ORIGINAL ARTICLE

EVALUATION OF PLASMA HORMONE CONCENTRATIONS USING ENZYME-IMMUNOASSAY/ENZYME-LINKED IMMUNOSORBENT ASSAY IN HEALTHY INDIAN MEN: EFFECT OF ETHNICITY

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ABSTRACT

The study involved three ethnic groups of India; Rajputs, Gorkhas and South-Indians. Each group consisted of ~40 healthy, male soldiers between 20–50 years. The reference ranges for cortisol, testosterone, prolactin, arginine vasopressin and proAtrial natriuretic peptide₁₋₉₈ were determined using Enzyme-Immunoassay (EIA) while plasma levels of thyroid-stimulating hormone, triiodothyronine, free-triiodothyronine, thyroxine and free-thyroxine were measured using Enzyme-linked immunosorbent assay (ELISA). The results indicated that plasma hormone concentrations were within physiological range and inter-ethnic differences were most prominent between north- (Rajputs and Gorkhas) and south- Indians. In comparison to Radioimmunoassay, the EIA method for prolactin, thyroid-stimulating hormone, free-thyroxine gave higher values while the ELISA method for triiodothyronine, free-triiodothyronine, and thyroxine gave lower values. These differences are due to differences in assay standards and design.

KEY WORDS

Indian population, Plasma hormones, Reference intervals, Ethnicity, EIA, ELISA.

INTRODUCTION

In clinical practice, measurement of plasma hormone concentrations is of importance for the evaluation and treatment of diseases. Hormone assays involve many methods; most popular had been Radioimmunoassay (RIA). The spectacular invention of enzyme immunoassay method (EIA)/enzyme-linked immunosorbent assay (ELISA) as a nonradioactive, less cumbersome variant has brought considerable benefits in terms of analytical, operational, and clinical outcomes (1). It is known that the results by RIA and EIA/ELISA are statistically different (2). Hence, the replacement of RIA with EIA/ELISA is likely to make values

obtained by the new method (EIA/ELISA) differ from those by RIAs. For each ethnic group, variance in outcomes using two different methods arises from two potential sources; factors affecting biological variation and factors affecting procedural-analytic variation (3). With the routine use of these assays being astronomical throughout the world, the need to establish reference hormone values for every ethnic group using EIA/ELISA is essential. The commonly encountered hormonal estimations in clinical practice usually include thyroid hormones, markers of stress, and reproductive hormones. In this perspective, little is known about the reference range for hormonal parameters in the Indian population which is polygenetic (4).

The aims of this study was to determine reference values for hormonal parameters in three different ethnic groups of the Indian population using EIA/ELISA and to compare the results obtained among the different ethnicities in subjects between 20 to 50 years of age.

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MATERIALS AND METHODS

Subjects: Healthy male soldiers (n=205) between age group 20-50 years were enrolled for the study after informed consent and approval from the ethics committee of the Institute. They belonged to the following ethnic groups: Rajput, Gorkha and South Indian. They represent different geographical, ecological and cultural settings of India. The Rajputs are from northwest India (Rajasthan), the Gorkhas are basically sub-mountainous people living in northern parts of India and South Indians are people from southern parts (Andhra Pradesh, Karnataka, Kerala, Tamil Nadu) of the country. Place of origin and age (i.e., date of birth) were self-reported. Based on morpho-linguistic classification of the Indian population (4); the subjects represent Caucasoid=Indo-European (Rajputs), Mongoloid=Tibeto-Burman (Gorkhas) and Australoid=Dravidian (South Indians) subtypes.

Diet consisted of authorized ration of the Indian army and food was consumed from common mess at respective locations. The average intake of the groups was 3,300 kcal (approximate composition: carbohydrate 60.5%, lipid 24.9% and protein 14.6%). There was no fluid restriction during the study. None of the study participants were on medication and just few subjects smoked occasionally and none reported daily consumption of alcohol.

Anthropometry: Body weight and height were measured using electronic weighing machine (Delmer, India) and an anthropometer. Height was measured to the nearest 0.5 cm and weight to the nearest 0.5 kg. Body fatness was estimated indirectly by calculating body mass index (BMI): body weight (kg) divided by the height squared (m^2).

Blood samples: Fasting, venous blood samples were collected from all these subjects, plasma was separated and stored at $-80^{\circ}C$ until analysis. Time of sampling was between 0700-0800 hours. The subjects were rested for half an hour in sitting position and then for 5 minutes in supine position before blood drawing. Commercially available EIA/ELISA kits were used for hormonal estimations. Plasma hormones measured were: cortisol, testosterone (T), prolactin (PRL), arginine vasopressin (AVP) and proAtrial natriuretic peptide (proANP₁₋₉₈), thyroid stimulating hormone (TSH), total triiodothyronine (TT₃), free triiodothyronine (fT₃), thyroxine (TT₄) and free tetraiodothyronine (fT₄).

Plasma cortisol was measured using EIA kits provided by IVDex Inc., USA (Range: 7-24 $\mu g/dL$). The intra- and inter-assay coefficient of variation was 6.2% and 6.7% respectively.

Plasma T was measured using EIA kits provided by IVDex Inc., USA (Range: 3 to 20 ng/mL). The intra- and inter-assay coefficient of variation was 7.5% and 8% respectively. Plasma PRL was measured using EIA kits provided by IVDex Inc., USA (Reference range: 3 to 20 ng/mL). The intra- and inter-assay coefficient of variation was 2.5% and 3.0% respectively. Plasma AVP was measured using EIA kits provided by Phoenix Pharmaceuticals Inc., USA (Reference range: 0 to 100 ng/mL). The intra- and inter-assay coefficient of variation was less than 4% and 15% respectively. Plasma proANP₁₋₉₈ was measured using EIA kits provided by Biomedica Gruppe, Austria (Reference range: 0 to 10 nmol/L). The inter- and intra-assay coefficients of variation were 4% and 2% respectively.

Plasma TSH was measured using ELISA kits provided by Calbiotech Inc, USA (Reference range: 0.39 to 6.16 $\mu IU/mL$). The inter- and intra-assay coefficients of variation were 5.9 to 9.3% and 6.6 to 8.1% respectively. Plasma T₃ was measured using ELISA kits provided by Calbiotech Inc, USA (Reference range: 0.52 to 1.85 ng/mL). The inter- and intra-assay coefficients of variation were 6.7% and 5.4% respectively. Plasma fT₃ was measured using ELISA kits provided by Calbiotech Inc, USA (Reference range: 1.4 to 4.2 pg/mL). The inter- and intra-assay coefficients of variation were 5.2% and 4.1% respectively. Plasma T₄ was measured using ELISA kits provided by Calbiotech Inc, USA (Reference range: 4.4 to 10.8 $\mu g/dL$). The inter- and intra-assay coefficients of variation were 3.7% and 3.0% respectively. Plasma fT₄ was measured using ELISA kits provided by Calbiotech Inc, USA (Reference range: 0.8 to 2.0 ng/dL). The inter- and intra-assay coefficients of variation were 6.01-10.81% and 3.25 to 10.99% respectively.

Statistics: Values are expressed as mean $\pm$ SEM (standard error of mean). A comparison between groups was done using analysis of variance with Bonferroni post-hoc analysis. Level of significance was $P < 0.05$.

RESULTS

The physical characteristics, represented by maximum number of subjects studied in each ethnic group are as shown in Table 1. The Rajputs were tall ($P < 0.05$) while Gorkhas were short and weighed less ($P < 0.05$) among the ethnic groups studied. BMI was less than 25 kg/m^2 and did not exhibit any ethnic variation.

Hormonal parameters as measured by EIA/ELISA and statistical differences for each variable among the ethnic groups are as shown in Tables 2-4. Plasma hormone concentrations of all the subjects for each ethnic group were

Table 1: Physical characteristics of the study subjects between 20 to 50 years of age

	Rajput (n=81)	Gorkha (n=81)	South Indian (n=43)
Age, Mean (years)	30.81 ± 0.78	28.54 ± 0.51	31.07 ± 1.15
Height, (cm)	174.14 ± 0.45	165.98 ± 0.53*	171.37 ± 0.75#
Weight, (kg)	67.08 ± 0.90	62.18 ± 0.85*\$	66.02 ± 1.01
BMI, kg/m ²	22.12 ± 0.27	22.54 ± 0.26	22.51 ± 0.35

Values are Mean ± SEM. Level of significance: P<0.05; Symbols: Rajput vs Gorkha (*), Rajput vs South Indian (#), Gorkha vs South Indian (\$)

within the normal range. Ethnicity related observations on plasma hormone concentrations were as follows: a) With the exception of plasma cortisol, differences among the three ethnic groups were observed for all other hormonal parameters. b) Significant differences were observed mainly between Rajputs and South Indians, Gorkhas and South Indians. Among the three ethnic groups: plasma AVP, proANP₁₋₉₈, and T₃ levels were high (P<0.05) in South Indians whereas plasma levels of fT₃, T₄ and fT₄ were low (P<0.05) in South Indians. Plasma T was higher (P<0.05) in South Indians than Rajputs. Also, plasma TSH was higher (P<0.05) in South Indians than Gorkhas. c) Differences between Rajputs and Gorkhas were observed only with respect to plasma PRL. Plasma PRL level was higher (P<0.05) in Rajputs than Gorkhas and South Indians. Reference values for various hormones by RIA in the Indian population and EIA/ELISA (present study) for comparison are as shown in Table 5.

DISCUSSION

The significance of reference hormonal levels lies in the fact that hormonal parameters are important factors to consider in clinical situations such as infertility, hypo/hyper-thyroidism. In developing countries like India, industrialization and urbanization with economic development have resulted in lifestyle changes and nutrition transition increasing the risk of chronic disease (11). Such changes are known to influence hormone levels. Further, international guidelines have recommended the establishment of reference intervals for every country and the lack of such studies in India has been recently reported (12). In the present study, the various reference hormone values measured using EIA/ELISA were within the physiological range for each ethnic group in subjects between 20 to 50 years of age. On the other hand, comparing the results among the 3 ethnic groups gave rise to new observations. Differences in plasma hormone concentrations were observed mostly between the north- (Rajputs, Gorkhas) and South- Indian ethnicities while hormonal variation between

Table 2: Plasma hormone measurements using EIA in the study group

	Cortisol, µg/dL	Testosterone, ng/mL			Prolactin, ng/mL			Arginine Vasopressin, ng/mL			proAtrial Natriuretic Peptide (1-98), nmol/L		
		Mean± SEM	No. of subject	95% CI	Mean± SEM	No. of subject	95% CI	Mean± SEM	No. of subject	95% CI	Mean± SEM	No. of subject	95% CI
Rajput	10.95 ± 0.58	6.21 ± 0.20	76	5.82 - 6.60	12.04 ± 0.44	49	11.16 - 12.92	3.63 ± 0.48	35	2.66 - 4.61	0.70 ± 0.05	48	0.60 - 0.79
Gorkha	9.84 ± 0.33	6.5 ± 0.18	81	6.14 - 6.86	9.8 ± 0.39	70	9.02 - 10.56	0.76 ± 0.04	39	0.68 - 0.83	0.55 ± 0.04	40	0.47 - 0.64
South Indian	9.25 ± 0.71	7.16 ± 0.44	43	6.27 - 8.05	7.77 ± 0.49	40	6.78 - 8.76	24.86 ± 1.77	41	21.28 - 28.44	1.54 ± 0.10	43	1.33 - 1.74

Table 3: Plasma hormone measurements using ELISA in the study group

	Thyroid Stimulating Hormone, µIU/mL	Total tri-iodothyronine, ng/mL			Free tri-iodothyronine, pg/mL			Thyroxine, µg/dL			Free thyroxine, ng/dL		
		Mean± SEM	No. of subject	95% CI	Mean± SEM	No. of subject	95% CI	Mean± SEM	No. of subject	95% CI	Mean± SEM	No. of subject	95% CI
Rajput	2.26 ± 0.13	0.91 ± 0.02	81	0.86 - 1.00	2.16 ± 0.06	61	2.04 - 2.28	7.00 ± 0.16	79	6.67 - 7.28	1.09 ± 0.05	53	1.00 - 1.19
Gorkha	2.05 ± 0.12	1.0 ± 0.03	81	0.94 - 1.07	2.21 ± 0.09	56	2.03 - 2.39	7.08 ± 0.21	57	6.65 - 7.50	1.0 ± 0.02	78	0.97 - 1.03
South Indian	2.62 ± 0.15	1.56 ± 0.04	36	1.49 - 1.63	1.51 ± 0.05	42	1.41 - 1.62	4.04 ± 0.28	42	3.47 - 4.60	0.78 ± 0.04	43	0.70 - 0.87

Table 4: Statistical significance for plasma hormone variation among the ethnic groups

Variables	Rajput vs Gorkha	Rajput vs South Indian	Gorkha vs South Indian
Cortisol	ns	ns	ns
Testosterone	ns	P<0.05	ns
Prolactin	P<0.001	P<0.001	P<0.01
Arginine Vasopressin	ns	P<0.001	P<0.001
proAtrial Natriuretic Peptide ₍₁₋₉₈₎	ns	P<0.001	P<0.001
Thyroid Stimulating Hormone	ns	ns	P<0.05
Total tri-iodothyronine	ns	P<0.001	P<0.001
Free-tri-iodothyronine	ns	P<0.001	P<0.001
Thyroxine	ns	P<0.001	P<0.001
Free-thyroxine	ns	P<0.001	P<0.001

Rajputs and Gorkhas was observed only with respect to PRL. But there was no variation in BMI among the three ethnicities. This is of importance from the fact that high degree of genetic differentiation exists among the Indian population (4).

Cortisol is the only plasma hormone that did not exhibit any ethnic variation. It has been observed that urinary stress hormones measured using high performance liquid chromatography did not differ between blacks and whites (13). However, ethnic variations in stress response have been

reported (14). The reproductive hormones, T and PRL also showed ethnic variation. Reports on plasma T variation in non-Western, non-clinical population is limited (15) and a detailed study on male reproductive physiology across different ethnicities of India is required.

On the other hand, PRL variation among men from different ethnic background has not been described previously. From the viewpoint of assay method, it can be observed (Table 5) that the values obtained by EIA for PRL is higher than RIA values while cortisol and T values are close to RIA values. An interesting finding is that the fluid regulating hormones-proANP₁₋₉₈ and AVP were significantly high in South Indians among the 3 ethnic groups. Ethnic difference in these hormones has been reported in other populations (16, 17). Although diet was similar in all the study subjects, it has been reported that dietary salt intake in urban South India is higher than current recommendations for the general population. In the Western society characterized by high sodium-low potassium intake; relatively blunted natriuretic responses in blacks than whites has been attributed to the greater prevalence of hypertension in blacks (18).

Plasma TSH and thyroid hormone measurements using ELISA showed that differences were mainly observed between north- (Rajputs and Gorkhas) and south- Indians. Environmental factors such as cold stress to which Rajputs and Gorkhas are exposed since birth than South Indians may also have contributed to such variation. These findings may help in our understanding of the prevalence and pattern of thyroid

Table 5: Plasma hormone concentrations using RIA and EIA/ELISA in the Indian population

Variables	Indian population RIA ^{5,6,7,8,9}	Present study (overall)	Conversion of conventional units to SI units	Reference range ¹⁰
Cortisol	238.38 ± 17.38 nmol/L	10.06 ± 0.30 µg/dL	277.56 nmol/L	140-690 nmol/L
Testosterone	24.61 ± 1.73 nmol/L	6.53 ± 0.14 ng/mL	22.64 nmol/L	10-35 nmol/L
Prolactin	5.76 ± 0.40 µg/L	9.98 ± 0.28 ng/mL	—	2-15 µg/L
Arginine Vasopressin	2.6 ± 0.22 [#] µU/mL	10.23 ± 1.21 ng/mL	0.09 pmol/L	0-2.8 pmol/L
proAtrial Natriuretic Peptide ₍₁₋₉₈₎	—	0.93 ± 0.06 nmol/L	—	0-2000 pmol/L
Atrial Natriuretic Peptide	22 ± 5 pg/mL	—	—	—
Thyroid-Stimulating Hormone	1.76 ± 0.37 µU/mL	2.26 ± 0.08 µIU/mL	—	0.5-5 mU/L
Total triiodothyronine	116 ± 7.9 ng/dL	1.07 ± 0.03 ng/mL	2.14 nmol/L	1.1-2.9 nmol/L
Free triiodothyronine	210 ± 15 pg/dL	2.01 ± 0.05 pg/mL	—	—
Thyroxine	8.46 ± 0.69 µg/dL	6.31 ± 0.15 µg/dL	81.21 nmol/L	64-154 nmol/L
Free thyroxine	0.50 ± 0.06 ng/dL	0.97 ± 0.02 ng/dL	12.48 pmol/L	9-26 pmol/L

#: Bioassay method of Yoshida and co-workers.

disorders that depends on age, ethnicity, gender and geographical factors (19). Further, thyroid disorders are amongst the most common endocrine disease in India (20). The values obtained by ELISA shows that TSH and fT_4 levels are higher than RIA while TT_3 , fT_3 and TT_4 values are lower than RIA (Table 5).

The study demonstrates the mean plasma hormone concentrations measured by EIA/ELISA in Rajput, Gorkha, South Indian ethnicities of the Indian population in subjects between 20 to 50 years of age. Difference in plasma hormones was most prominent between north- (Rajputs and Gorkhas) and south- Indians. In addition, as the level of physical training and diet were uniform for all the subjects, effects of these factors could not have contributed to the apparent hormonal variations among the ethnic groups studied. In comparison with RIA, values obtained by EIA/ELISA for PRL, TSH and fT_4 were higher while values for TT_3 , fT_3 and TT_4 were lower. These differences are due to differences in assay standards and design. However, interpretation of data requires careful consideration of the method used for hormonal estimation, small sample size, lack of higher age groups and other ethnicities, physically activity. Further studies involving hormones and their gene polymorphisms as a possible basis for ethnic variation involving various age groups may help in understanding the prevalence and pattern of endocrine disorders in India.

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