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Need for Reference Ranges for Liver Function Tests in Infants and Children from Taita Taveta County, Kenya

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Abstract

Reference data for clinical chemistry on reference values of children in Taita Taveta County are fundamental for clinical diagnosis, management and research. In such a study, a target sample population of 120 respondents is usually required. In this study, a total of 577 healthy subjects from Taita Taveta population were employed to generate the normal reference data on eight clinical chemistry markers. The data obtained will provide needed information to medical practitioners with regards to patient management, as well as conducting research studies on disease.

Keywords: Clinical chemical parameters; Reference values; Liver function test; Children

Background

Established normal laboratory reference values are critical in the interpretation of patient's test results, proper patient's management as well as in research work. Normal Reference ranges are established from a reference population which is selected on the basis of gender, feeding habits and nutrition environmental conditions, race and age [1-3]. It is essential that reference values are established from appropriate and relevant populations [3,4]. Studies on reference values in Kenyan adults and Tanzanian populations, showed a remarkable differences when compared to normal ranges from European countries [1,5].

The normal laboratory reference values currently employed in clinical and research institution in most of African countries, Kenya included, are referred from textbooks and local research works some of which, are decades old [6,7].

These referred reference values are based on European populations where noticeable and significant differences have been scientifically proven from limited researches that have been done in south of Sahara [1,2]. The Clinical Laboratory and Standards Institute (CLSI) recommends that normal laboratory reference values should be established from a specific population and which should be verified every 5 years [8,9]. This research was conducted to establish liver function tests normal reference values for 1- 17 years old residents of Taita Taveta district, Kenya.

Materials and Methods

Study area

This study was undertaken at Moi County Referral Hospital in Voi-Taita Taveta region, Kenya. The research was done in Voi which located in sisal farming region approximately 591 meters above sea level in Coastal zone of Kenya.

Participants

Reference population consisted of healthy female and male population of ages between 1 and 17 years old citizens residing in Taita Taveta region for more than half a year. The study participants were drawn from different regions of Taita Taveta County of Coast region. Infants and children (with written consent from parents and/or guardians), were recruited into the study. Only those who met the required criteria were involved in the study.

Ethical consideration

Ethical approval was obtained from Kenyatta Ethical Review Committee (KUERC) and authorization to carry out research was granted by Health department Wundanyi, Kenya.

Inclusion as well as exclusion criteria

Healthy children of between 1 to 17 years residents in Taita Taveta region for more than half a year were incorporated in the research.

All serum samples that tested positive either for HIV, Hepatitis B antigen, Syphilis and pregnant adolescents were all excluded from this study.

Immunochromatographic reagent strip (Determine HIV-1/2, Tokyo, Japan) screened for HIV antibodies. A 50 µL of serum sample was introduced on to sample pad. This was left for a minute before a chase buffer was applied. The results were read after 15 min. Appearance of two red bars on the strip indicated the sample was Positive for HIV, while a single red bar on the control meant the sample under study was negative for HIV antibodies.

HbsAg were screened using single step hepatitis B Surface Antigen Strip (HBsAg, from Beijing, China). This is a qualitative immunoassay test, where by a test pad with immersed serum is put under test for 10-15 min. Then the strip is then placed on a flat surface. Positive result were indicated by appearance of double distinct red lines on

control and test regions on the strip while Negative results were indicated by the appearance of only one red bar at the control window.

The presence of *Treponema pallidum* antibodies was tested with ultra-rapid method which is a qualitative test strip that is immunoassay based (Beijing, China). A 50 ul of test sample was introduced on to a sample pad and was allowed to stand for a minute before addition of buffer. Those samples that had two red bars were Positive with syphilis while a single line on the control window indicated absence of *treponema pallidum* antibodies.

Specimen collection

Blood used in the study was sampled from a healthy population of children and infants at day time between July 2012 and November 2012.

Three milliliters of whole blood was bled using five mills syringe and needle for those children who were above five years of age and a younger ones scarp vein was used to bleed them with aid of a syringe.

Then the collected blood sample was transferred into plain vacutainer, labelled with subject's name, age and research number. Then the blood specimens, in ice-cool box were transported to analyzing laboratory. In the Laboratory, the blood samples were centrifuged, separated and were screened for Hepatitis A and C antibodies, human immunodeficiency virus (HIV), Syphilis antibodies and pregnancy for adolescent girls.

Specimen transportation, processing and storage

In the Laboratory, the blood samples were centrifuged, separated and were screened for Hepatitis A and C antibodies, human immunodeficiency virus (HIV), Syphilis antibodies and pregnancy for adolescent girls. Samples that were negative from any of the tested disease conditions were frozen awaiting analysis. Analysis was done at Moi County Referral hospital located in Voi Kenya.

Laboratory analysis

Nine liver function markers were analysed on the separated sera, they were: total proteins, albumin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Gamma-glutamyl transferase (GGT), lactate dehydrogenase, total and direct bilirubin. Standard procedures (SOPs) were followed during analysis as they are developed and maintained at the Moi District Hospital, Voi. Cobas Integra® 400 chemistry auto analyzer was used, that is manufactured in Germany (Roche Diagnostics).

Calibration of the machine

Calibration for Autoanalyzer systems (C.f.a.s) was used to perform calibrations automatically.

Quality assurance (QA)/Quality control (QC)

To guarantee accuracy and precision of results, precautions were observed though out the analytical phase. Internal quality control sera were from Roche diagnostics; that are responsible with Precipath and Precinorm external quality control samples from American Institute of proficiency (API) that is responsible in monitoring machine performance at Moi District Hospital Laboratory. Manufacturers' instructions in addition to quality control protocols were followed throughout the analytical period as per Ohmann, 1997 specifications.

Data management

Data was entered in a Microsoft Excel spreadsheet, cleaned and exported to Statistical Package of Social Sciences. Then the data was visually inspected and all the values found to be physiologically impossible were removed. Outliers in the remaining data were identified by use of Box plots as outlined by Horns and Pesce [10].

Statistical analysis

The first quartile ($Q_{.25}$), the median ($Q_{.50}$) in addition to third quartile ($Q_{.75}$) were determined, then interquartile range was calculated (IQR) from the differences of third and first quartiles ($Q_{.75}$-$Q_{.25}$). Data that was observed to be lower than 1.5 × IQR of first quartile, or higher than 1.5 × IQR of third quartile was considered as outliers and was manually deleted. These exclusions led to some parameter results missing hence difference in sample sizes for different parameters.

The data was subjected to normality test and was not normally distributed hence Kolmogorov-Smirnov plus Shapiro-Wilk statistical methods were varied in this study. This necessitated the use non-parametric (NCCLS, 2000) [2]. Non-parametrically, reference intervals, and median values were obtained from analyzed results separately for females and males at 95% confidence limit (2.5^{th} and 97.5^{th} percentiles as outlined by Horns and Pesce [10].

P-values for disparity between female and male participants were determined with use of Mann-Whitney test. Values that had ($p \leq 0.05$) were considered to be significantly different. Comparison for reference interval for diverse age sets and gender was determined by use One-Way-ANOVA, while Dunnetts Multiple tests for Comparisons since none of age categories had the minimum sample population of 120 as required in determination of reference intervals (NCCLS, 2000) [2]; $p \leq 0.05$ were considered significant.

Results

Established reference ranges for infants and children

Liver function panel parameters (LFTs) TP, ALT, ALB, AST, LDH, BIL-T and BIL-D demonstrated significant gender differences ($p \leq 0.05$ values) except ALT, ALP and GGT (Table 1). Throughout the entire analysis, daily quality control was observed and all standard deviations (SD) noted. All daily quality control that was run were within acceptable ranges of ± 2SD of target values (Figures 1 and 2).

Analyte (Unit)	Sex	N	Median	Percentiles		Reference value	RI	Difference between M&F	
				2.5^{th}	97.5^{th}	Z-value		Sig	

TP (g/L)	M/F	553	73.7	59.8	85.7	59.8-85.7	26.0	-2.431	0.015*
	F	270	74.2*	60	86.6	60.0-86.6	26.6		
	M	282	73	58.8	84	58.8-84.0	25.2		
ALB (g/L)	M/F	553	46	35.6	53	35.6-53.0	17.4	-2.431	0.025*
	F	270	46.4*	37.4	53	37.4-53.0	15.6		
	M	282	45.8	32.8	53	32.8-53.0	20.2		
ALP (U/L)	M/F	553	271.7	74.7	566.6	74.7-566.6	419.9	-1.498	0.134
ALT (U/L)	M/F	552	11	3.1	31.1	3.1-31.1	28	-1.413	0.158
AST (U/L)	M/F	553	25*	10	52.1	10.0-52.1	42.1	-2.718	0.007*
	F	270	27.2	11	54.5	11.0-54.5	43.5		
	M	282	23.8	10	51.1	10.0-51.1	41.1		
GGT (U/L)	M/F	362	19	10	44	10-44	34	-0.434	0.664
BIL-D (µmol/L)	M/F	551	7.07	2.59	16.58	2.59-16.58	13.99	-2.576	0.01*
	F	269	7.42*	3.12	16.6	3.12-16.6	13.48		
	M	281	6.8	2.3	16.81	2.3-16.18	13.88		
BIL-T (µmol/L)	M/F	551	11.2	4.49	27.04	4.49-27.04	23.5	-2.718	0.034*
	F	270	11.75*	4.79	27.76	4.79-27.76	24.8		
	M	280	10.5	3.81	26.48	3.81-26.48	23.6		

Results are expressed as Medians for the number of subjects indicated under the column labelled N. The sex difference is significant at $p \leq 0.05$, Sig=Significance..... (What does the*mean?). Include the meaning on the legend.

Table 1: Establishment of Reference Ranges for TP, ALB, ALP, ALT, AST, GGT, LDH, BIL-D and BIL-T for infants and children of Taita Taveta County, Kenya.

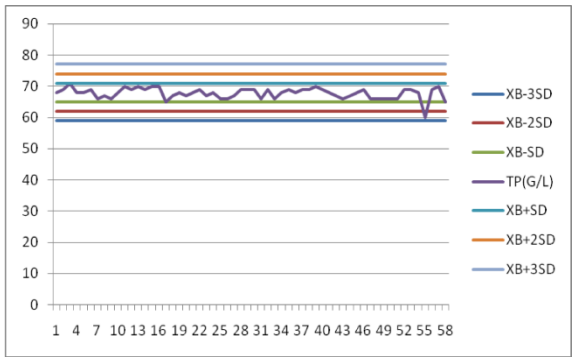

Figure 1: Levy-Jennings normal control chart for Total protein.

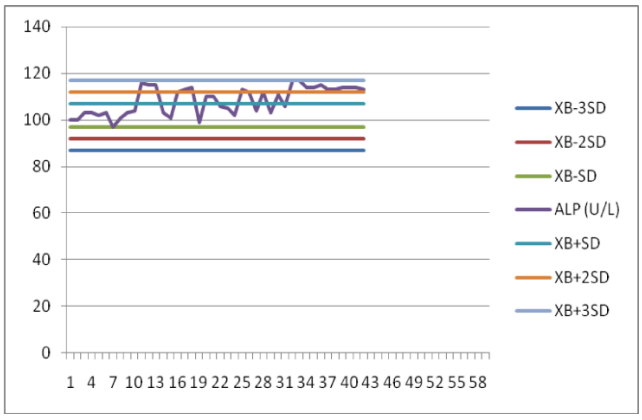

Figure 2: Levy-Jennings normal control chart for ALP.

Reference Intervals for Different Age Sets in Healthy Infants and Children of Taita Taveta County of Coast region, Kenya

The study population was grouped into four categories: Category I (1-5 years); Category II (6-10 years); Category III (11-15 years); and Category IV (>15 years). Reference interval between females and males for each gender was estimated by use of t-test, while ANOVA and post ANOVA was used to determine the differences between four age sub-groups. Statistically significant values were those that had P less than 0.05, ($P \leq 0.05$) (Table 2).

In age category I, males recorded significantly higher values of BIL-D than female of corresponding age, while in age category II and IV; female children had a significantly higher level of the same metabolite than males. In age category II, males had significantly decreased values for AST and TP (Figures 3 and 4). In age category III, AST values for male were significantly lower, and significantly elevated levels for LDH than those of females of same age category (Table 2) (Figure 5).

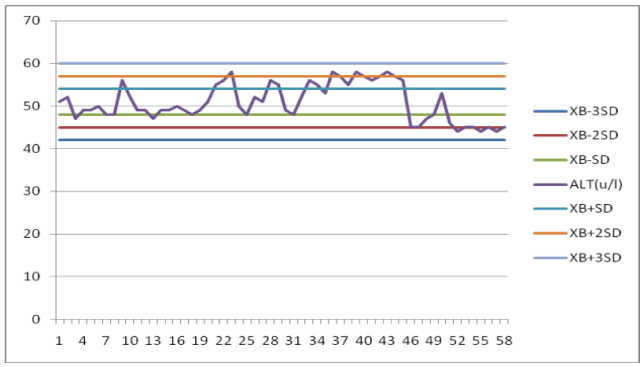

Figure 3: Levy-Jennings for normal for control chart ALT.

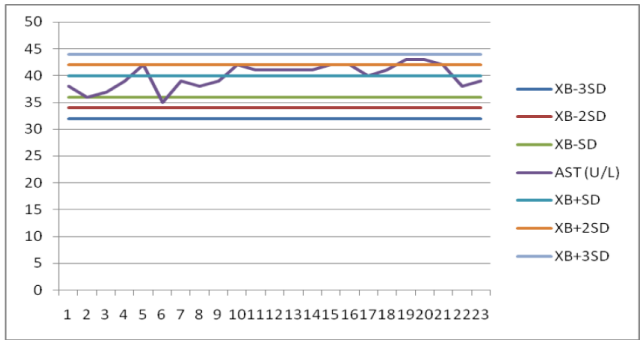

Figure 4: Levy-Jennings control chart for AST (normal).

There was a significance decrease in LDH in male : significantly decreased level of LDH in age category II compared to age category I; a significantly decreased level of LDH in age category III compared to age category I; a significantly decreased level of BIL-T, AST, ALP, LDH, and GGT in age category IV compared to age category I; and significantly decreased values for BIL-D, BIL-T, AST, ALP, GGT, and LDH in age category IV compared to age category III (Table 2).

Female under fifteen years of age had: a significantly decreased level of AST and LDH in age category III compared to age category I; a significantly decreased level of AST, ALP and LDH in age category IV compared to age category I; a significantly decreased level of AST in age category III compared to age category II; a significantly decreased level of AST, ALP, and TP in age category IV compared to age category II; a significantly decreased level of AST, ALP and LDH in age category IV compared to age category III (Table 2).

The biochemical valuables for ALB, and ALT ii the four age categories were age independent (Table 2).

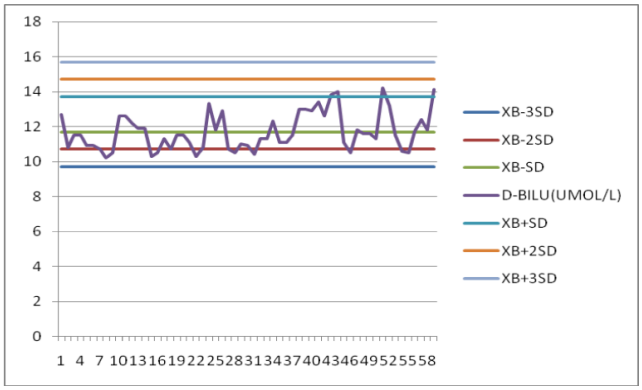

Figure 5: Levy-Jennings control chart for BIL-D (normal).

Analyte (Unit)	Sex	Reference range at varying age intervals							
		1-5 years	N	6-10 years	N	11-15 years	N	>15 years	N
TP (g/L)	M	73.06 ± 4.42	36	72.75 ± 6.51*	75	73.46 ± 6.43	86	71.21 ± 5.07	82
	F	72.93 ± 7.05	27	75.47 ± 6.78	75	74.71 ± 8.72	88	71.81 ± 6.31e	72

ALB (g/L)	M	44.51 ± 3.13	36	44.69 ± 4.37	75	45.16 ± 5.07	86	45.05 ± 5.76	82
	F	45.66 ± 3.76	27	45.84 ± 3.29	75	45.35 ± 3.94	88	46.97 ± 3.62	72
ALP (U/L)	M	322.78 ± 91.92	36	315.90 ± 100.22	75	302.45 ± 147.79	86	207.91 ± 162.00^{cef}	82
	F	321.69 ± 63.87	27	308.43 ± 91.97	75	301.50 ± 132.65	88	136.41 ± 74.00^{cef}	72
ALT (U/L)	M	11.86 ± 5.58	36	13.46 ± 10.62	75	13.51 ± 15.72	86	12.73 ± 7.85	82
	F	12.56 ± 5.71	27	12.89 ± 7.07	75	11.55 ± 5.74	87	11.40 ± 7.23	72
AST (U/L)	M	34.53 ± 11.67	36	29.64 ± 9.23*	75	26.28 ± 14.12*	86	17.76 ± 9.76^{cef}	82
	F	36.49 ± 9.39	27	34.34 ± 13.47	75	29.02 ± 10.06^{bd}	88	18.96 ± 8.73cef	72
GGT (U/L)	M	23.06 ± 7.65	19	20.81 ± 7.12	41	21.38 ± 8.84	58	18.96 ± 9.92^{ce}	74
	F	22.58 ± 8.45	17	20.12 ± 5.87	36	24.55 ± 26.75	47	24.55 ± 6.84	60
BIL-D (µmol/L)	M	8.04 ± 3.41*	36	6.82 ± 2.82*	75	7.87 ± 3.55	86	6.44 ± 3.27^{*f}	81
	F	7.94 ± 3.12	27	8.06 ± 3.34	75	8.00 ± 3.80	87	7.89 ± 3.56	72
BIL-T (µmol/L)	M	13.68 ± 6.54	36	11.59 ± 5.00	75	12.75 ± 5.73	85	10.13 ± 5.68^{cf}	81
	F	13.57 ± 6.00	27	13.47 ± 6.02	75	13.33 ± 6.57	88	11.98 ± 6.69	72
∗abcddef									

Table 2: Establishment of age and sex related reference ranges for TP, ALB, ALP, ALT, AST, GGT, LDH, BIL-D and BIL-T for infants and children of Taita Taveta County, Kenya.

Discussion

This research provides the primary established clinical liver function test reference intervals for both males and females of 1-17 years for Taita Taveta District in Kenya. Similar research studies on respondents aged 18-55 years demonstrated variation in reference ranges [1,3,5,6,8,9,11,12].

Variation in BIL-D, BIL –T, AST, ALP, GGT and LDH showed decreased levels. This is thought to be due to variance in sex hormonal changes as well as difference in body mass. Variations in biochemical parameters of different ages and sex do suggest that some of parameters are dependent on age. The rise in the established serum reference levels for Bilirubin among males and females with advance in age could be attributed to general increase in body weight as they advancing age as explained by Kuzuya et al. [13]. This was also noted in AST, whereby there was statistically notable difference between females and males of age group of 6 to 10 years, 11 to 15 years and those above 15 years, this might be partly owed to decline in liver functions as well as integrity with genetics and age. These finding are in conformity with other similar research findings [14].

Evaluation of liver function parameters TP, ALB, AST, BIL-D, BIL-T, ALP.ALT and GGT all varied with manufactures reference values [1,12,15], these variations may perhaps be attributed to liver not being fully functional. From this study, it was evident there were variation in TP, ALB, AST and BIL-T per sex (Table 1). There was significance difference in TP, ALB, ALT, BIL-D and BIL-T with reference in use at Moi District Hospital values. In Table 2, different was also noted in varying age intervals in 6-10 for TP for males and females and over 15 years females and those of ages 11-15 years (Figures 6 and 7). For ALP, there was significance difference between 6-10 years, 11-15 years and those above 15 years. AST and BIL-D, while in BIL-T there were difference age intervals 6-10 years, 11-15 years and above 15 years for male only. The above variations could be due to environmental conditions, diet, [16] and analytical method [2].

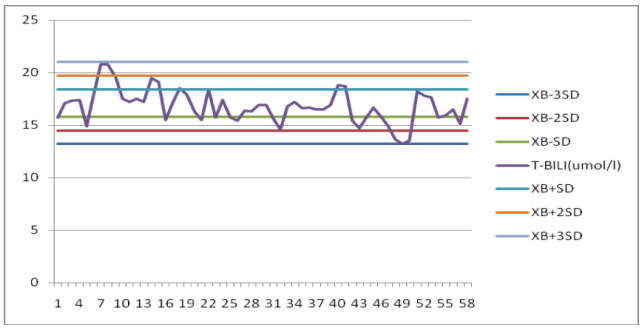

Figure 6: Levy-Jennings control chart for BIL-T (normal).

Different genetic composition and lifestyles of different groups of people may also explain the differences that exist within members of the same population in terms of gender as tabulated in table 1 as well as in table 2. Other counties have reported similar differences in other age categories [3,5,8].

From the study its evident that diet, physical, genetics and social conditions have an impact on physiological functions in the same population [17,18].

The reference ranges that were established in this research study, most of them varied within the same population. This proves the need to establish age and gender reference values which are applicable to

exact populations for diverse geographical regions, with various diets and mineral compositions in soils instead of using biochemical reference ranges that are determined from different populations and geographical locations to be applied for all populations [1,2,9,17].

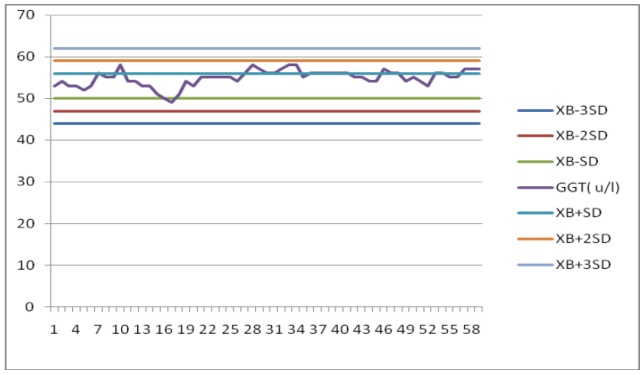

Figure 7: Levy-Jennings control chart for GGT (normal).

Conclusion

This research study established; liver function parameters for ages 1 to 17 years of Taita Taveta-Kenya, which are independent from quoted in literature and different reagent's manufacturers. There was evidence of variation of some parameters from reported literature. This concur with other research findings in other parts of the world [1,2,5,8,9,12,19,20].

Acknowledgement

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