, BJ, AN, SA, NM, RS, AN.
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Manuscript first draft writing: RR, IAS, HR, TA, MAG, AS.
Manuscript review and approval: All authors.

Data sharing statement

Any data generated in this study is available on a reasonable request to the corresponding author.

Declaration of interests

All authors declare that they do not have any conflict of interest/competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lansea.2023.100226.

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