Letter to the Editor

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Reference values for alanine aminotransferase, α-amylase, aspartate aminotransferase, γ-glutamyltransferase and lactate dehydrogenase measured according to the IFCC standardization during uncomplicated pregnancy

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To the Editor,

In the clinical context, laboratory test results are compared to the relevant reference values, therefore accuracy of reference values is essential for appropriate clinical management decisions. Adequacy of reference intervals may be biased by the presence of physiological changes and by analytical variation, a disturbing method-dependent phenomenon particularly evident in the measurement of serum enzymes. Physiological changes of biochemical parameters occurring during pregnancy may be not accounted for by reference values if these are based on healthy men or non-pregnant women. However, the adoption of analytical procedures providing results traceable to an appropriate reference measurement system may allow the method-dependency of test results to be minimized [1]. In recent years, the IFCC has re-assessed the optimization of methods for the measurement of the catalytic activity concentration of several enzymes in human serum. The assay of some of these enzymes may be useful for the evaluation of pathological complications of pregnancy, which may be detrimental for mother or fetus, and for the diagnosis of intercurrent diseases affecting pregnant women. The aim of this work was to define the reference intervals for alanine aminotransferase (ALT), α-amylase (Amy), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT) and lactate dehydrogenase (LDH) in uncomplicated pregnancy, using methods providing results traceable to the corresponding IFCC standardized reference measurement procedures.

From March 2008 to March 2009, 252 women aged 17–43 years in the 22nd–26th week of an uncomplicated pregnancy were recruited. Exclusion criteria were the presence of diabetes mellitus, alcohol consumption and the use of therapeutic drugs. Blood was collected in plain tubes after overnight fasting. Serum samples leftovers, after routine clinical laboratory investigations, were de-identified and stored at –80°C until measurement; jaundiced, lipemic and hemolyzed sera were excluded. Analyses were performed using a Beckman Coulter AU680 analytical system (Beckman Coulter, Cassina De' Pecchi, Italy) according to the manufacturer's instructions. Reportedly, all the analytical methods provided results traceable to the corresponding IFCC primary reference measurement procedures; however, for ALT, AST, GGT and LDH assays we verified the traceability of analytical data through the analysis of three serum pools that

had concentration values assigned by the corresponding reference procedures [1]. Reference intervals (2.5th and 97.5th percentiles) with 90% confidence interval were calculated for each assay using non-parametric techniques [2]. For outliers exclusion, the Tukey method was used [3].

In Table 1, results for ALT, AST, GGT and LDH obtained with the AU680 system in the analysis of three serum pools are compared to results measured by the corresponding IFCC standardized reference measurement procedures. For ALT, GGT and LDH the calculated total error, at all tested concentrations, fulfilled the optimum quality specification for total error derived from biological variation [4]; for AST the optimum quality specification was fulfilled for 28 U/L and 52 U/L concentrations, whilst for the lowest concentration (18 U/L) the desirable quality specification was met [5].

No statistically significant correlation between age and the catalytic activity concentration was found for any of the five enzymes, therefore the data were not partitioned to produce age-related reference intervals. After exclusion of outlier data, sets of 226–251 analytical values remained for calculation of reference intervals; the catalytic activity concentration values did not fit the Gaussian distribution on the basis of skewness and kurtosis [3]. Table 2 reports the proposed reference intervals for ALT, Amy, AST, GGT and LDH for women in the 22nd–26th gestational week of uncomplicated pregnancy. Overall, the listed reference limits are lower than the corresponding limits for females in the general population [6, 7]: our data confirm that this is clear especially as regards GGT. Speculatively the exclusion criteria adopted for recruitment of reference population might be advocated to justify this fact: alcohol consumption and use of some therapeutic drugs may cause an increase of GGT serum concentration by induction of new enzyme activity, whilst raised GGT concentrations are associated with gestational diabetes mellitus [8]. Not only the reference values in uncomplicated pregnancy for GGT, but also the reference values for ALT, AST and LDH were lower than the respective non-pregnant reference values: pregnancy-specific reference values may allow earlier and more accurate recognition of abnormal liver function in women with pregnancy complications, such as pre-eclampsia. Reference values for Amy in uncomplicated pregnancy were similar to reference values usually adopted for non-pregnant women.

There are strengths and weaknesses to our study. In the attempt to produce reference values traceable to the primary reference measurement procedure in accordance with the IFCC recommendations, we used commercial methods reportedly traceable to the IFCC standardization. Furthermore, to stay on the safe side, we provided direct confirmation of the traceability of analytical data and reported a good agreement between values from the commercial methods and values from the relevant primary reference measurement procedures in the analysis of serum pools. Such direct confirmation was available for ALT, AST, GGT and LDH, while it was not possible for Amy, owing to unavailability to these authors of the reference measurement procedure. All women involved in this study were in the 22nd–26th gestational week, therefore the herein proposed reference intervals primarily concern this clear-cut period; however, it was shown that changes of ALT, Amy, AST, GGT and LDH concentrations during the whole pregnancy are clinically insignificant [9, 10].

Table 1 Differences between the commercial methods employed for analysis of the reference population samples and the corresponding IFCC standardized reference measurement procedures, evaluated on the basis of quality specifications derived from biological variation [4].

Assay	Reference procedure value[a], U/L	Commercial method value[b], U/L	Difference, %	Quality specification
ALT	14.6	13.5	−7.4	Optimum
	25.3	24.5	−3.3	Optimum
	67.3	64.5	−4.2	Optimum
AST	18.4	16.5	−10.3	Desirable
	28.0	27.0	−3.5	Optimum
	52.1	50.5	−3.1	Optimum
GGT	17.5	17.0	−3.1	Optimum
	34.6	34.0	−1.7	Optimum
	57.3	55.5	−3.2	Optimum
LDH	132.5	127.5	−3.8	Optimum
	196.6	195.5	−0.5	Optimum
	337.2	345.0	2.3	Optimum

[a]Mean of four replicates (two replicates in each of two runs);
[b]mean of two replicates.

Table 2 Proposed reference intervals for women in the 22nd–26th gestational week of uncomplicated pregnancy.

Assay	2.5th percentile (90% CI)	97.5th percentile (90% CI)
ALT, U/L	7 (6–8)	30 (29–31)
Amy, U/L	33 (23–35)	110 (108–114)
AST, U/L	10 (9–11)	28 (27–30)
GGT, U/L	4 (4–4)	16 (15–17)
LDH, U/L	116 (110–120)	207 (202–221)

Furthermore, the number of subjects recruited for this study widely exceeds the minimum sample size of 120 individuals for calculation of reference values recommended by the IFCC [2].

To our knowledge, this is the first study reporting reference values for ALT, Amy, AST, GGT and LDH assuredly traceable to the IFCC standardization in uncomplicated pregnancy. The proposed reference intervals may be adopted by laboratories employing such standardized analytical methods.

Conflict of interest statement

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