Short Communication

Establishment of reference ranges for Serum Leptin and C-Peptide using enzyme-linked immmunosorbent assay method: a preliminary report

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Abstract: Reference ranges (RR) or Reference Intervals (RIs) are very important values for accurate interpretation of clinical laboratory test results. Any test result that is not accompanied by a valid RR value is less informative and may not be interpreted correctly. However, reference values of most analytes for Nigerian population are not readily available. The present study aims at establishing reference values of Serum Leptin and C-Peptide for Dutse, Jigawa State. In a cross sectional study, eighty (80) reference individuals were recruited. Serum Leptin and C-Peptide were analysed using ELISA methods. Following an accepted guideline, population specific reference intervals were established for these analytes and found to be 3.13ng/mL to 14.09ng/mL for Leptin and 0.56ng/mL to 5.64ng/mL for C-Peptide, respectively. Populations sharing similar physical and socio-economic characteristics may adapt these intervals if validated and considered suitable for their laboratory methods.

1. Introduction

By definition, Reference Range (RR) for a particular measurement is defined as Mean ± 2SD and is the predictive interval between which 95% of values of a reference group falls into, in such a way that 2.5% of the time a sample value will be less than the lower limit of this interval, and 2.5% of the time it will be larger than the upper limit of this interval, whatever the distribution of these values (Stephen *et al*., 2008).An RR "is an interval that, when applied to the population serviced by the laboratory correctly includes most of the subjects with characteristics similar to the reference group and excludes the others" (Ceriotti, 2007). Methods for establishing reference ranges are mainly based on assuming a normal distribution (Sterne and Kirkwood, 2003) or a log-normal distribution (Patrick *et al*., 1992), or directly from percentages of interest (Winkle *et al*., 1979).
In this part of the world, Leptin and C-peptide are biochemical analytes that are new and have potentials of being introduced into the routine investigations for improvement of patient care. Therefore their RRs values needs to be established for accurate test results interpretation. Methods for establishing these ranges are expensive and often difficult to perform (Alex *et al*., 2010), as such most laboratories depend on RRs that have been reported by the manufacturer or as established by another laboratory. For example, a survey of 500

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laboratories in 2001 by College of American Pathologists (CAP) found that 390 laboratories (78%) adopted manufacturers' values for reference intervals (Friedberg *et al*., 2007).

In the above mentioned survey, 163 laboratories conducted an internal study of healthy subjects to establish reference intervals. Of these laboratories, about one half tested between 21 – 50 samples and one quarter tested more than 100 samples. Methods for verifying reported RRs for adoption are relatively more simple studies and require only 20 healthy subjects to recruit (CLSI, 2008). However, it has been recommended that RRs be established for each and every Population, community and/or laboratory (Alex *et al*., 2010) becausethere are some biological differences in serum analytes levels of populations as a result of several factors including ethnic, racial, socio-economic and/or physical (El-Hazmi and Warsy, 2001).

Leptin is a 16KDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism (Tracy et al., 2002). It is a hormone made by adipose cells that helps to regulate energy balance by inhibiting hunger. The biological effects of leptin action suggest its potential diagnostic and therapeutic utility in a variety of pathologic states, including obesity, diabetes and their co-morbid diseases (Martins, 2006).

C-peptide is a short 31-amino-acids polypeptide that connects insulin's A-chain to its B-chain in the proinsulin molecule (Wahren *et al*., 2000). Patients with diabetes mellitus may have their C-peptide levels measured as a means of distinguishing type 1 diabetes from type 2 diabetes or Maturity Onset Diabetes of the Young (MODY) (Jones and Hattersy, 2013). C-peptide and insulin are secreted in equimolar amounts; however, C-peptide does not undergo significant hepatic extraction but is renally eliminated and therefore persists longer in the peripheral circulation. Thus, C-peptide has longer half-life (> 30 minutes) and less fluctuation serum levels compared to insulin (5 minutes half-life) (Rubenstein *et al*., 1969). Hence, measurements of C-peptide more accurately reflect pancreatic insulin secretion rates than insulin (Clark, 1999). Moreover, C-peptide concentration is independent of exogenous insulin and is not subject to interference from insulin auto-antibodies induced by insulin therapy (Schultess *et al*., 2009). However, this study aims at establishing reference ranges of serum Leptin and Serum C-Peptide levels for the inhabitants of Dutse, North-West Nigeria.

2. Materials and Methods

The study was conducted in Dutse, Jigawa State, North-West Nigeria. A total of eighty (80) reference subjects were recruited. These included individuals of both sexes (forty adult males and forty adult females), chosen from Dutse, Jiagawa state, North-West Nigeria. The subjects were selected using a direct method as described by the United States National Committee on laboratory Standards (NCCLS) Guidance Document C28A2. Accordingly, for most analytes, the lower and upper reference limits were assumed to demarcate the estimated 2.5th and 97.5th percentiles of the underlying distribution of values (Sasse *et al*., 2000). Ethical approval for the study was given by the Ethics and Research Committee of the Rasheed Shakoni Specialist Hospital, Dutse, Jigawa state. Informed consent was obtained from each subject prior to the commencement of study. From each selected subject, a total of five millilitres (5.0ml) of venous blood specimen was collected into a sterile vacutainer and allowed to clot at room temperature (25°C). Each sample was centrifuged at 300r.p.m. for five (5) minutes and the clear unhaemolysed sera were harvested and rapidly kept frozen at -20°C until analysed for serum Leptin and C-peptide levels, respectively.

Serum Leptin was estimated using the leptin ELISA Kit (WKEA Med Supplies Company, China, a commercially prepared kit based on Sandwich Principle, as developed by Engvall *et al*.(1971).Serum C-Peptide was measured by C-Peptide ELISA kit from Monobind Inc, USA, based on Sandwich Principle, as modified by Wang *et al*. (2012).

Data entry and analysis were performed using MS Excel (2007) and Epi Info 7 (CDC, 2015). Reference intervals were determined using the methods described in the NCCLS guidelines (NCCLS 1992). The underlying assumption is that the 2.5th and 97.5th percentiles will contain 95% of the distributions of normal values in the reference population.

3. Results

Figures 1 and 2 display the Orgive of Leptin (ng) and C-peptide (ng/L) respectively of the eighty (80) reference individuals, while Table 1 summarizes the lower (2.5%) and upper limits (97.5%) of the two analytes.
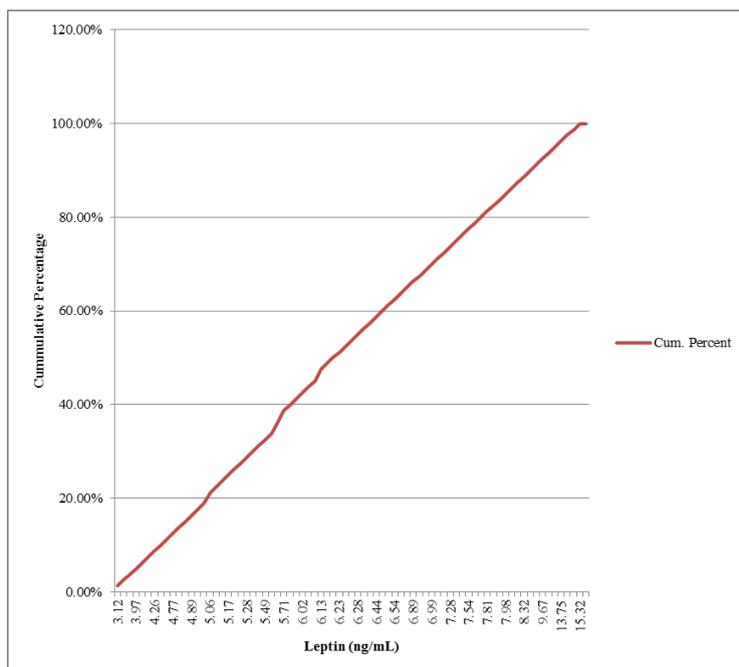
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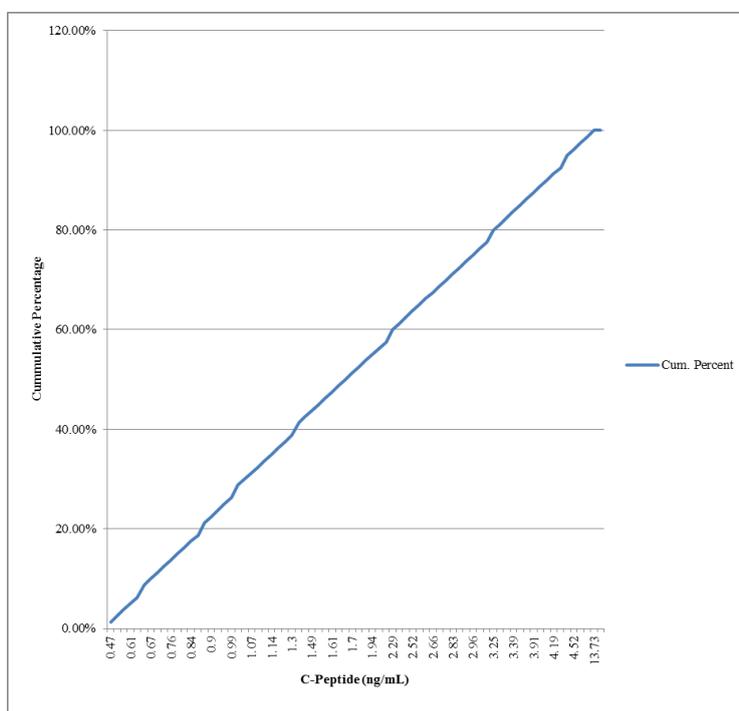**Figure 1. Serum Leptin (ng/mL) Orgive of the reference population.**

Figure 2. Serum C-Peptide (ng/mL) Orgive of the reference population.

Table 1. Reference values of Serum Leptin and C-Peptides in reference subjects (2.5 percentile lower limits and 97.5 percentile upper limits)

Variables	Leptin (ng/mL)	C-Peptide (ng/mL)
Low	3.13	0.56
High	14.09	5.64

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4. Discussions

Several researches have recommended the inclusion of serum Leptin (Abdelgadir *et al*., 2002; Sweeney, 2002; Van Gaal *et al*., 1999) and serum C-peptide (Jones and Hattersley, 2013) in the routine tests for management of diabetic and related patients. This prompted the need to establish reference ranges for these analytes. The present study established the lower and upper reference values of Serum Leptin and C-peptide for the studied population to help ininterpretation of laboratory results.

From the study, serum leptin lower and upper limit reference values were found to be 3.13 ng/mL and 14.09 ng/mL, respectively. Leptin reference values (3.13 – 14.09 ng/mL) agrees to some extent with 0.7 ng/mL (lower limit) and 18.3 ng/mL (upper limit) reported by Mayo Clinic US (1995 -2016). There was however, wide difference when compared with 8.95 ng/mL (lower limit) and 23.75 (upper limit) reported on the Spanish population (Teresa *et al*., 2015).

Similarly, Serum C-peptide lower and upper reference values were 0.56 and 5.64 ng/mL respectively. This is closely relatedto 1.1ng/mL – 4.4 ng/mL reported by Mayo Clinic US (1995 -2016). It however differs significantly (especially in the higher reference limit) from the 0.51ng/mL - 2.72 ng/mL reported by Global Diabetes Community, UK (2016).

It is clear from the present study that it is important to establish reference values that are applicable to specific community/laboratory instead of adopting a reference value that is meant for another different community/laboratory. This is because, reference values as a measure of physiological function may be affected by several factors that varies from population to population or from one community to another in relation to several variables including diet, socio-economic conditions, genetics and physical environment; and to a large extent the laboratory method(s) for the estimation of such analyte (ADA, 2015).This further buttressed the statement of Alex *et al. (*2010).

5. Conclusions

The reference values generated from this study can be used in Jigawa and its environs for proper interpretation of laboratory result of Leptin and C-Peptide in clinical management of diabetes and other related illness. It can also serve as a guide during some other health related activities such as screening population for diabetes etc. Populations sharing similar physical and socio-economic characteristics may adapt these intervals if validated and considered suitable for their laboratory methods.

Conflict of interest

None to declare.

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