

Full Length Research Paper

Evaluation of the determination of reference ranges for reproductive hormones (prolactin, FSH, LH, and testosterone) using enzyme immuno assay method

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Accepted 30 March, 2007

The study involved a total of three hundred (300) subjects; 100 women aged 17 - 40 years, 50 women aged 17 - 40 years, 50 women aged 55 - 68 years, 50 men aged 17 - 45 years, and 50 men aged 55 - 70 years. The reference ranges for some reproductive hormones (Prolactin, FSH, LH and testosterone) were determined using the enzyme-immunoassay method. The values were compared with previously existing values, which were obtained by radio immunoassay method. The results indicated that for the polypeptide hormones (Prolactin, LH and FSH), the enzyme immunoassay method gave lower values and narrower ranges than radio immunoassay. On the other hand, the enzyme immunoassay method gave higher values and wider ranges for the steroid hormone, (testosterone), than the radioimmunoassay method. These differences are due to differences in assay standards and design. Hence, replacing the traditional radioimmunoassay with enzymeimmunoassay, since the enzymeimmunoassay is cheaper and more accessible, will unavoidably cause some analytical shifts and affect the interpretation of the results of endocrine investigation in reproductive medicine.

Key words: Reference ranges, reproductive hormones and enzyme immunoassay method.

INTRODUCTION

Reference range is a range of values to which the result of an investigation in question can be compared (Baron and Whicher, 1989). A reference value may be defined as a value obtained by observation or measurement of a particular type of quality on a reference individual. A reference individual is an individual selected for comparison using defined criteria. The strategy to achieve this involves using sampling method, which could be by either (i) direct/indirect method or (ii) random/non-random method. For more specific grouping, further stratification into age, sex, genetic and socio economic groups can be done. Reference values can be influenced by some factors; these include the analytical technology, the selection of so-called healthy population as reference individuals, and the statistical method (Nakayama, 1992). The reference values are sometimes referred to as "normal" values, as they indicate the boundaries between normal and abnormal values.

In reproductive endocrinology reference hormonal values are needed, especially in the clinical evaluations of cases of infertility in both males and females, erectile dysfunction in males and menstrual disturbances in females. For instance Kuku et al. (1988) measured levels of gonadotropins, prolactin and testosterone in infertile men and found that about 60% of them had abnormal serum levels of one or more of the hormones. Mikhail (2006) investigated the role of testosterone in erectile dysfunction and concluded that testosterone is essential for proper erection. All these were possible because of availability of reference hormonal values. Other workers (Stricker et al., 2006) had a study reemphasizing the need to establish reference values for reproductive hormones during different phases of the menstrual cycle.

There are many methods for hormone assay; most popular had been the Radioimmunoassay (Matched Reagents Radio Immunoassay). In 1992, the WHO Scientific and Technical Advisory Group proposed the enzyme immunoassay (EIA) as an alternative. The EIA kits were field tested and satisfactory result was obtained (Cekan and Arcangues, 1996). The EIA method is cheaper, more

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Table 1. Experimental procedure showing the group from which blood was sampled.

Group	No. of Subjects	Sex	Age range (years)	Frequency of blood sampling for each subject
Group A ₁	100	Females	17 – 40	Bled once either on: m, m+1, m+2, m+29
Group A ₂	50	Females	17 – 40	Bled daily from day m+11 – day m+18
Group B	50	Females	55 – 68	Bled only once
Group C ₁	50	Males	17 – 45	Bled only once
Group C ₂	50	Males	55 – 70	Bled only once

Group A₁ Subjects – History of normal menstrual cycles, of 24 – 30 days cycle length.

Group A₂ Subjects – as for A₁.

Group B Subjects – Menopausal.

m = Day 1 of menstrual cycle.

Table 2. The mean levels of prolactin, FSH and LH during days of menstrual Bleeding in A₁ and the mid cycle serum levels of these hormones in study groups A₁ and A₂ combined.

Hormone	Mean level during day 1-day 5 of menstrual cycle in A ₁	Mid cycle level in A ₁ and A ₂ combined (13 th -14 th days)
Prolactin		
Mean ± SD	229.6 ± 72.6 miu/L	408.0 ± 17.2 miu/L
95% confidence Interval	172.8 – 293.3 miu/L	384.2 – 431.8 miu/L
FSH		
Mean ± SD	7.2 ± 2.8 iu/L	7.8 ± 0.36 iu/L
95% confidence interval	2.4 – 9.3 iu/L	7.31 – 8.30 iu/L
LH		
Mean ± SD	5.0 ± 0.79 iu/L	16.1 ± 1.23 iu/L
95% confidence interval	4.3 – 5.7 iu/L	14.5 – 17.8 iu/L

accessible, and the reagents have good stability. Apparently, the replacement of RIA with EIA is likely to make values obtained by the new method (EIA) differ from those by RIAs. For every ethnic group, the type of deviation to expect between two assay methods consists of two parts; the analytical variation and the between-subject (biological) variation. Therefore, the replacement of radio immunoassays with enzyme immunoassays requires the re-establishment of reference hormonal values for every ethnic group. The aims of this study are therefore to determine valid reference values for reproductive hormones in the study population using the enzyme immunoassay method, and to compare the results obtained in this study (EIA) with results previously generated through radio immunoassay (RIA).

MATERIALS AND METHODS

The study was carried out in Sagamu, Ogun State, Nigeria. A total of three hundred (300) subjects were recruited. These included individuals of sexes, some chosen within the Olabisi Onabanjo University Teaching Hospital (medical students and members of staff) and others were chosen outside the teaching hospital (young adults on their service year for the National Youth Service Corps,

traders, farmers and civil servants). The subjects were grouped into five groups with group specification as below (Table 1)

Approval for the study was given by the Olabisi Onabanjo University Teaching Hospital Ethical Review Committee on research involving human subjects, and all the subjects gave informed consent for the study.

Between 8 – 10 ml of venous blood was collected from each subject, with most collections done between 9.00 a.m. and 11.00 a.m. The blood was collected into plain containers and allowed to clot. Each sample was centrifuged at 1000 rpm for 10 min to achieve separation. The serum obtained was put into aliquots in each case, labeled and stored at – 20°C. One aliquot of each specimen was taken at a time, to avoid repeated freezing and thawing, and the samples were analyzed for hormone estimation using enzyme immunoassay (EIA), according to the World Health Organization (WHO) matched reagent programme protocol (manual) for EIA kits (protocol/version of December 1998 for Prolactin, LH, FSH; and protocol/version December 1999 for Testosterone). The various assay results obtained for the various groups were then subjected to descriptive statistics, and are as given in the tables below.

RESULTS AND DISCUSSION

The various values obtained for the hormone estimations in the various groups are as shown in the tables below (Tables 2 - 4). The significance of reference hormonal values lies in the fact that hormonal disorders are

Table 3. Serum concentrations (mean ± SD and 95% confidence interval) of prolactin, FSH and LH in 50 postmenopausal women aged 55 - 68 years (B group).

Hormones	Mean ± SD	95% Confidence interval
Prolactin	367.9 ± 142.1 (miu/L)	328.5 – 407.3 (miu/L)
FSH	67.4 ± 15.1 (iu/L)	63.2 – 71.6 (iu/L)
LH	37.8 ± 19.1 (iu/L)	32.5 – 43.1 (iu/L)

Table 4. Serum concentrations of prolactin, LH, FSH and testosterone (mean ± SD and 95% confidence interval) in men in groups C₁, and C₂.

Group	No. of Subject	Sex	Age range (years)	Hormone	Mean ± SD	95% Confidence interval
C ₁	50	Males	17 – 45	Prolactin	218.5±102 (miu/L)	190.20-246.81 (miu/L)
				LH	5.9 ± 2.33	5.34 – 6.54 (iu/L)
				FSH	5.6 ± 2.26 (iu/L)	4.92 – 6.22 (iu/L)
				Testosterone	33.2 ± 18.38 (nmol/L)	28.1 – 38.3 (nmol/L)
C ₂	50	Males	55 – 70	Prolactin	203.7 ± 187 (miu/L)	151.6 – 255.8 (miu/L)
				LH	13.5 ± 9.75 (iu/L)	10.8 – 16.2 (iu/L)
				FSH	19.8 ± 14.1 (iu/L)	15.9 – 23.7 (iu/L)
				Testosterone	25.0 ± 6.08 (nmol/L)	23.3 – 26.7 (nmol/L)

Table 5. Comparison of serum hormone levels of men in the age group 17 – 45 years (Group C₁) in this study (EIA) with previous studies by WHO, 1991 and Obwaka et al. 1982 in which fertile men were used (RIA).

Hormone	Reference ranges (95% Confidence interval)		
	Present Study (Group C1)	WHO (1991)	Obwaka et al. 1982)
Prolactin (miu/L)	190.20-246.81	110 - 510	96 – 489
LH (iu/L)	5.34-6.54	2.5 - 9.8	0.94 – 8.88
FSH (iu/L)	4.92 – 6.22	1.2 – 5.0	0.35 – 4.5
Testosterone nmol/L)	28.1 – 38.3	13 – 33	7.84 – 30.87

important factors to consider in specific clinical situations. In Nigeria oligo/azoospermia is quite common in patients with sickle-cell disease (Mobede, 1989), and the improvement of clinical care makes more sickle cell patients to reach reproductive age which will invariably make sickle cell disease to contribute more proportionately to the etiology of oligo/azoospermia in Nigerians (Kuku and Osegbe, 1989). A lot of other cases of infertility, especially from infections (Alausa and Osoba, 1978) are all over in our environment. Many cases of erectile dysfunction, which require hormonal assays, are seen in clinical practice (Mikhail, 2006). Past works had justified the need for hormone assays in infertile men (Kuku et al., 1988) and women (Emokpae, 2005).

All these call for widespread availability of hormonal assays which will be of great help both in separating untreatable (primary testicular disease) from the treatable (hypothalamic/pituitary) diseases (Kuku and Osegbe, 1981), as well as facilitate the requirement for hormone replacement therapy provided reference range is established (Iwamoto et al., 2004). In this study, the various reference hormonal values for the various age groups

and sexes involved in the study are as presented in Tables 2, 3, and 4. While Tables 2 and 3 show the values for the younger females and older females respectively, Table 4 shows the values for the younger males and older males. All the values, including the LH peak observed in the mid cycle for the female age group 17 - 40 years, are keeping with physiological expectations.

However, comparing the various results of this study (by enzyme immunoassays) with values from past studies (by radioimmunoassay), a number of observations was made. In 1981, Dada et al. (1981) carried out a study on the serum levels of prolactin, FSH and LH on normally menstruating women, using radioimmunoassay. The results of their study are compared with those of normally menstruating women, aged 17 - 40 years, in this study (by EIA) as shown in Table 5 and 6. It is clear from above that with the enzyme immunoassay, the values obtained for these peptide hormones (Prolactin, LH, FSH) were lower and the ranges were narrower, though FSH was not markedly affected. Subjecting the results by RIA and EIA to statistical analysis, using the student's t-test there is a significant difference (P < 0.001).

Table 6. Comparison of the RIA results of Dada et al. (1981) with those of normally menstruating women, aged 17 - 40 years in this study (by EIA).

Hormone	Results of study by Dada et al	Result of this study
	(1981; RIA)	(EIA)
Prolactin	200.0 – 800.0 iu/L	157.0 – 302.2 iu/L
FSH	2.0 – 7.0 iu/L	4.4 – 10.0 iu/L
LH	4.0 – 30.0 iu/L	4.2 – 5.8 iu/L

Mathur et al. (1986) reported a reference range of 9.5 – 30.0 nmol/L for serum testosterone, as control values for all the participants in their study of the effect of exercise on cortisol and testosterone on male athletes and non-athletes using radio immunoassay. The results in this present study (EIA) gave values of 14.82 – 51.58 nmol/L for serum testosterone. This indicated that the enzyme immunoassay yielded a higher value with a wider range for the steroid hormone compared with the radioimmunoassay. The results by RIA and EIA are significantly different ($P < 0.001$) when subjected to statistical analysis using the student t-test.

The findings in this study on the hormone estimations with regards to assay method (EIA or RIA) are further reflected by comparing the values obtained in this study for men, aged 17 - 45 years, with the results of previous studies by WHO (1991) and Obwaka et al. (1982). Their results using radio immunoassay showed wider range and higher upper extreme values for the hormones prolactin, LH and FSH than the results of this study (EIA) and the reverse holds for testosterone. This study however gains support from a similar study done using EIA method in caucaseans (Merino and Carranza et al., 1997). Incidentally, despite the fact that EIA is becoming popular, the RIA is still very much in use (Hackney and Zack, 2006).

The variations observed in the reference hormonal values by these 2 assay method is not a peculiar phenomenon, as evaluation of different assay methods by other researchers also showed variations of results (Sikaris, 2005; Christina, 2004).

Conclusion

The study demonstrated that for the polypeptide hormones (Prolactin, FSH, and LH) the enzyme immunoassay method gave lower values with narrower reference range than the radio immunoassay. For the steroid hormone (testosterone) the EIA gave higher values with wider reference range. The differences are due to inherent differences in the assay method and design, which are influenced by the assay reagents and standards as well as methodologies. Clinicians, laboratorians, and researchers as well should bear in mind these expected variations when using these assay methods in evaluating

endocrine problems in the study environments and elsewhere in the world.

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